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Editorial Information

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EXTENDED ABSTRACT

Development of Preliminary Classification System for Drug Related Problems in Patients Receiving Radiopharmaceuticals

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SUMMARY

The identification and management of drug related problems (DRP) are essential components of the pharmaceutical care process. Therefore, a specific classification system for DRP associated with radiopharmaceuticals (DRPr) is needed to optimize patient care. In this study, a systematic searching of peer-reviewed English literature was performed and 62 types of DRPr were identified. A preliminary classification system was developed consisting of 11 categories and 17 subcategories of problems and causes of DRPr, which can lead to misinterpretation of diagnosis, repetition of procedure, delay of patient treatment, and increase radiopharmaceutical expenditure. Thus, the documentation of DRPr is important to identify, manage and prevent them from occurring.

Keywords: Classification system, Drug related problem, Radiopharmaceuticals, Nuclear medicine, Pharmaceutical care

INTRODUCTION

Radiopharmaceuticals are drugs that contain radioactive compounds or radioisotopes. The use of radiopharmaceuticals for diagnostic and therapeutic purposes is widely recognised and is expected to increase in the future. Therefore, DRPr should be identified and properly managed to optimize patient care. However, data on DRPr are limited, and implementation of pharmaceutical care for patients receiving radiopharmaceuticals is often overlooked. DRP classification systems designed to assist healthcare practitioners are not suitable for radiopharmaceutical use, as reported by Petel et al. Besides, there is no universal classification system for DRP available currently. Due to the nature of radiopharmaceuticals, some categories are missing from the available classification system, making it difficult to document DRPr. Therefore, the development of a specific classification system for radiopharmaceutical patients is necessary to allow systematic DRPr documentation and evaluation of drug therapy in the nuclear medicine department. The aim of this study was to identify the types of DRPr and to suggest items for the classification system for DRP in patients receiving radiopharmaceuticals.

MATERIALS AND METHODS

A systematic searching of peer-reviewed English literature was performed for all periods using electronic databases: Web of Science, MEDLINE, and Google scholar. The search terms used include "radiopharmaceutical*", "radionuclide*", "problem*", "false-imaging", "altered biodistribution", "error", "adverse event", "side effect", "drug interaction", "pharmacologic intervention", "therapy", "treatment" and "diagnostic". The published journal articles that described the DRPr were included in the study. PRISMA guideline was followed for the article selection. Three reviewers (NDI, MFO and NI) were involved in the screening processes to ensure the included articles meet the eligibility criteria. The identified DRPr in the literature were then coded according to PCNE System V9.1 to generate the items for the preliminary classification system for DRP in patients receiving radiopharmaceuticals. Two primary domains of the PCNE System which are 'Problem' and 'Cause' were used to code the DRPr. This classification system was adapted for usability of DRPr documentation by giving suitable operational definition to each code. Any DRPr that misfitted with the coding system was put under the 'other' category.

RESULTS AND DISCUSSION

A total of 70 articles were retrieved from the literature search. Following the screening processes, 18 articles were finally included. Of these, 62 types of DRPr had been identified. The most common DRPr was administration problems (24.9%) which involves misadministration, inappropriate route of administration, infiltrated

injection or extravasation and wrong administration time. Administration problems of radiopharmaceuticals have been emphasized the most in literatures perhaps because of its impact to patient management such as repetition of procedure, delay of patient treatment, patient apprehension with the clinical activities and increase health expenditure. Several intervention strategies had been done to prevent the occurrence of DRPr focusing more on technical part such as staff training, implementation of bar-code system, and dose calibration. The involvement of pharmacist with other healthcare practitioners in direct patient care such as in detecting, documenting and preventing DRPr may be needed to reduce the occurrence.

For coding of system, most of the DRPr were misfit with the modified PCNE V9.1 (56.6%) showing the lack of items for radiopharmaceutical use in the system. The missing items are due to several DRPr that are only unique to radiopharmaceuticals such as imaging problems (11.3%), altered biodistribution (14.9%), errors in preparation of radiopharmaceuticals (12.9%), and errors in dosimetry measurement (3.2%).

Each code of items in the classification system should be specific and lead to one choice only. Therefore, items cannot be redundant in each code of system. Due to the missing of items in the classification system for DRPr, the misfitted DRPr were pooled together to form new categories and subcategories. A preliminary classification system was developed consisting of 11 categories and 17 subcategories of problems and causes of DRPr. Table 1 shows the items of the preliminary classification system. The pools of items may need further refinement for the classification system to ensure suitability for use in the clinical setting.

CONCLUSION

This study identified 62 types of DRPr that can occur in the nuclear medicine department. Different to other pharmaceuticals, the DRPr identified also involves technical aspect of the preparation to its administration hinting at the needs for dedicated classification for radiopharmaceutical use. This preliminary study could be useful for documentation of DRPr and pharmaceutical care research area.

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Table I: A preliminary classification system based on PCNE System V9.1

Code	Categories and Sub-categories for DRPr
P1	*Treatment effectiveness*
P1.3	Untreated symptoms or indication
P2	*Treatment safety*
P2.1	Adverse drug reaction (possibly) occurring
P3	*Other*
P4	*Imaging problem*
P5	*Altered biodistribution*
C1	*Drug selection*
C1.1	Inappropriate drug according to guidelines/formulary
C6	*Drug use process*
C6.1	Inappropriate timing of administration or dosing intervals by a health professional
C6.2	Drug under-administered by a health professional
C6.3	Drug over-administered by a health professional
C6.5	Wrong drug administered by a health professional
C6.6	Drug administered via wrong route by a health professional.
C6.7	Extravasation/dose infiltration
C6.8	Drug administered to wrong patient
C7	*Patient related*
C7.5	Patient take food that interacts
C9	*Other*
C9.2	Other causes
C10	*Preparation and formulation procedure*
C10.1	Exceeded percentage of radionuclide/radiochemical/particulate impurities from the pharmacopoeia/guideline
C10.2	Errors in labelling of radiopharmaceuticals vial / container
C10.3	Pregnancy form missing/incomplete
C11	*Dosimetry*
C11.1	Dosimetry too high
C11.3	Dosimetry too low

P=Problem; C=Cause

EXTENDED ABSTRACT

Factors Associated with Second-Hand Smoke (SHS) Exposure among University Students in Malaysia

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SUMMARY

A cross-sectional study was conducted among university students to investigate the prevalence and factors that contribute to second-hand smoke (SHS) exposure. Almost half of the 387 participants reported they had been exposed to SHS within the past 30 days with higher exposure found among students age between 19 to 24 years old, male, taking business and management related courses, residing in an urban area and apartment-type housing. SHS exposure was lower in those who had a current illness and rated their health as 'Poor.' Further analysis showed a significant association between gender and SHS exposure ($p = 0.048$).

Keywords: Multi-unit housing, Second-hand smoke exposure, Smoking, Socio-demographic, University students

INTRODUCTION

Secondhand smoke is defined as the involuntary inhalation of tobacco smoke by non-smokers near smokers. Associated with increased mortality risk from cardiovascular, respiratory disease, and cancer, SHS exposure contributed to 1.2 million deaths worldwide annually and a 2019 national survey highlighted 1 in 2 Malaysians was exposed to SHS. Parental and peer smoking, poor knowledge, residing in multi-unit housing were among factors related to higher SHS exposure (1-3). In 2003, Malaysia introduced a 100% smoke-free policy in universities aiming to reduce the smoking prevalence and SHS exposure among university students. Considering the COVID 19 lockdown situation, where most students are confined at home, SHS exposure for those with smoking family members is expected to be higher in this period (2). Therefore, this study aimed to investigate the prevalence of SHS exposure and its associated factors among Malaysia's university students.

MATERIALS AND METHODS

A cross-sectional study was conducted between April to May 2021 among 387 university students who are either non-smokers or ex-smokers (at least six months' abstinence period). Data were collected using a validated online questionnaire adapted from past studies (3-4). Following a pilot study among 20 university students, the questionnaire was disseminated via social media platforms such as WhatsApp, Facebook and Twitter. The questionnaire consisted of three sections. Section one contained the participant's demographic information, while second and third section contained questions which evaluate the prevalence and characteristics of participant's SHS exposure. IBM SPSS version 26 software was used in the data analysis. Chi-square was used to determine the association between participants' variables and SHS exposure. All the variables that were significant at $p < 0.05$ level in the chi-square tests were tested in multivariate regression analysis. This study has obtained ethical approval from UiTM Research Ethics Committee (REC/03/2021-UG/MR/230).

RESULTS AND DISCUSSION

Almost half (49.9%) of the participants reported they had been exposed to SHS within the past 30 days with significantly higher exposure among students age between 19 to 24 years old, male, taking business and management related courses, residing in an urban area and apartment-type housing. Further analysis revealed a significant association between gender and SHS exposure, with male students having a higher prevalence of SHS exposure (60.3 %) than female students (47.5 %) ($p = 0.048$). The higher SHS exposure probably due to social relationships with other male friends, many of whom are smokers. It may also be associated with

their lower level of SHS avoidance compared to the female students (5). The primary areas of exposure were in public places (64.8%) and at home (57 %). High SHS exposure at home is unavoidable particularly in students with smoking parents or family members. In this study, 51.7% of the participants reported having family members who are current smokers. Metabolites of tobacco-specific lung carcinogens associated with SHS have been detected in non-smokers with smoking household member. The constant exposure to SHS can lead to increased risk of coronary heart disease, stroke and lung cancer in exposed adults, and increased risk of respiratory infections among exposed children. Apart from the health risks, individuals are more prone to smoking initiation following frequent SHS exposure at home with each day of increased SHS exposure would result in a 16 % higher likelihood of smoking initiation (2). SHS exposure was lower in those who had a current illness and rated their health as 'Poor' while exposure rate was nearly identical between those with and without ill family members. Further research with a longer-term follow-up and larger sample is needed to confirm the findings and to establish cause-and-effect relationships between SHS exposure and the associated factors.

Table I: Univariate analysis of SHS exposure by socio-demographic and health-related characteristics (n =387)

Variable		Exposed		Non-Exposed		P value[a]
	n	n	%	n	%	
Gender						
Female	304	149	47.5	165	52.5	0.048
Male	73	44	60.3	29	39.7	
Ethnicity						
Malay	379	187	49.3	192	50.7	0.151
Others	8	6	75.0	2	25.0	
Faculty of study						
Science & Technology	322	158	49.1	164	50.9	0.355
Social Science & Humanity	25	11	44.0	14	56.0	
Business & Management	40	24	60.0	16	40.0	
Type of residency						
Landed house	290	146	50.3	144	49.7	0.398
Flat/Apartment	30	19	63.3	11	36.7	
Condominium/Serviced apartment	8	4	50.0	4	50.0	
Place of residency						
Urban	173	97	56.1	76	43.9	0.082
Rural	155	72	46.5	83	53.5	

CONCLUSION

The prevalence of SHS exposure among university students in Malaysia is high at 49.9% with higher exposure reported among students who were male, residing in urban area and apartment-type housing. Implementation of smoke-free home policies and ongoing awareness campaign on SHS are expected to enhance university students' protection against its exposure.

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EXTENDED ABSTRACT

Generational Differences of Knowledge, Attitudes and Practices on Herpes Simplex Virus (HSV) Infection

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SUMMARY

Herpes zoster (HZ) or shingles refers to an infection that causes a painful rash due to reactivation of the varicella-zoster virus (VZV), the same virus that causes chickenpox. The majority of respondents reported that they had heard of herpes zoster. The older generations mainly had misconceptions towards HZ, while the students have more knowledge regarding herpes zoster compared to their parents. From this study, it can be concluded that extensive public education regarding herpes zoster is needed, together with effective prevention strategies to establish awareness and knowledge of herpes zoster among people of various ages.

Keywords: Herpes zoster, Shingles, Knowledge, Attitude, Practice

INTRODUCTION

Herpes zoster (HZ) or shingles refers to an infection that causes a painful rash due to reactivation of the varicella-zoster virus (VZV), the same virus that causes chickenpox. As the incidence of HZ is increasing globally and with the current treatment, doctors can only lessen the duration of the disease and reduce the pain (1). Other than modern medicines, traditional Chinese medicines and apple cider vinegar was used to treat HZ, which may help reduce pain and discomfort (2). The elderly prefers to practice their ways of treating the disease than the younger generation, who believes in modern medicine, and there has been limited research conducted in Malaysia to assess the knowledge, attitudes, and practices towards HZ. This study explores generational differences in knowledge, attitude, and practice in HZ among the pharmacy students representing the younger generation versus their parents.

MATERIALS AND METHODS

This study is a cross-sectional study involving 200 respondents consisting of pharmacy students of Universiti Teknologi MARA (UiTM) Puncak Alam Campus and their parents. Based on the inclusion criteria, the respondents were recruited by convenience sampling to participate in this study. Both parents and pharmacy students who had the competency to understand English or Bahasa Melayu were also recruited. Respondents that unable to return the complete questionnaire were excluded. In the questionnaire, HZ was referred to as 'shingle'. The questionnaire was adapted from previous studies, and the final version of the questionnaire consisted of four sections: demographic, knowledge (8 items), attitude (5 items), and practices (6 items) (3, 4). A 5-point Likert scale, closed-ended questions, and multiple choices were used for knowledge, attitude, and practice sections. This study was approved by the Universiti Teknologi Mara Research Ethics Committee before the commencement of the study with reference number REC/03/2021 (UG/MR/219).

RESULTS AND DISCUSSION

A total of 200 respondents completed the survey distributed through email and instant messaging applications consisting of 100 responses each from parents and pharmacy students with a mean age of 38.69 ± 16.44 years ranging from 21 to 66 years old. Of the 200 respondents, 183 reported that they had heard of herpes zoster. The most known symptoms recognised by students and parents were rash, blisters, and neuropathic pain. 80% of the students have more knowledge compared to their parents (68%) regarding the disease caused by VZV, similar to a global survey conducted by Paek & Johnson in 2010 (4). The majority of the respondents agreed that shingles have a significant effect on health and are worried about being infected. The treatment choices of students and their parents were almost similar and in line with the standard medical practices, except the older respondents preferred

allopathic medicine/hospital specialists compared to corticosteroids as the youngsters did. However, 22% of parents did not want to be vaccinated compared to 7% among students. This outcome is similar to the findings in a cross-sectional study in South Korea by Yang et al., where 85.8% of the public were willing to be vaccinated or vaccinate their parents aged more than 50 years old against HZ (5). It is important to highlight that this study contains a few limitations. Firstly, the participants were pharmacy students in UiTM Puncak Alam, and their parents were recruited using convenience sampling. Therefore, this study could be subjected to selection bias. Secondly, the findings of this study also may have limited generalisability to the general population. Thus, representative samples from a broad range of people in terms of age, ethnicity, education, or medical history may be recruited in future studies to attain a more diversified group of people and further corroborate other associations with socio-demographic respondents.

Table I: Demographics of respondents

Demographic	No. (%) of respondents	
	n	%
Age (years)		
Mean ± SD		38.69 ± 16.44
Gender		
Male	36	18.0
Female	164	82.0
Educational attainment		
Primary or below	1	0.5
Secondary	39	19.5
Tertiary or above	160	80.0
History of chickenpox		
Yes	151	75.5
No	33	16.5
Unsure / Do not know	16	8.0
Have you ever heard about "herpes zoster" or "shingles"?		
Yes	183	91.5
No	17	8.5
History of shingles		
Yes	12	6.0
No	163	81.5
Unsure / Do not know	25	12.5

CONCLUSION

From this study, it can be concluded that students have more knowledge regarding HZ than their parents. Extensive public education regarding herpes zoster and effective prevention strategies are needed to establish awareness and knowledge of herpes zoster among people of various ages. These may eventually improve quality of life and health and, most importantly, correct the misconceptions of HZ.

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EXTENDED ABSTRACT

Knowledge and Practise Towards Sunscreen Application among Pharmacy Students: A Cross-sectional Study in Universities

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SUMMARY

The first line of defence against skin cancer and sun-damaged skin is sunscreen. The goal of this study is to ascertain the knowledge and practise of pharmacy students regarding the use of sunscreen. Descriptive statistics in the form of percentages and frequencies were used. For inferential statistics, Fisher's exact test, independent sample t-test and one-way ANOVA were used. 296 students took part in the study. Students' knowledge scores were often higher for females than for males. Most pharmacy students (83.1%) who use sunscreen are female (76.4%). In terms of knowledge, being female and having a higher CGPA show significant results. In terms of sunscreen use, female and Malay students were found to use it more. Widespread education about sunscreen application may improve the public's knowledge regarding sunscreen.

Keywords: Sunscreen, Skin pigmentation, Sun protection factor, Ultraviolet rays, Pharmacy

INTRODUCTION

The greatest organ of the human body is the skin, which is constantly exposed to ultraviolet radiation (UVR) and other risks from the sun. Chronic skin disorders can result from prolonged exposure to UVR (1). The frequency of human skin disorders caused by UVR can be significantly reduced by using sunscreen (2). Hence, one of the strategies to avoid skin disorders is by applying sunscreen, which can shield UVR. Use of sunscreens will lead to a tolerance of the skin towards UV (3). However, the utmost priority is to ensure the effectiveness of sunscreen. Knowledge and practise in the application of sunscreen can influence its use and effectiveness. Thus, the purpose of this research is to determine the knowledge and practise of sunscreen application. This study is aimed to be done among pharmacy students at three different universities. As pharmacists, specifically community pharmacists, also play a role in the management of skin disorders, the study would like to determine the knowledge of these future pharmacists during their undergraduate years.

MATERIALS AND METHODS

This study is a cross-sectional study that used a validated questionnaire adapted by Awadh et al. (4). This study used a convenience sampling strategy. The questionnaire was distributed via Google Form among pharmacy students at the three universities, which are Universiti Brunei Darussalam, UiTM Puncak Alam, and KPJ Healthcare University College. The questionnaire contained information regarding demographic profile, knowledge, and practise regarding sunscreen application. The data was analysed using Statistical Package for the Social Sciences (SPSS) version 25.0. The data on demographic background, knowledge, and practise of sunscreen were analysed in terms of frequencies and percentages. Fisher's exact test was used to examine the association between categorical independent variables and dependent variables. The differences in the pharmacy student's knowledge scores were compared using an independent sample t-test for two groups of data and a one-way analysis of variance (ANOVA) for data with three groups and above.

RESULTS AND DISCUSSION

In terms of knowledge score, only gender and differences in CGPA show a statistically significant result with a p-value<0.05. Females had higher knowledge about sunscreen compared to males ($p = 0.000$), and those with CGPA > 3.5 had the highest knowledge. Other variables such as ethnicity, family education, and current year of study did not have any significant differences.

Examining the correct response rate, most respondents answered incorrectly for the question about the amount of sunscreen needed to cover the entire body. Most of them are unaware that the effectiveness of sunscreen is

only assured if it is applied on a regular basis with a sufficient amount, which is 2.0 mg/cm^2 (5). Regarding SPF (sun protection factor) value, the majority of students understood that greater protection would be provided by a higher SPF value. Nevertheless, the students were unaware that this protection is only for UVB. An indication for UVA protection is represented by the PA symbol, which has more + to indicate higher protection against UVA.

In terms of practise, female (p = 0.000) and Malay students (p = 0.014) were found to significantly use sunscreen compared to their counterparts. In terms of gender, it is consistent with the knowledge that females also used sunscreen more than males. However, for CGPA, those with a CGPA of 3.1–3.5 used sunscreen more. Awareness by female students of the associated risks of sun exposure could be one of the reasons contributing to this finding. This study also found that most respondents used sunscreen only on their faces. There are many other exposed areas to the sun; hence, the appropriate application should not be limited to the face.

CONCLUSION

The present study showed that knowledge and practise differences were associated with several factors as discussed. Some aspects of knowledge and awareness about sunscreen usage still need to be improved in the future and can be incorporated into undergraduate pharmacy education.

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EXTENDED ABSTRACT

Knowledge, Behavior and Practise of Self-Medication among Young Adults in Malaysia

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SUMMARY

The practise of self-medication has become a huge concern to consumers and the public health due to the rise in potential adverse drug effects and rampant irrational use of medicine in general. The right knowledge, behaviour, and practise towards self-medication should be well-taught within the population at the earliest point possible. The study aimed to assess the young adult's knowledge, behaviour, and practise towards self-medication. A questionnaire was disseminated to young adults in Malaysia through online platforms. 83.3% out of 222 respondents showed good understanding of the rational practise of self-medication, although they view the undesirable effects as not serious. Many stakeholders should be involved in devising a comprehensive strategy to increase health awareness among the public.

Keywords: Self-medication, Knowledge, Behavior, Practice, Young adults

INTRODUCTION

Globally, the practise of self-medication has been reported as drastically increasing. Self-medication (SM) is a human behavioural action in which a person uses medications to treat self-diagnosed minor symptoms or conditions, with the ability to do both good and harm (1). In most cases, self-diagnosis, inappropriate treatment, and limited knowledge of the medicine being taken could be detrimental to health as many would experience adverse drug reactions (2). The purpose of this study was to assess young adults aged 20 to 34 years old's knowledge, behaviour, and practise of SM.

MATERIALS AND METHODS

This is a prospective, cross-sectional, questionnaire-based study that was conducted from March to May 2021 among young adults in Malaysia, ages 20 to 34. An e-survey using a self-administered questionnaire was disseminated to 222 local respondents through social media such as Facebook, WhatsApp, Twitter, and Instagram. Data were processed by descriptive and inferential analysis and analysed using SPSS version 20 statistical software, an ANOVA one-way, and Pearson's Chi-square test of independence. It was conducted at $\alpha \leq 0.05$ is taken as statistically significant.

RESULTS AND DISCUSSION

Among the survey respondents (n = 222), most are Malay (95.5%) and female (84.7%). 83.3% of respondents were between the ages of 20 and 24; 91% were single; and 87.8% were students. 50.9% had a graduate degree, and 52.3% were in B40 of total house income with a monthly household income below RM4360. 49.5% of respondents mentioned they were practising SM to save time. Only 36.5% of participants admitted they had never sought a health professional's consultation before self-medicating. Most of the participants were aware of the consequences of SM. Hence, 72.0% of participants mentioned they only use the medication for a short time, and 51.4% would read patient information leaflets before consuming medications. High knowledge of self-medication levels was found to be prevalent since most of the participants were from educated groups. The level of knowledge regarding SM was 76.3%. This finding contradicts a similar study by Azhar et al. that showed the knowledge level to be low to moderate (3). Advances in the health care system and services, as well as established awareness campaigns of the rationale use of drugs, may explain the current finding (4). 6.3% of respondents practise SM due to poor access to healthcare facilities. Sambakusi CS et al echoed the finding, mentioning consumers from suburban locations

would prefer to use traditional herbal medications due to economic difficulties and a lack of trust in modern drugs (5). 68.9% of the respondents have never experienced side effects after practising SM, 29.3% have rarely experienced it, and 1.8% reported having experienced adverse drug reactions such as drug-drug interactions. 65.8% of the respondents self-medicated as their first action when experiencing minor illnesses such as cough and fever. Antipyretics and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to self-medicate, thus proper counselling by healthcare professionals is required to avoid potential major health complications. The continuous education of the public on the quality use of medicine concerning SM and awareness movements by MOH should be implemented.

CONCLUSION

Young adults' knowledge and behaviour towards self-medication is at a satisfactory level, despite its high practise. Community empowerment and a high health literacy level could be achieved through a comprehensive strategy directed at promoting quality and safe use of medicine during public education and awareness programmes.

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EXTENDED ABSTRACT

Open and distance learning (ODL) during the COVID-19 Pandemic: Factors Affecting Students' Emotional Health

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SUMMARY

The abrupt changes in learning mode to open and distance learning (ODL) during the COVID-19 pandemic affect the students' emotional health. The aims of the study are to assess the emotional health of the students' and the factors that affect their emotional health. A Pandemic Emotional Impact Scale (PEIS) revealed that students from urban areas had statistically significantly higher PEIS scores compared to those from rural areas (H (1) = 4.10, p = 0.043). Male students (p = 0.11), those from the Business & Management faculty (p=0.044) and those with a CGPA between 3.0 and 3.5 before the pandemic (p=0.037) were reported to be more isolated compared to other groups.

Keywords: distance learning, COVID-19 pandemic, students, emotional health

INTRODUCTION

During the COVID-19 pandemic, a Movement Control Order (MCO) was initiated. Following the MCO, the Ministry of Higher Education announced the implementation of ODL for university students (1). Students' stress levels rise as they transition from face-to-face learning to ODL, and other stressors at home may have an impact on their emotional health. However, information and/or studies about students' emotional health and the factors affecting it are limited. This study aims to assess the emotional health of students at Universiti Teknologi MARA (Puncak Alam campus) as a result of the COVID-19 pandemic and determine the ODL factors (generic skills, academics, social lecturers and accessibility issues) that affected their emotional health.

MATERIALS AND METHODS

Respondents were recruited using convenience sampling. A questionnaire was distributed via WhatsApp, Twitter, and Instagram using Google Forms. The instrument consisted of Section 1; demographic data, Section 2; a validated 16-item PEIS instrument (2). A higher total PEIS score indicates a greater emotional impact. The instrument comprises pragmatic concerns and emotional effects. Section 3 had 19 items on ODL related factors (social, academic, lecturer, and accessibility issues, and generic skills) (3). Both the PEIS instrument and ODL factors used a 5-point Likert scale, and responses range from not at all (0) to extremely (4) and strongly disagree (1) to strongly agree (5), respectively. Using the sample size calculator by Raosoft, the sample size needed was 377. The data was collected for four weeks and analysed using IBM SPSS for Windows version 21. Descriptive data, the normality test, Spearman's rho correlation, and the Kruskal-Wallis test were used in the study.

RESULTS AND DISCUSSION

Majority of the respondents were female (82.0%), third-year students (35.3%), and lived in urban areas (58.4%) with their parents (78.0%). Most of them had a CGPA between 3.0 – 3.5. The number of students with a CGPA of 3.0–3.5 increased by 8.5% after the pandemic, while the number of students with a CGPA of more than 3.5 decreased by 3.2%. The proportion of students with a CGPA of 2.0–2.9 falls from 11.14% prior to the pandemic to 5.84% as their most recent CGPA.

The highest PEIS scores (ranging from 0 = 'not at all' to 4 = 'extremely') indicated that they were less productive (2.92), had more difficulty concentrating (2.82), and were more bored (2.70), indicating the three most significant contributors to their emotional health. Emotional effects items also had a higher mean (2.41) than pragmatic concerns (2.18), indicating that students were more concerned with anxiety, depression, boredom, or frustration than with health, finances, or grief. A higher total PEIS score was strongly correlated with the following items: 'feeling more grief or sense of

loss', (rs = 0.80, N = 377, p ≤ 0.01), 'feeling more angry or irritated', (rs = 0.76, N = 377, p ≤ 0.01) and 'feeling that the future seems darker or scarier than before', (rs = 0.76, N = 377, p ≤ 0.01). Students from urban areas had significantly higher PEIS scores than those from rural areas (H (1) = 4.10, p=0.043).

In Table I, the ODL-related factors that most affected the student were social issues (3.63) followed by academic issues (3.54). Lack of effective communication skills and feeling isolated were the top concerns of the students. Male students (p=0.111), from the Business and Management faculty (p=0.044) as well as those with a CGPA between 3.0 and 3.5 prior to the pandemic (p=0.037) were found to be more isolated than other groups. Students from urban areas were significantly more emotionally affected compared to their rural peers. In contrast, studies have shown that female students (4) and those from rural areas (5) were the most emotionally affected. Similarly, a systematic review reported that college students felt more anxious and distressed than prior to the pandemic (5).

Table I: Mean score (1-5) on factors affecting students' emotional health, n=377

ODL related factor that affects student's emotional health	Mean (SD)
Social issue	**3.63**
Learner feels isolated	3.66 (1.11)
Lack of group discussions during assignments	3.60 (1.12)
Online learning is too indirect	3.58 (1.07)
Online learning is too personal	3.37 (1.09)
Academic issues	**3.54**
Inadequate effective communication skills	3.81 (1.00)
Lack of reading skills	3.27 (1.16)
Generic skill	**3.27**
Inadequate good writing skills	3.28 (1.11)
Lack of vocabulary acquisition	3.25 (1.10)
Lecturer issues	**3.06**
Lack of clear learning expectations from lecturers	3.59 (1.12)
Course materials will be delayed online	3.17 (1.16)
Inadequate instructors to assist in lesson delivery	3.06 (1.17)
Lower quality of materials online	3.05 (1.18)
Lecturers or instructors are not trained to teach	2.42 (1.12)
Accessibility issue	**3.04**
Internet bundle cost is too high	3.60 (1.22)
Inadequate internet access	3.37 (1.23)
Issues with correct browser for learning	2.95 (1.20)
Incompatibility of some phones and laptop	2.92 (1.25)
Unavailability of the required technological devices	2.38 (1.14)

Mean range between1- 5, 1 is strongly disagree and 5 is strongly agree

CONCLUSION

In conclusion, this study revealed that students were affected emotionally due to the pandemic and its subsequent stressful ODL implementation, suggesting that the emotional health of university students should be monitored during a pandemic. The university can help alleviate the impact by promoting preventive measures and providing psychological support to those affected.

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EXTENDED ABSTRACT

Perceived Challenges of Healthcare Professionals in Providing Diabetes Care for Young People with Type 2 Diabetes Mellitus: A Qualitative Study

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SUMMARY

This study aimed to explore the perceived challenges of multidisciplinary health professionals (HCPs) in providing diabetes care for young people with type 2 diabetes mellitus (T2DM) in Malaysia. Maximum variation and snowball sampling methods were used to recruit the HCPs. Sixteen HCPs from two tertiary hospitals were interviewed. All interviews were audio-recorded and analysed using thematic analysis. The identified challenges were: (1) challenging interactions, (2) patients underestimate the severity of T2DM, (3) lack of social support and (4) limitations in healthcare budget. This study highlighted that the HCPs' perspective and concerns associated with diabetes care provision need to be addressed for improving the glycaemic control among the young people with T2DM.

Keywords: Young people, Type 2 diabetes mellitus, Healthcare professionals, Challenges, Qualitative study

INTRODUCTION

The young onset type 2 diabetes mellitus (T2DM) requires continuous medical management and patient-driven diabetes self-management activities as T2DM progression in young people is more aggressive than adult-onset T2DM (1). Both international and Malaysian guidelines advocate multidisciplinary healthcare team in providing comprehensive diabetes care and self-management support to young people with T2DM (2, 3). Previous study findings showed that HCPs' attitudes and perspectives towards T2DM management can influence the uptake of diabetes self-management activities among people with T2DM (4). To date, the HCPs perspectives and perceived hurdles in providing diabetes care among young people in Malaysia remain scarce. Therefore, this study aimed to understand the perceived challenges of multidisciplinary HCPs in diabetes care of young people with T2DM in Malaysia using qualitative approach.

MATERIALS AND METHODS

This study was conducted at the endocrinology clinics, Hospital Putrajaya and Hospital Pulau Pinang after ethics approval from the Medical Research and Ethics Committee, Ministry of Health, Malaysia [NMRR-18-3476-44989 (IIR)] and UiTM Research Ethics Committee [UiTM600-IRMI (5/1/6)]. In-depth face-to-face interviews were conducted from November 2019 to September 2020 among the HCPs for their experience in dealing with young patients with T2DM. Maximum variation and snowballing sampling methods were used to recruit the HCPs with more than 6 months of diabetes management experience. Of the 16 interviewed from HCPs, they were diabetes nurse educators (n=3), pharmacists (n=3), staff nurses (n=3), occupational therapists (n=2), dietitians (n=2), assistant medical officers (n=2) and a medical officer. Thematic analysis was carried out after all transcripts were checked by

the informants and supervisory team to ensure data confirmability and trustworthiness. The codings and themes were regularly discussed and reviewed by all authors to ensure study credibility. Data saturation was achieved at the 14th interview with two additional interviews to confirm no new theme emerged.

RESULTS AND DISCUSSION

This study identified the four challenges that HCPs need to embrace in providing optimal diabetes care for young people with T2DM (Table I). First, challenging interactions between HCPs and young people pose difficulties in tailoring T2DM management. The difference in ethnicity between HCPs and young patients leads to language barrier. Furthermore, HCPs' poor communication skills coupled with patients' unwillingness to disclose information about themselves impede effective communication. Good communication and empathetic interaction are important for building trust in the delivery of effective diabetes self-management support. Secondly, the patients underestimate the severity of T2DM and therefore neglected the need for self-management activities. This situation could be due to a lack of awareness about the importance of well-controlled T2DM or its complications. Third theme described the lack of social support from the family. Parental denial in their children T2DM diagnosis could hinder the young people's efforts to make necessary behavioural changes related to diabetes self-management. Additionally, the stigma among peers and lack of community support for healthy lifestyles were the potential barriers to optimal diabetes self-management. Alternatively, increased public's knowledge about T2DM, hypoglycaemia management, and concerted support from peers, family and community to young people could result in positive outcomes in T2DM self-management thus improving young patients' glycaemic outcomes and quality of life (5). Fourth theme identified the limited healthcare budget faced by the HCP in diabetes care caused doctors to have limitations to switch from insulin to newer oral medications that are relatively more expensive than insulin. Moreover, the HCPs perceived that lack of subsidised glucose monitoring equipment may contribute to the poor uptake of self-monitoring blood glucose among patients. The restriction in prescribing due to rising cost of medicines and financial constraints in the health system is not confined to our setting, but also frequently reported in other high- and low-income countries.

Table I: Selected quotes for four themes of healthcare professional- perceived challenges in the provision of diabetes care to young people with type 2 diabetes mellitus

Themes	Informants' quotes
Challenging interactions	*"I felt different ethnicity is a barrier for me. For example, if the patient is Indian, I will pass the case to an Indian dietitian. It is much easier to communicate. Food is culture, so sometimes I cannot understand their food culture."* (H#07, dietitian) *"… sometimes the timeframe is short, as they were called by the doctor. So I don't have enough time for the counselling, as for some patients, we need to "psycho" them first in order to get information from them."* (H#02, physiotherapist) *"One young patient, she did not tell anything about herself, and cry while the doctor talked to her. Maybe she is scared as I can see from her face expression."* (H#11, physiotherapist)
Patients underestimate the severity of T2DM	*"...They are knowledgeable. I cannot understand how they think about themselves and their illness. We told them: 'Ok, your disease is like this...' Then they reply, 'I already know'. But for me, they still do what they want to do, even though it is wrong." (H#05, staff nurse)* *"There were patients who took medication but did not think of other things. For example, they still take carbonated drinks, cola, and do not limit their rice portion. For me, they are still not able to take care of themselves."*(H#01, staff nurse and diabetes educator) *"At the beginning of their diagnosis, they did not feel anything as they are still young. As time goes by, after few years with diabetes, they felt they had enough, refused to take any medicine. They felt down,"* (H#08, assistant medical officer and diabetes educator)
Lack of social support	*"…diabetes management also related to how the adult perceives the disease. Certain adults don't believe that their children have diabetes, they don't believe that it is a progressive disease that cannot be cured. Some of them might find it very difficult to accept. Parental support is very important for the young patient. Parental support means the parents take care of their diet, encourages them to perform SMBG. Things like that, for those that have very good support from their family then I think it will be easier. Otherwise, it is more difficult"* (H#14, pharmacist) *"Some of the friends brought sweet drinks and food while visiting their friends. So, I told them about their friend's condition and asked them to be more careful and avoid the sweet things if possible."* (H#06, nurse) *"In the last two weeks, a 19-year-old girl presented with T2DM. The patient was on insulin and seemed scared when she met me. She was reluctant to take medication and insulin because she mentioned that she was bullied at school."* (H#11, physiotherapist)
Limitations in healthcare budget	*"Quota issue. We are getting towards trying to get rid of insulin slowly. We have more options orally. Of course, those medications are more expensive, so not easily available."* (H#09, medical officer) *"I mean we can provide good service to the patient but sometimes we have to choose our patient to give certain medication as we fall under quota. If we cannot provide certain medications for this year due to this quota system, then we need to allocate them for the new following open quota. So, I feel like the patient's treatment is delayed but no choice."* (H#13, pharmacist) *"Since you know the majority of Malaysians are with diabetes, there should be funding system for SMBG. I think this is the cause most of the patients don't comply with SMBG. After all, they don't have funds. Funds to buy the strips and needles." (H#13, pharmacist).*

CONCLUSION

The study findings suggested that good communication is important for HCPs in delivering comprehensive diabetes care and engaging young patients to disease self-management. Furthermore, education and awareness on the importance of optimal diabetes self-management in achieving glycemic control among patients and patients' social network need to be addressed.

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EXTENDED ABSTRACT

Epidemiology of Thromboembolic (TE) complications in COVID-19 Patients: A Review on Incidence

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SUMMARY

Cardiovascular complications classified as thromboembolic (TE) complications can be classified into arterial thromboembolism (ATE): [myocardial infarction (MI) and stroke] and venous thromboembolism (VTE): [deep vein thrombosis (DVT) and pulmonary embolism (PE)]. These events were associated with increase in morbidity and mortality in COVID-19 patients. Based on the articles reviewed, the most common TE complication in hospitalized COVID-19 patients was VTE (n=24) which was reported as PE (1.0%-40.0%) and DVT (0.4%-84.2%) compared to arterial ATE complications (0.5%-15.2%). Hence, every patient should undergo thorough risk factor assessment for TE complication and allow individualized optimal thromboprophylaxis management to improve patient's outcome.

Keywords: Thromboembolic complication, Venous thromboembolism, Arterial thromboembolism, COVID-19

INTRODUCTION

By the end of 2019, cases of pneumonia with unknown aetiology were detected in Wuhan, China caused by a new coronavirus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), later known as COVID-19 (1). During the early phase, the clinical manifestation of COVID-19 primarily affected the respiratory system, with severe cases causing alveolar damage and respiratory failure (2). As more COVID-19 cases were reported, the episodes of thromboembolic (TE) complications classified as venous thromboembolism (VTE) and arterial thromboembolism (ATE) were commonly observed causing increase in morbidity and mortality (3).

MATERIALS AND METHODS

A literature search was performed using ScienceDirect and Pubmed database. The search strategy was completed using keywords and subject heading related to "COVID-19", "thromboembolic complication", "venous thromboembolism", "arterial thromboembolism", "deep vein thrombosis", "pulmonary embolism", "myocardial infarction" and "stroke". The search filters based on the date of publications from March 2020 to September 2022 which included the keywords related. The EndNote 20 was used as a reference manager in this review.

Based on the keywords, a total of 5,010 research articles were found. Among these articles, only those reporting on the incidence of TE events in COVID-19 populations were included. The remaining articles which were considered as the irrelevant, duplicated, incomplete or article that is not published in English language were excluded. Finally, 33 studies were included in this review for the incidence of TE complications in hospitalised COVID-19 patients.

RESULTS AND DISCUSSION

Thirty-three studies that reported on the incidence of TE complications in hospitalized COVID-19 patients (Table I) were included in this review. Most of the studies were performed in Europe (n=18), United Stated (n=9), Asia (n=2), North Africa (n=2) and United Kingdom (n=2). The study populations were hospitalised COVID-19 patients ranging from 23 to 5,966 patients, who were admitted in either intensive care unit (ICU) (n=8), general wards (n=12) or in combination of both ICU and general wards (n=13).

VTE, reported as DVT or PE, was the most common TE complication in hospitalized COVID-19 patients. Most articles reported VTE complications separately as PE (n=27) and DVT (n=22), with incidence rates ranging from 1.0% to 40% and 0.4% to 84.32%, respectively. Stroke or myocardial infarction (MI) were the common ATE complications seen in the included studies. Thirteen studies reported stroke incidence ranging from 0.5% to 15.2%, while only eleven studies observed MI incidence ranging from 0.8% to 8.7% in their study population.

Studies show wide variation in the incidence of TE

Table I: Literature included for TE complications among hospitalised COVID-19 patients

Author (Year)	Number of populations	VTE		ATE	
		PE	DVT	Stroke	MI
ICU					
Klok, F. A. et. al., (2020)	184	13.6%	1.6%	1.6 %	
Gonzalez-Fajardo, J.A. et. al., (2021)	261	22.2%	7.7%	5.7%	5.0%
Bozzani, A. et. al., (2020)	38	-	84.2%	-	-
Elboushi, A. et. al., (2022)	198	14.6%	7.6%	-	-
Brandao, A. et. al., (2021)	243	7.8%	3.7%	1.2%	2.5%
Haksteen, W. E. et. al., (2021)	188	43.1%		-	-
Hekms, J. et. al., (2020)	150	16.7%	2.0%	0.6%	-
Fraisse, M. et. al., (2020)	92	20.1%	6.5%	2.2%	1.1%
General Ward					
Mohamud, M F. Y. et. al., (2022)	46	13.0%	4.3%	15.2%	8.7%
Jimenez-Guiu, X. et. al., (2021)	57	-	10.5%	-	-
Garcia-Ortega, A. et. al., (2021)	73	35.6%	-	-	-
Chen, B. et. al., (2021)	23	-	82.6%	-	-
Rali, P. et. al., (2021)	147	10.9%	9.5%	-	-
Erben, Y. et. al., (2021)	915	9.0%		-	-
Valle, C. et. al., (2021)	114	57%	-	-	-
Silva, B. V. et. al., (2021)	300	15.3%	-	-	-
Vivan, M. A. et. al., (2022)	697	32.4%	-	-	-
Chang, H. et. al., (2021)	183	-	31.7%	-	-
Chaudhary, R. et. al., (2021)	102	1.0%	2.9%	1.0%	2.0%
Cueto-Robledo, et. al., (2022)	26	34.6%	3.8%	3.8%	-
ICU and General Ward					
Al-Samkari, H. et. al., (2020)	400	2.5%	2.3%	0.5%	2.3%
Kaptein, F. et. al., (2021)	947	10.2%	0.7%	1.3%	0.8%
Martinot, M. et. al., (2021)	600	2.7%	2.2%	-	1.3%
Munoz-Rivas, N. et. al., (2021)	1127	3.9%	0.5%	1.2%	0.5%
Kajoak, S. et. al., (2022)	445	8.1%	-	-	-
Tholin, B. et. al., (2021)	550	3.6%	0.6%	0.7%	2.0%
Martнnez Chamorro, E. et. al., (2021)	342	26%	-	-	-
Filippi, L. et. al., (2021)	267	18.7%	-	-	-
Arribalzaga, K. et. al., (2021)	5966	2.6%	0.4%	-	-
Whyte, M. B. et. al., (2020)	214	37.4%	-	-	-
Bruggemann, R. et. al., (2020)	60	40.0%	-	-	-
Fujiwara, S. et. al., (2021)	628	1.8%	2.1%	-	-
Lodigiani, C. et. al., (2020)	388	2.6%	1.3%	2.3%	1.0%

complications among hospitalized COVID-19 patients, which may result from limited data collection due to the absence of a diagnosis for asymptomatic patients (4). Studies published since the end of 2020 show a high trend of TE complications, possibly due to increased awareness of their prevalence as a major clinical presentation in COVID-19 patients, leading to increased screening for TE episodes.

Furthermore, different study settings, either from the general ward or ICU, may have different COVID-19 prognoses. Critically ill patients, especially those in the ICU, have a higher risk of a hypercoagulable state due to immobilization, mechanical ventilation, central venous access devices, and nutritional deficiencies, leading to a poorer prognosis (5). Therefore, all COVID-19 patients with risk factors such as cardiovascular disease, active cancer, diabetes, or a previous history of TE complications should be closely monitored for the development of TE complications.

CONCLUSION

This review found that VTE is more common than ATE among hospitalized COVID-19 patients in both ICU and general wards. Therefore, every patient should undergo a thorough risk factor assessment for TE complications upon admission. This strategy helps identify those at risk of TE complications and allows for individualized optimal thromboprophylaxis management to improve patient outcomes.

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EXTENDED ABSTRACT

Emotional Intelligence, Vaccination Knowledge, and Vaccination Attitudes among UiTM Cawangan Selangor Students during COVID-19 Pandemic

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SUMMARY

Emotional intelligence (EI) is essential to positively promote vaccination knowledge, eventually influencing an individual's vaccination attitudes. This research evaluated respondents' demographic profiles, EI, vaccination knowledge, and attitude, also the correlation between the variables. This research involves 387 undergraduate students, from the first year to the fourth year, from 12 faculties in UiTM Cawangan Selangor (UCS). Research revealed that most respondents have high EI, vaccination knowledge, and positive vaccination attitudes. A positive correlation was found between the level of EI, vaccination knowledge, and vaccination attitudes indicating EI is the factor that increases students' vaccination knowledge, subsequently impact on positive vaccination attitudes.

Keywords: Emotional Intelligence, Vaccination Knowledge, Vaccination Attitudes, COVID-19, Pandemic

INTRODUCTION

Emotional intelligence (EI) is vital for individuals to comprehend, use and positively control emotions such as surprise, confusion, and curiosity that promote knowledge-seeking behaviour (1). During the COVID-19 pandemic, a lack of vaccination knowledge resulted in vaccine hesitancy in the community. Vaccine hesitancy arises when concerns about vaccines' side effects, efficacy, and safety could influence a person's views and attitudes toward COVID-19 vaccination (2). Vaccination attitude refers to the acceptance or hesitancy of the person to take the vaccine. Therefore, this study evaluated respondents' EI, vaccination knowledge, and vaccination attitudes. The relationship between respondents' demographic profiles, EI levels, vaccination knowledge level, and vaccination attitudes during the COVID-19 pandemic has been determined.

MATERIALS AND METHODS

This online research used a quantitative cross-sectional correlational design involving 387 undergraduate students registered under UiTM Cawangan Selangor (UCS), including UiTM Puncak Alam, UiTM Puncak Perdana, and UiTM Sungai Buloh. It involves first-year to fourth-year students from 12 faculties, including the medical and non-medical fields, from the first to the third week of April 2022. The questionnaire includes demographic backgrounds, USM Emotional Quotient Inventory (USMEQ-i), Level of Vaccination Knowledge, and Vaccination Attitudes Examination (3-5). The mean EI was calculated and categorised into low (0.00-1.20), moderate (1.21-2.80), and high (2.81-4.00). A total score of more than 4.50 was categorised as high knowledge, meanwhile, a score of less than 4.00 indicates having a positive vaccination attitude. The data were analysed for descriptive and inferential analysis. Descriptive statistics were presented in means, standard deviation, and percentages. Meanwhile, inferential statistics were used to determine the relationship between variables using Chi-square and Spearman's rank-order correlation.

RESULTS AND DISCUSSION

Most respondents were female (n = 293, 75.7%), third-year students (n = 124, 32%), from the Faculty of Pharmacy (n = 94, 24.3%). Most respondents (n = 152, 39.3%) trusted healthcare professionals, government agencies, and international organisations for vaccine information. Most respondents (n = 156, 40.30%) received the PfizerBioNTech vaccine, while only 2 (0.5%) were not immunised for the COVID-19 vaccine. Most respondents had high EI, including medical (n = 129,

70.1%) and non-medical (n = 156, 76.8%) backgrounds, indicating they were skilful in comprehending, using, and positively controlling emotions. There were associations between EI and year of study [X 2 (3, N = 387) = 14.440, p < 0.01] and faculty [X 2 (11, N = 387) = 21.182, p < 0.05]. First-year students have high EI than other study years; meanwhile, the Faculty of Dentistry recorded higher EI. We presume academic setting and social pressure could affect the association between the variables.

Most respondents had high knowledge of COVID-19 vaccination, including medical (n = 169, 91%) and non-medical (n = 150, 74%) fields students, indicating they were concerned and knowledgeable about the COVID-19 vaccine. The medical field students (mean = 2.96 ± 0.83) have less hesitancy towards vaccination than the non-medical field (mean = 3.33 ± 0.84, p < 0.05. Perhaps, their formal education syllabus exposes medical field students to advanced knowledge of viruses, immunisation, and vaccination. Meanwhile, non-medical field students are educated mainly through reading and listening to mainstream media.

EI influenced vaccination knowledge, and there was a significant weak positive correlation between these variables, rs (387) = 0.111, p < 0.05. There was a significant weak correlation between vaccination knowledge and vaccination attitudes, rs (387) = 0.149 p < 0.05, indicating that students with high vaccination knowledge showed positive attitudes towards the COVID-19 vaccine.

CONCLUSION

Most respondents had high EI, vaccination knowledge, and positive vaccination attitudes. A positive correlation was found between EI level and vaccination knowledge, and also, between vaccination knowledge and vaccination attitudes. EI is an essential factor in increasing vaccination knowledge and high vaccination knowledge influences on positive vaccination attitudes.

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

A Descriptive Study on the Efficacy and Safety of Sodium Glucose Co–Transporter 2 Inhibitors as Add on Therapy in Patients with Type 2 Diabetes

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SUMMARY

Sodium-glucose co-transporter 2 inhibitors (SGLT2i) are associated with fair glomerular filtration rate (GFR) control, weight loss, and blood pressure control. The aim of the study was to describe the effectiveness and safety of SGLT2i as add on therapy in patients with Type 2 Diabetes Mellitus (T2DM) at Malaysian Armed Forces (MAF) hospitals. Records of HbA1C, serum creatinine, body weight, and blood pressure were traced from the date of initiation of SGLT2i and the subsequent clinic follow-ups, and hospitalization or clinical event related to cardiovascular, renal, or adverse effects were recorded. SGLT2i have shown significant HbA1C reduction, GFR control, and weight loss.

Keywords: Sodium–glucose co-transporter 2 inhibitors (SGLT2i), Empagliflozin, dapagliflozin, Type 2 Diabetes Mellitus

INTRODUCTION

Dapagliflozin and empagliflozin have been utilized in the MAF hospitals since 2016 and 2017, respectively (1). Limited data available regarding the effects of SGLT2i among the local populations with regards to the impact in HbA1C reduction, weight loss, glomerular filtration rate (GFR) progress, and blood pressure control. Thus, we examined the effectiveness and safety of SGLT2i in Malaysian Armed Forces (MAF) hospital patients with Type 2 Diabetes Mellitus (T2DM).

MATERIALS AND METHODS

Patients who were started with dapagliflozin 10mg or empagliflozin 25mg from January 2016 to December 2021 and fulfilled the inclusion criteria were selected from four MAF hospitals. Demographic data and changes in HbA1C, serum creatinine, weight, and systolic blood pressure (SBP) were retrieved from the patient's file. Each patient had their parameters recorded at baseline, 3rd-7th month and 8th-15th month. Any history of cardiovascular or renal events, and adverse effects within the therapy period were also recorded. SPSS v.28.0 was used for descriptive and inferential analysis. Ethical approval was sought from the Research Ethics Committee of Universiti Teknologi MARA (REC (PH) /PG/030/2022) and Armed Forces Health Services Ethics Committee (PKAT/JKE/23-03).

RESULTS AND DISCUSSION

The data for 109 patients consuming either empagliflozin (n=45) or dapagliflozin (n=64) were collected. All parameters of interest were comparable at baseline between the 2 groups. It was found that the mean HbA1C was reduced for both empagliflozin (1.22%, P<0.001) and dapagliflozin (1.37%, P<0.001) within 15 months. It was noted that the first reading recorded greater HbA1C reduction compared to the second reading. Mean GFR for both agents was significantly reduced within the first seven months, but subsequently increased back before the 15th month. Weight loss was seen for both agents, with a mean difference of -3.18 kg for empagliflozin (P<0.001) and -2.48 kg for dapagliflozin (P<0.001) within 15 months. It was noted that there was no significant difference in the effect of SGLT2i on patients' SBP within 15 months of therapy (Table I & II).

For clinical event, 2 cardiovascular events: myocardial infarction (n=1) and thromboembolism (n=1); 7 renal events: new cases of stage 3 chronic kidney disease (n=5), acute kidney injury (n=1), and nephrolithiasis (n=1); and 2 adverse events: headache (n=1) and pyelonephritis (n=1) were reported. No new incidence of HF or stroke, nor recorded hospitalization of patients with pre-existing HF or stroke were reported. No new incidence of stage 4 chronic kidney disease or end-stage

Table I: Change in Clinical Parameters After 3-7 Months of SGLT2i Treatment

SGLT2i			Mean	SD	Mean Difference	*P*-value
Empagliflozin	HbA1C (%)	Baseline	9.34	1.73	-1.17	<0.001
		1st reading	8.17	1.31		
	GFR (mL/min/1.73 m^2)	Baseline	86.58	17.44	-5.30	0.004
		1st reading	81.28	17.94		
	Weight (kg)	Baseline	83.51	10.68	-1.63	0.001
		1st reading	81.88	11.09		
	SBP (mmHg)	Baseline	137.35	13.62	-3.19	0.261
		1st reading	134.16	15.39		
Dapagliflozin	HbA1C (%)	Baseline	9.55	1.93	-1.07	<0.001
		1st reading	8.47	1.71		
	GFR (mL/min/1.73 m^2)	Baseline	85.13	21.30	-3.97	0.030
		1st reading	81.16	18.18		
	Weight (kg)	Baseline	82.50	10.78	-1.87	<0.001
		1st reading	80.63	11.20		
	SBP (mmHg)	Baseline	133.89	17.86	1.00	0.676
		1st reading	134.89	18.16		

Table II: Change in Clinical Parameters After 8-15 Months of SGLT2i Treatment

SGLT2i			Mean	SD	Mean Difference	*P*-value
Empagliflozin	HbA1C (%)	Baseline	9.26	1.73	-1.22	<0.001
		1st reading	8.04	1.48		
	GFR (mL/min/1.73 m^2)	Baseline	86.31	17.09	-3.60	0.048
		1st reading	82.71	18.00		
	Weight (kg)	Baseline	84.16	11.87	-3.18	<0.001
		1st reading	80.98	11.53		
	SBP (mmHg)	Baseline	137.75	13.06	-2.34	0.248
		1st reading	135.41	12.23		
Dapagliflozin	HbA1C (%)	Baseline	9.55	1.92	-1.37	<0.001
		1st reading	8.17	1.52		
	GFR (mL/min/1.73 m^2)	Baseline	85.08	21.89	-2.02	0.513
		1st reading	83.06	24.37		
	Weight (kg)	Baseline	82.52	10.74	-2.48	<0.001
		1st reading	80.05	12.05		
	SBP (mmHg)	Baseline	133.40	17.66	0.38	0.860
		1st reading	133.78	12.33		

renal failure were reported. No cardiac or renal related death was reported.

CONCLUSION

SGLT2i as add-on therapy for T2DM management appeared to have been beneficial in terms of HbA1C reduction, GFR control, and weight loss in patients from MAF hospitals.

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EXTENDED ABSTRACT

The Evaluation of the Risk Factors Contributing to Vancomycin Toxicity Incidence Among the Elderly: A Retrospective Study

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SUMMARY

Elderly patients have different pharmacokinetic and pharmacodynamic properties than younger patients. The current recommended vancomycin adult daily dose often causes unexpected vancomycin toxicity. The estimated creatinine clearance by the standard Cockcroft-Gault method tends to overestimate the renal function of elderly population, leading to inappropriate vancomycin dosing hence causing vancomycin toxicity. Therefore, this study will evaluate the risk factors contributing to vancomycin toxicity incidence among the elderly. The findings of this study will be used as a guide to develop recommendations for standard daily vancomycin doses for Malaysian elderly population as vancomycin toxicity is associated with increased mortality, hospitalization, and medical costs.

Keywords: Vancomycin, Elderly, Toxicity, Malaysian

INTRODUCTION

Vancomycin is widely used in clinical practice to treat Methicillin-resistant Staphylococcus aureus (MRSA) infections despite limitations such as poor tissue penetration, slow antibacterial effects, and the potential for toxicity (1). MRSA infection is more prevalent in the elderly compared to younger patients because they have weakened immune systems, multiple comorbidities and decreased physiologic reserves to fight infection (2). Currently, the National Antimicrobial Guideline (NAG) is being used to assist physicians and pharmacists in determining antibiotic dosage regimen. NAG is adopted from the Infectious Diseases Society of America (IDSA) guideline, which does not have a specific antibiotic dosing guideline for the elderly. Due to pharmacokinetic and pharmacodynamic variability in the elderly population as well as high prevalence of renal impairment, effective dosing and safe monitoring remain challenging (1,3,4,5). The current recommended vancomycin adult daily dose often causes unexpected vancomycin toxicity in this population (2). Hence, the purpose of this study is to evaluate the risk factors contributing to vancomycin toxicity incidence among the elderly.

MATERIALS AND METHODS

This study is a retrospective observational study that will be performed in two local public tertiary care hospitals in Central Malaysia. Purposive sampling method will be applied in this study. This study will include all elderly patients aged 60 years old and above with no renal impairment who are being treated with intravenous vancomycin, whereas patients with incomplete medical records will be excluded. Elderly patients receiving intravenous vancomycin from January 2019 to December 2022 will be identified using the Pharmacy Information System (PhIS), a system that integrates pharmacy-related services in the government sector. The data collection form will be designed into three parts in which data such as demographic characteristics (age, body weight, gender, body mass index and height), comorbidities, pathogens involved, sites of infection, indication for vancomycin therapy, and exposure to other known potential nephrotoxic therapy will be recorded. The statistical analysis will be conducted using IBM Statistical Package of the Social Sciences (SPSS). The summarization of the method used for the objective is illustrated in Table I.

SIGNIFICANCE OF STUDY

This study aims to guide physicians and pharmacists in deciding dosage regimen that are suitable for the elderly population depending on the pharmacokinetic and pharmacodynamic considerations. A comprehensive

Table I: Summarization of the method used for the objective

Objective of study	Method of data analysis	Source of data
To identify the risk factors of vancomycin toxicity incidence among elderly patients	Binary logistic regression	Medical records, PhIS database, laboratory database and TDM request forms

evaluation of the risk factors that contribute to vancomycin toxicity incidence in elderly Malaysian patients will be investigated further. To recommend an appropriate vancomycin dose for Malaysia's elderly population, it is necessary to first understand and identify the dosage regimen that can cause vancomycin toxicity in the elderly population in Malaysia. The results of this study will aid in the prevention of vancomycin toxicity in the elderly, which can lead to increased mortality, hospitalization, and medical costs. As a result, these findings may improve the quality of life of elderly patients who are being treated with vancomycin.

NOVELTY

There are limited studies conducted to identify suitable vancomycin dosage regimen for the elderly population. Therefore, findings of this study will benefit the elderly population especially in Malaysia that are treated with vancomycin.

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CONCLUSION

SGLT2i as add-on therapy for T2DM management appeared to have been beneficial in terms of HbA1C reduction, GFR control, and weight loss in patients from MAF hospitals.

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EXTENDED ABSTRACT

Design and Development of Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) Loaded with Xanthorrhizol

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SUMMARY

Xanthorrhizol was incorporated into a self-nanoemulsifying drug delivery system (SNEDDS) to overcome the solubility problem. In addition, a pseudo-ternary phase diagram was constructed and employed as a tool to optimise the composition of the SNEDDS formulation. The SNEDDS nano formulations were subjected to a characterization study that included the mean globule size, zeta potential, and polydispersity index (PDI). Cremophor RH40, Tween 80, and Grape seed oil were selected as the surfactant, co-surfactant, and oil phases, respectively. Based on the results, the mean globule size, zeta potential, and PDI of the optimum SNEDDS formulation were 65.10 nm, -25.06 mV, and 0.290, respectively, and the self-emulsification time of the selected SNEDDS formulation was less than 1 minute (rapidly forming clear nanoemulsion).

Keywords: Xanthorrhizol, SNEDDS, Polydispersity index (PDI), Zeta potential, Ternary phase diagram

INTRODUCTION

Xanthorrhizol is a bisabolene-type sesquiterpenoid compound found in plants, including turmeric and ginger, that has been studied for its potential pharmacological benefits (1). Despite the potential health benefit that xanthorrhizol may offer in treating and managing diseases, the administration of this compound is indeed challenging due to its poor aqueous solubility and low bioavailability. In order to circumvent these issues and reap the potential health benefits of xanthorrhizol, several promising delivery systems, including nanoparticles, liposome encapsulations, cyclodextrin complexation, and self-nano emulsifying drug delivery systems (SNEDDS), may be considered. In this study, SNEDDS was chosen as a potential delivery system for xanthorrizol due to the potential for the system to form a stable oil-in-water nanoemulsion upon contact with aqueous media such as gastrointestinal fluids (2). In doing so, this will enhance the solubility of the compound, which may aid in augmenting the absorption of xanthorrhizol across the gastrointestinal mucosa, which would culminate in an improvement in bioavailability (3).

MATERIALS AND METHODS

The solubility studies of xanthorrhizol (Javaplant, Indonesia)-loaded SNEEDS formulations were carried out using shake flask methods (3). A ternary phase diagram was constructed using different percentage compositions of grape seed oil (Trade Lane Inc., USA) as oil, cremophor RH40 (BASF, Germany) as surfactant, and Tween 80 (Zulat Pharmacy, Malaysia) as cosurfactant, as shown in Figure 2. Following this, nanoemulsion region was selected to be incorporated into the xanthorrizol-SNEDDS formulation. The characteristics of SNEDDS nanoemulsion were evaluated based on the rate of emulsification upon being introduced into water under gentle agitation. The mean globule size, zeta potential, and polydispersity index (PDI) were measured by using a Malvern zeta sizer 1600 (United Kingdom).

RESULTS AND DISCUSSION

The solubility studies of xanthorrhizol in selected oils (grape seed oil, sunflower oil, castor oil, olive oil, palm kernel oil, jojoba oil, and avocado oil), surfactants, and co-surfactants (cremophor RH40, Tween 80, cremophor EL, tween 20, PEG 400, propylene glycol, and transcutol HP) are depicted in Fig 1.

The selection of surfactants was governed by emulsification potential and compatibility with oils (3). Cremophor EL, cremophor RH40 and transcutol HP were selected as surfactant candidates and the highest %transmittance value was shown by Cremophor RH40 (99.95±1.23%). In addition, cosurfactant is essential

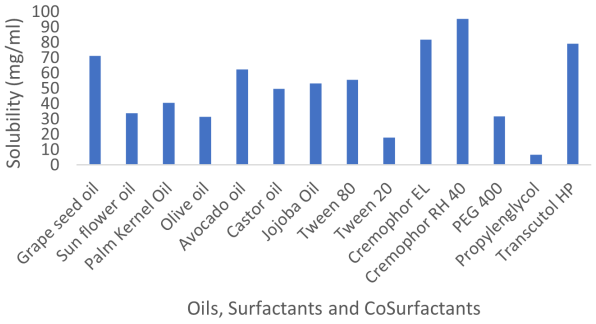

Figure 1: The solubility of xanthorrhizol in various oils, surfactants and cosurfactants

to enhance the surfactant's emulsification efficiency to produce nanoemulsion (4). In this study, Tween 80 was selected as a cosurfactant as it is capable of improving the nano-emulsification of Cremophor RH40 as revealed by 99.95±0.72% %transmittance value when investigated for their emulsification in combination with cremophor RH40. On the other hand, the maximum solubility of xanthorrhizol in the oils was achieved when solubilized in grape seed oil (71.18±0.20 mg/ml).

A ternary phase diagram was constructed to optimise the composition of the grape seed oil, cremophor RH40, and Tween 80 for SNEDDS formulations and to identify the nanoemulsion area. As shown in Figure 2, the percentage of grape seed oil, cremophor RH40 and Tween 80 were optimised in the range of 2-25%, 20-50% and 33-68%, respectively.

Following this, the selected formulation was subsequently loaded with xanthorrhizol to develop the xanthorrhizol-loaded SNEDDS formulation (xanthorrhizol-SNEDDS). The xan-SNEDDS formulation was characterised for mean globule size, zeta potential, PDI and time for self-nanoemulsification. The mean globule size of xanthorrhizol-SNEDDS was 65.10±0.58 nm with a PDI value of 0.29±0.30, indicating narrow size distribution while, the zeta potential value of the xanthorrhizol-SNEDDS was -25.06±1.71, showing good stability dispersion. The time for self-nanoemulsification was evaluated through visual observation based on grade systems (5). The xanthorrhizol-SNEDDS formulation was rapid (less than 1 minute), forming a clear nanoemulsion.

CONCLUSION

The present study demonstrated that the ternary phase diagram was successfully constructed with an optimised range of grape seed oil, cremophor RH40 and Tween 80 which were 2-25%, 20-50% and 33-68%, respectively. The SNEDDS loaded with xanthorrizhol was successfully

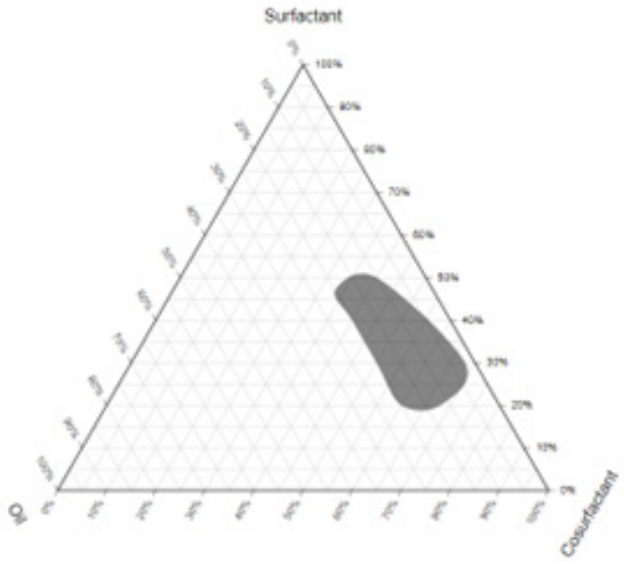

Figure 2: The ternary phase diagram

formulated with a mean globule size of 65.10±0.58 nm and PDI value of 0.29±0.30. This study suggests the potential of SNEEDS as a promising delivery system for xanthorrizol.

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EXTENDED ABSTRACT

Effect of Solubilizing Agents on the Pharmacokinetic Profile of a BCS Class II drug, Griseofulvin, in Rats

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SUMMARY

Griseofulvin is classified as a class II drug according to the Biopharmaceutics Classification System (BCS), which has low solubility and high permeability characteristics. The low solubility of griseofulvin can lead to poor bioavailability. Therefore, in this study, solubilizing agents including propylene glycol, Transcutol® P, Tween 80, 2-hydroxypropyl β-cyclodextrin (2HP-β-CyD), polyethylene glycol (PEG) 400, labrasol and hydrogenated castor oil-60 (HCO) were used to improve the solubility of griseofulvin and enhance the pharmacokinetic profile of griseofulvin after oral administration in rats. Based on the results, 10% (v/v) Transcutol® P significantly increased ($p<0.05$) the oral absorption of griseofulvin by 1.8-fold compared to the griseofulvin solution.

Keywords: Griseofulvin, Pharmacokinetic profile, BCS Class II drug, Transcutol® P, Solubilizing agents

INTRODUCTION

Advancements in pharmaceutical chemistry, in tandem with our ever-growing understanding of human physiology, have led to the development of novel and efficacious drug candidates. Nevertheless, a majority of these candidates exhibit poor aqueous solubility, which causes variability in the absorption profile of the drug as well as limiting its bioavailability (1). In order to obviate this issue, formulators often utilise various strategies to augment the solubility of these drug candidates, whether via the use of solubilizing agents or by exploring alternative formulation approaches. Among these strategies, the use of solubilizing agents offers the simplest yet most effective approach to improving drug solubility, leading to enhanced oral bioavailability. Undeniably, there is an armamentarium of solubilizing agents that formulators may utilise to enhance the solubility of poorly soluble drugs. This includes (1) water-soluble complexation carriers such as cyclodextrins, (2) water-soluble organic (co)solvents (e.g: PEG400, propylene glycol), and (3) surfactants (e.g. Tween 80, labrasol, HCO-60, Transcutol®P). In this study, we utilised 10% (v/v or w/v) of a range of solubilizing agents in an attempt to improve the solubility and pharmacokinetic profile of griseofulvin following oral administration in rats.

MATERIALS AND METHODS

Griseofulvin suspension was prepared as a control group in Hepes-tris buffer (pH 7.4). The other formulations consist of griseofulvin that was dissolved in 20% (v/v) DMSO (0.5mg/ml) or giseofulvin (0.5mg/ml) solubilized in the presence of 10% (w/v or v/v) of solubilizing agents either propylene glycol, Transcutol® P, Tween 80, 2-hydroxypropyl β-cyclodextrin (2HP-β-CyD), polyethylene glycol (PEG) 400, labrasol and hydrogenated castor oil-60 (HCO). For the pharmacokinetic study, the rats were fasted overnight prior to the experiment. Griseofulvin solution (6.25 mg/kg BW) in the presence or absence of 10% (v/v) solubilizing agents was administered via oral gavage to the rats (2,3). Then, blood samples were collected at predetermined time points via the jugular vein into heparinized syringes for up to 240 min to determine the drug concentration using HPLC. The area under the curve (AUC) was calculated by the trapezoidal method from zero to the final sampling time in order to elucidate the pharmacokinetic profile of the drug.

RESULTS AND DISCUSSION

The effects of several solubilizing agents on the intestinal absorption of griseofulvin are shown in Figure 1 and Table I. As shown in Figure 1, the absorption of griseofulvin (6.25 mg/kg) in control was low throughout the experiment. However, the intestinal absorption of griseofulvin was significantly enhanced by 10.5-fold when griseofulvin is dissolved in 20% (v/v) DMSO (Table I). DMSO was reported to be inert towards other chemicals, easily penetrates cell membranes without

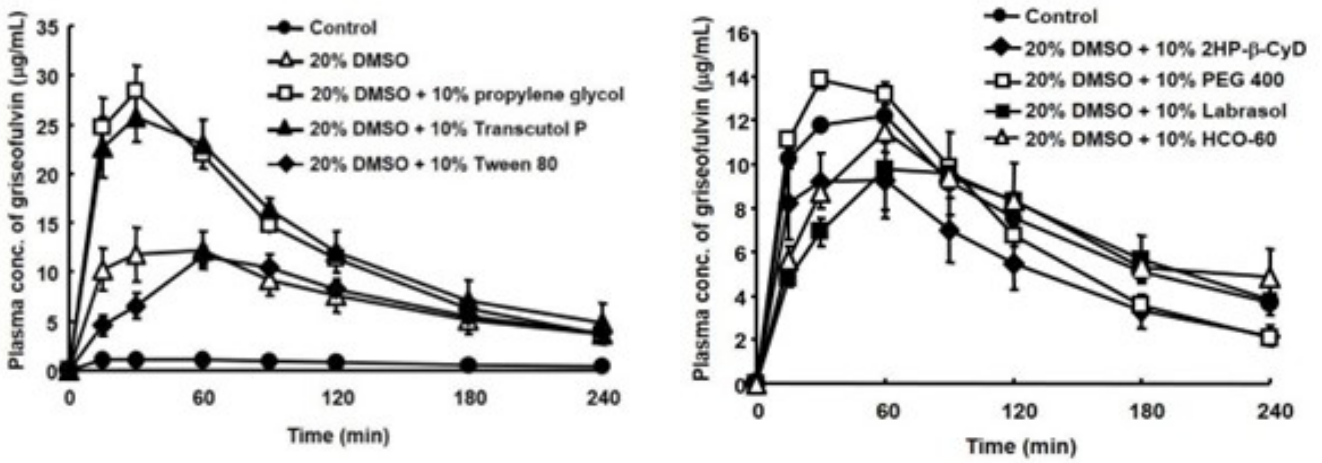

Figure 1: Plasma concentration-time profiles of griseofulvin (6.25 mg/kg) in the presence and absence 10% (v/v, w/v) solubilizing agents by an *in vivo* oral absorption method. Results were expressed as the mean ± S.E. of at least 3 experiments.

Table I: Effect of solubilizing agents on the absorption profile of griseofulvin (6.25 mg/kg) by an in vivo oral absorption method

	Cmax (µg/mL)	Tmax (min)	AUC_{0-240} (µg/mL·min)	F (%)
Control	1.15 ± 0.1	25.0 ± 5.0	176.3 ± 9.3	1.3
20% (v/v) DMSO	14.1 ± 2.0	45.0 ± 6.7	1816.4 ± 349.8*	13.6
20% (v/v) DMSO + 10% (v/v) Propylene glycol	28.4 ± 2.6	30.0 ± 0.0	3105.5 ± 74.9**,N.S.	23.2
20% (v/v) DMSO + 10% (v/v) Transcutol® P	26.9 ± 2.2	25.0 ± 5.0	3203.0 ± 469.7**,#	24.0
20% (v/v) DMSO + 10% (v/v) Tween 80	11.8 ± 0.9	60.0 ± 12.0	1693.8 ± 182.6*,N.S.	12.7
20% (v/v) DMSO + 10% (w/v) 2HP-β-CyD	9.5 ± 1.5	50.0 ± 10.0	1326.5 ± 258.8*,N.S.	10.0
20% (v/v) DMSO + 10% (v/v) PEG 400	13.9 ± 0.4	40.0 ± 10.0	1747.8 ± 82.6*,N.S.	13.1
20% (v/v) DMSO + 10% (v/v) Labrasol	10.4 ± 2.1	70.0 ± 10.0	1634.3 ± 280.5*,N.S.	12.2
20% (v/v) DMSO + 10% (v/v) HCO-60	11.5 ± 0.9	60.0 ± 0.0	1744.0 ± 67.6*,N.S.	13.1

Results were expressed as the mean ± S.E. of at least 3 experiments.
*P,0.05, **P<0.01, compared with the control. #P<0.05 compared with 20% (v/v) DMSO, N.S. no significant difference, compared with 20% (v/v) DMSO.

causing irreversible damage and has been widely used because of large solubilization capacity and low toxicity (4). Moreover, our study demonstrated that 10% (v/v) Transcutol® P further enhanced the intestinal absorption of griseofulvin as shown by AUC value of 3203.0 ± 469.7 compared to griseofulvin solution with AUC of 1816.4 ± 349.8. Besides, the presence of 10% (v/v) propylene glycol in the formulation seemed to increase the pharmacokinetic profile of griseofulvin albeit insignificant. Conversely, the other solubilizing agents did not give a significant effect on the intestinal absorption of griseofulvin as compared with griseofulvin solution in 20% (v/v) DMSO. It is worth noting that, in our preliminary studies, all these solubilizing agents at the concentrations used did not cause any membrane damage to the intestinal epithelium (5).

CONCLUSION

Transcutol® P with a concentration of 10% (v/v) significantly ($p<0.05$) enhanced the pharmacokinetic profile of griseofulvin after oral administration in rats. Thus, the addition of Transcutol® P was shown to act as an enhancer in the oral delivery of griseofulvin. However, other solubilizing agents did not significantly affect the absorption profile of griseofulvin.

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EXTENDED ABSTRACT

Formulation and Characterization of Philippine Lime (*Citrofortunella microcarpa*) Pectin and Chitosan-Based Beads for Colon Targeting

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SUMMARY

The study aimed to formulate pharmaceutical beads from the extracted pectin of Philippine lime peels and crosslink them with commercial chitosan. The formulated beads were subjected to pH swelling, morphology by Scanning Electron Microscope (SEM), functional groups identification using Fourier Transform Infrared Spectroscopy (FTIR) and drug release analysis. The findings showed no significant differences between the pH swelling percentages, thus indicating that the pH does not affect the swelling of the formulated beads. The SEM analysis presented irregularly shaped beads with a rough surface typical among complexes. FTIR analysis showed relatively similar transmittance peaks and similar functional groups with commercial pectin. The mean concentrations across various pH were not significantly different, that pH did not affect the release of loperamide but the pectin-chitosan beads were found to have a high drug release at pH 7.4 which is the pH of the colon, thus suggesting that it can be a colon-targeted oral drug delivery.

Keywords: Philippine lime, Pectin, Chitosan, Beads, pH-dependent swelling, Colon targeting

INTRODUCTION

Mindanao is bountiful in resources and known as the "fruit basket of the Philippines" and Philippine lime or Citrofortunella macrocarpa is one of them. One method of interest is using its peels as a pectin source, which is used in many colon-targeted biomedical systems for its non-toxicity, biodegradability, and biocompatibility. The primary goal of the targeted medication delivery system is to direct the therapeutic agent's pharmacological effect towards the afflicted organs. However, pectin as the sole drug carrier is insufficient for colon-targeted drug delivery due to its water solubility, thus necessitating cross-linking with another compound. This study aims to formulate pectin-chitosan crosslinked beads using loperamide as the model drug. The formulated beads are characterised using observation under SEM, FTIR, pH swelling, and in vitro drug release.

MATERIALS AND METHODS

The extracted pectin from the peel of Philippine lime was characterised using FTIR: TENSOR II + TGA - IR STA: 2500 REGULUS FTIR to identify the characteristic functional groups. The SEM: QUANTA 250 was used to characterize the surface morphology of soft-capsule. The cross-linking process was done by injecting the pectin-loperamide mixture dropwise using a tuberculin syringe above the calcium chloride-chitosan solution. The chitosan-coated pectin beads were removed from the solution via filtration and left to air-dry. The surface morphology of the beads was examined using SEM. The structures of pectin, chitosan, and pectin-chitosan crosslinked were determined using FTIR. Its pH swelling capacity was also tested. The changes in the weight of the samples after exposure to simulated gastrointestinal conditions indicated the degree of swelling of the beads. The in-vitro drug release was also determined.

RESULTS AND DISCUSSION

The percentage yield of pectin from Philippine lime peels was 63.65 %. On characterization, the SEM images of the formulated pectin-chitosan beads are irregularly shaped and have rough surfaces, which is typical among complexes (Fig. 1).The spectra of the commercial pectin and the extracted pectin were compared, showing relatively similar transmittance peaks and the presence of similar functional groups, thus confirming the identity of the pectin extracted. The FTIR analysis was also done on chitosan.

The FTIR analysis shows the functional groups in pectin-chitosan beads (Fig.2). It showed that the -NH stretch

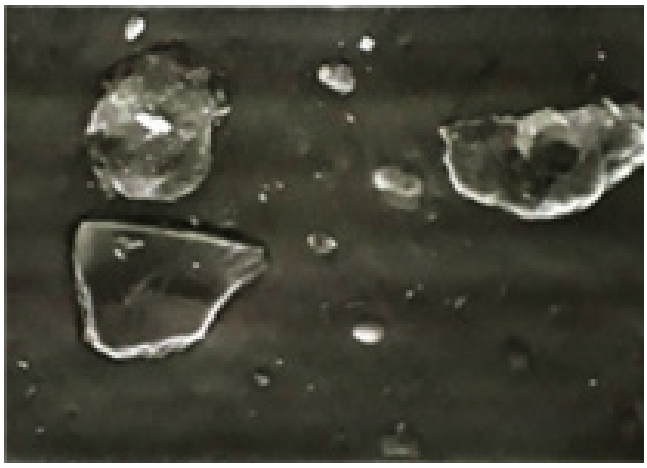

Figure 1: SEM images of Philippine lime peel pectin-chitosan beads. *Note: magnification of 1200x (left), 2,500x (right)*

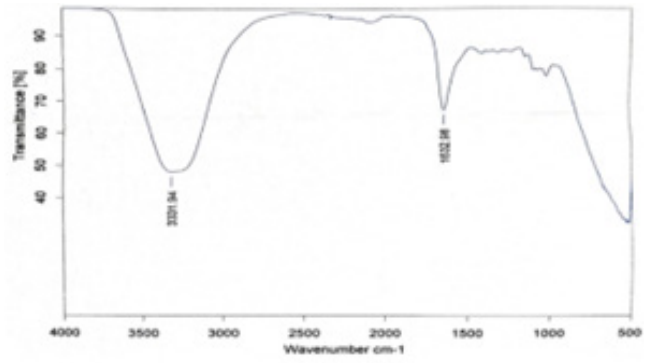

Figure 2: FTIR Spectra of Philippine lime Pectin-Chitosan Beads

of chitosan disappeared, and the -OH stretch increased. The peaks of -C-O and -C-H bands along the spectra also decreased. A medium and sharp stretching peak -C=C appeared in 1632.98 cm^{-1}. This would indicate that there were alterations in the environment of the chitosan due to its interaction with the pectin. The shifting in the spectrum of the formulated pectin-chitosan beads and other changes, such as the decrease and increase of the transmittance peaks, indicated changes due to the interaction of pectin and chitosan.

On percent drug release of loperamide at pH 1.2, 4.5 and 7.4, the mean concentrations across various pH were not significantly different, indicating that pH did not affect the release of loperamide but the pectin-chitosan beads were found to have a high drug release at pH 7.4 (Table I) which is the pH of the colon, thus suggesting that it can be a colon-targeted oral drug delivery.

Table I: Percent Drug Release of Loperamide across different pH levels

pH	Initial concentration (ppm)	Final concentration after 6 hours	% drug release
1.2	10000	512	5.12
4.5	10000	761	7.61
7.4	10000	9.39	9.39

**ppm- parts per million*

CONCLUSION

The pectin extracted from Philippine lime was crosslinked with chitosan, and pharmaceutical beads were formulated with the capacity for drug delivery in the colon. The drug released is highest at pH 7.4, having a transit time of 6 hours which indicates its possible capacity for colon targeting drug delivery systems.

ACKNOWLEDGMENTS

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EXTENDED ABSTRACT

Celecoxib-loaded Nanoemulsion: Solubility of Celecoxib in Various Fractionated Medium Chain Triglycerides and Surfactants

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SUMMARY

Drug loading in nanoemulsion (NE) formulation is highly dependent on the drug's solubility in oil and surfactant. Hence, the study aimed to examine the solubility of celecoxib (CXB) in fractionated medium chain triglycerides (FMCTs) oils, namely palm kernel oil (PKO), palm kernel olein (PKOlein) and medium-chain triglycerides (MCTs), with the surfactants Span 80, Span 20, Tween 80, and Tween 85. CXB was mixed in oils and surfactants before being centrifuged at 4000 rpm for 15 mins. As a result, higher solubility of CXB was seen in MCTs and mixed PKOlein/MCT oil, and surfactants of Tween 80 and Tween 85, as indicated by the formation of a clear solution.

Keywords: Celecoxib, Fractionated medium chain triglycerides (FMCTs), Nanoemulsion, Solubility, Surfactant

INTRODUCTION

Celecoxib (CXB) is a Biopharmaceutics Classification System (BCS) Class II drug with high permeability but low water solubility, resulting in poor bioavailability which limits its oral usage. Thus, transdermal delivery could be a potential alternative to overcome this problem. Nevertheless, the skin barrier function restricts its permeation into the skin. Nanoemulsion (NE) is an optically isotropic colloidal dispersion of oil, surfactants, and water with droplet sizes ranging from 20 to 500 nm. NE as a transdermal carrier system not only improves the drug's permeation but also increases the drug's solubility. Medium-chain triglycerides (MCTs) containing medium-chain fatty acids (carbon 6 to 12) are also gaining interest due to their excellent solubilising potential. Drug loading in each NE formulation is highly dependent on the solubility of the drug in both oil and surfactants. Hence, this study aimed to screen the solubilising capability of FMCTs and non-ionic surfactants for further use in developing a NE formulation for transdermal delivery of CXB.

MATERIALS AND METHODS

The fatty acids (FA) composition of each FMCTs was determined by gas chromatography (GC). Various FMCTs oil (PKO, PKOlein and MCTs), mixed FMCTs oils; all in 1:1 ratio (PKO/MCTs, PKOlein/MCTs and PKO/PKOlein/MCTs), and non-ionic surfactants (Span 20, Span 80, Tween 80, and Tween 85) were used for solubility determination of CXB. An excess amount of CXB was dissolved in 2 g of each excipient followed by vortex mixing to homogenise and allowed to settle in a water bath at 45-50°C for 10 mins before centrifugation for 15 min at 4500 rpm (1). Any changes to the mixture, either becoming clear (soluble) or turbid (insoluble) were observed and recorded.

RESULTS AND DISCUSSION

CXB, a highly permeable drug but less soluble in water, was selected for the experiment. This kind of lipophilic drug can be delivered by incorporating it into oil droplets to form oil-in-water (O/W) NE, which should be small enough to allow passive diffusion through skin barriers. In addition, the solubilising capacity of the oil is crucial for the development of stable NE because it indirectly demonstrates the drug's loading capacity (2). Compared to other oils, MCTs and PKOlein/MCTs were found to have excellent solubilising performance (Figures 1c and e). This might be due to the higher content of medium chain fatty acids (MCFAs) of caprylic acid (C8) and capric acid (C10) at 55.8% and 44%, respectively, as shown in

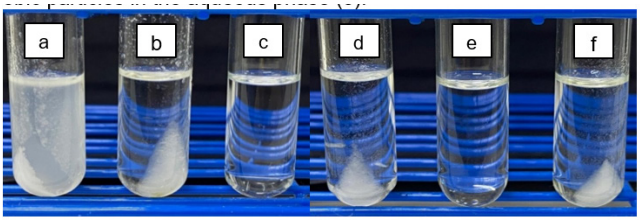

Figure 1: Solubility of CXB in FMCTs oils. PKO (a); PKOlein (b); MCTs (c), PKO/MCTs (d), PKOlein/MCTs (e); and PKO/ PKOlein/MCTs (f)

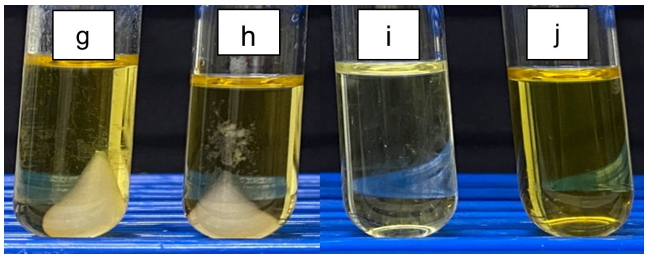

Figure 2: Solubility of CXB in non-ionic surfactants. Span 20 (g); Span 80 (h); Tween 80 (i); and Tween 85 (j)

Table I. According to Shahba et al. (3), carbon 8 and 10 are able to act as lipophilic solubilisers to enhance the solubility of a highly lipophilic drug.

A good surfactant should promote drug solubilisation while being non-irritating on the skin. Non-ionic surfactants (Span 20, Span 80, Tween 80, and Tween 85) are preferred over other surfactants because they cause less skin irritation and toxicity (4). As in Figure 2, Tween 80 and Tween 85 gave clear solution, indicating that CXB have good solubility in both. The hydrophilic-lipophilic balance (HLB) values of Tween 80 and Tween 85 are 15.00 and 11.00, respectively. In general, hydrophilic surfactants with HLB values larger than 10 are ideal for formulating (O/W) NE to disperse hydrophobic particles in the aqueous phase (5).

Table I: Compositions of FA in FMCTs oils

FA	PKO (%)	PKOlein (%)	MCTs oil (%)
Caprylic acid C8-0	2.9	3.4	55.8
Capric acid C10-0	3.0	3.2	44.0
Lauric acid C12-0	46.0	41.7	0.2
Myristic acid C14-0	16.4	13.8	-
Oleic acid C18-1	17.3	22.3	-

*FA= fatty acids, FMCTs= fractionated medium chain triglycerides, PKO, palm kernel oil, PKOlein= palm kernel olein, MCTs=medium chain triglycerides

CONCLUSION

In conclusion, MCTs and mixed PKOlein/MCT oils, along with Tween 80 and Tween 85 demonstrated good CXB solubilisation capability and can potentially be used for phase studies in the development of NE formulations.

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

Development and Effectiveness of Alcohol-Based and Non-Alcohol-Based Hand Sanitiser Cream Formulations

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SUMMARY

Developing hand sanitiser cream containing moisturising agents may overcome skin problems and increase consumer compliance. Alcohol-based (F1-F2) and non-alcohol-based (F3-F4) hand sanitiser creams were developed and tested with a 1-month accelerated stability study. The stability study parameters include homogeneity, pH, droplet size, zeta potential and microbial enumeration. As for the efficacy study, the Kirby-Bauer method was employed. Overall, all formulations were stable, as no significant changes were seen in the formulated creams throughout the stability study. All formulations showed antimicrobial activities, with F3 showing the highest diameter of inhibition against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus epidermidis with diameters of 11.5mm, 10mm, 8mm and 13.5mm respectively. Hence, F3 was the best formulation since it demonstrated the highest zeta potential values and inhibition diameter.

Keywords: Hand sanitiser cream, Zeta potential, Antimicrobial activities, Stability study, Efficacy

INTRODUCTION

Hand sanitisers in liquid and gel forms cause dryness and dermatitis due to the lack of moisturising agents (1). Therefore, we developed cream-based hand sanitiser incorporated with moisturising ingredients to prevent skin dryness and irritation. However, it may be unsuitable to develop hand sanitiser cream with an effective ethanol concentration of 60-95%, since emulsions containing more than 40% ethanol have been reported to be unstable (2). This study was conducted to investigate the development of stable and effective hand sanitiser creams. The formulations were tested for their droplet size, zeta potential and antimicrobial activities. The research could lead to the development of hand sanitisers that can be used as both hand lotion and hand sanitiser, protecting consumers against pathogens while maintaining skin health.

MATERIALS AND METHODS

Hand sanitiser creams were formulated as oil-in-water emulsions containing materials listed in Table I for both alcohol-based and non-alcohol-based cream formulations using double-boiling and homogenisation techniques. A one-month preliminary accelerated stability study was conducted at 40°C±2 C with a relative humidity of 75%±5%. The parameters evaluated were homogeneity, pH evaluation, droplet size, zeta potential, microbial limit and specific microbial tests. Microbial limit tests were performed according to the USP 40 which includes total aerobic microbial count (TAMC), total yeast and mould count (TYMC) and specific microbial tests (*S. aureus* and *P. aeruginosa*) (3). The antibacterial activity was determined using the Kirby-Bauer method by measuring inhibitory zone diameters.

Table I: Hand sanitiser cream formulations

Ingredient	Maximum allowed concentration (%)	Function	The concentration used (%)			
			F1	F2	F3	F4
Isopropyl alcohol (IPA)	90	Actives	60			
Ethanol (EtOH)	95			60		
Benzalkonium chloride (BKC)	0.1				0.1	
Chloroxylenol (CXL)	1.0					1.0
Water	100	Solvent	4.8	4.8	64.9	64
Glycerin	79.2	Humectant	16	16	12	12
Glycerol monostearate	5	Emulsifier	5	5		
Olivem	5				5	5
Carbopol	25	Thickener	4	4	7.5	7.5
Olive oil	83	Emollient	8.2	8.2	9	9
Phenoxyethanol	1	Preservative	1	1	1	1
Fragrance	2	Fragrance	1	1	0.5	0.5

RESULTS AND DISCUSSION

In this study, four different actives, including IPA (F1), EtOH (F2), BKC (F3), and CXL (F4) were compared for stability parameters such as homogeneity, pH, droplet size, zeta potential and microbial stability. The correct selection of surfactants, co-surfactants, and oils, determines the functional qualities of emulsions. Throughout 1-month accelerated stability study, all formulations were homogeneous, and pH values were within the acceptable range (pH 4.1-5.8) with average pH of 4.25±0.01, 4.36±0.01, 5.86±0.08 and 5.10±0.03 for F1-F4, respectively. In addition, the droplet size of all formulations were within the range of macroemulsion (1-50 µm) (Figure 1). The droplet size decreased from 0-day to week 4, indicating stability and adequate surfactant concentration. Next, the creams were tested for zeta potential to determine the degree of attraction and repulsion among colloidal particles. High zeta potentials of -32.3 - -49.13mV were shown in Figure I, indicating strong electrostatic repulsion and stability of physical properties (4). However, higher alcohol concentrations tend to increase droplet size and lower zeta potential measurements.

Pharmaceutical products can harbour harmful microorganisms that cause product degradation and diseases (5). This study found no growth for TAMC, TYMC, *P. aeruginosa*, and *S. aureus* in the tested cream formulations after one-month storage, indicating their microbiological stability and safety. F3 was found to be the most effective against different types of bacteria as shown in Table II. Nonetheless, F1 and F2 showed less effectiveness but were comparable to commercial hand sanitiser. No interaction was found between alcohol and other ingredients that adversely affect the alcohol effectives as a hand sanitiser.

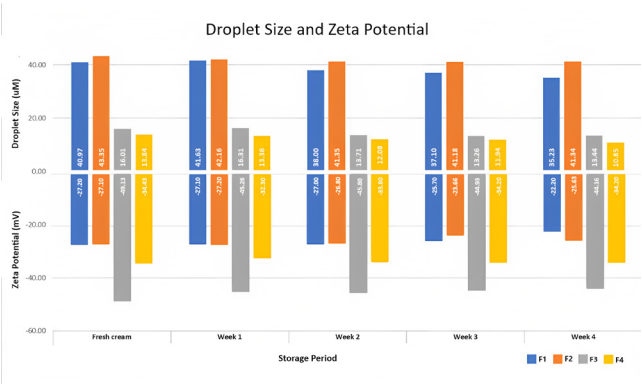

Figure 1: The droplet size and zeta potential values of cream formulations

CONCLUSION

All formulations were homogenous, pH suitable for skin and showed antimicrobial activity against gram-negative and gram-positive bacteria. Non-alcohol-based hand sanitiser is better than alcohol-based hand sanitiser in terms of droplet size and zeta potential parameters. The best formulation was F3 which had high zeta potential values and inhibition zone diameter compared to other formulations.

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Table II: Antimicrobial activities of cream formulations including positive and negative controls. Results are expressed as the mean±SD, n=3.

Sample	Actives	Concentration (%)	Diameter of inhibition(mm)			
			E.coli	*S.aureus*	*P.aeruginosa*	*S.epidermidis*
F1	Isopropyl alcohol	60	7.25±0.04	10.5±0.71	8	8.75±0.35
F2	Ethanol	64.8	7.75±0.35	7.25±0.35	8	8.75±0.35
F3	Benzalkonium	0.1	9	12±1.41	9.5±0.71	13±1.41
F4	Chloroxylenol	0.6	7.75±0.35	8.5±0.71	9	9.75±0.35
Positive control	Denatured alcohol (commercial)	68	11.5±0.71	8	10	13.5±0.71
Negative control	Sodium chloride	0.9	0	0	0	0

EXTENDED ABSTRACT

Impact of Homogenisation Time on Droplet Size Distribution and Rate of Separation of Emulsions Formulated with Various Oils and Olivem 1000 as an Emulsifier Agent

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SUMMARY

The purpose of this research is to examine the influence of various oils on the droplet size and stability of emulsions that use Olivem 1000 as an emulsifying agent. Nine formulations were prepared at different homogenisation times of 5, 10 and 15 minutes using the hot mixing technique. Each formulation was evaluated for droplet size distribution, polydispersity index (PDI), and stability study for over a month. Formulation 6, comprising 10% grape seed oil, 7% Olivem 1000 and 83% water with homogenisation time of 10 minutes is considered the best formulation as it produced the smallest droplet size, low PDI, and good stability with no separation upon one-month storage.

Keywords: Emulsion, Particle size distribution, Olivem 1000, Oils, Stability study

INTRODUCTION

Emulsions are dispersed in multiphase systems in which the dispersed phase is present in the form of droplets in a continuous phase. The droplets' diameter lies between 0.1 µm and 0.1 mm, depending on the emulsification process (1,2). The selection of oils and emulsifiers is critical to ensure the emulsions prepared are stable. The objective of this study was to formulate a series of emulsions using various oils (jojoba, grape seed, olive, and Waglinol) in varying proportions of oil, water, and emulsifying agent. Olivem was used as an emulsifier for the emulsification process with a hydrophilic-lipophilic balance (HLB) value of 8-9. In oil-in-water (O/W) emulsions, the emulsifiers with HLB in the range of 8-18 shall be considered (3) as they are more hydrophilic and thus better at stabilising O/W emulsions. The droplet size, PDI, and stability of all formulations were assessed at homogenization times of 5, 10, and 15 minutes.

MATERIALS AND METHODS

The emulsions were prepared by mixing Olivem 1000 (B&T SRL, Italy) into the oil phase. The composition of emulsifying agents and oil is shown in Table I. There were four different oil types used: jojoba oil (Desert Whele Jojoba, US), grapeseed oil (Better World Sdn. Bhd, Malaysia), olive oil (Zulat Pharmacy, Malaysia), and Waglinol (Lasemul Corporation). The mix was heated at 80 C until Olivem 1000 melted. On the other hand, water was also heated at the same temperature. These two mixtures were mixed at different homogenisation times of 5-, 10-, and 15-minutes using Benchtop homogeniser (Pro Scientific Inc., USA). Subsequently, the droplet size distribution analysis was performed using Malvern Mastersizer 2000 (Malvern Instruments Co. Ltd, UK) set at 1500 rpm. Then, Stability Analyser LUMiFuge was used to determine their stability. The test was conducted for 45 minutes to determine the stability of the formulations for one-month storage.

Table I: Composition of nine (9) emulsion formulations

Formulation	1	2	3	4	5	6	7	8	9
Oil (%)	5	5	5	10	10	10	15	15	15
Olivem (%)	3	5	7	3	5	7	3	5	7
Water (%)	92	90	88	87	85	83	82	80	798

RESULTS AND DISCUSSION

Formulation 6 demonstrates the lowest PDI range for each oil and homogenisation time (0.4 to 0.79 µm) as compared to other formulations. The optimum formulation comprised of grape seed oil set at 10 minutes homogenisation time. This formulation have the smallest droplet size of 3.46 µm with PDI of 0.41 µm (Table II). This is attributed to grape seed oil being composed of 55% linoleic acid (C18:2), a polyunsaturated fatty acid that forms a rigid and dense viscoelastic film at the interface.

(4,5). This makes Olivem 1000 adsorb better onto the interface, preventing the emulsion from agglomeration. Table II shows the effect of homogenisation time on droplet size and uniformity of Formulation 6.

Additionally, as shown in Table III, it can be seen that Formulation 6 remains stable without separation for a month when using oils such as jojoba oil homogenized for 10 minutes, grape seed oil homogenized for 5, 10, and 15 minutes, and olive oil homogenized for 10 and 15 minutes. The most stable preparation is grape seed oil with a homogenisation time of 15 minutes (0.0104 mm of separation over a year).

Table II: Effect of homogenisation time on droplet size and uniformity of Formulation 6 (10% of oil, 7% of Olivem 1000 and 83% of water). Results are expressed as mean ± SE at least 3 experiments

Homo-genisation time	Droplet size (µm)			Uniformity (µm)		
	5 min	10 min	15 min	5 min	10 min	15 min
Jojoba oil	12.944 ± 2.793	8.081 ± 0.102	9.154 ± 0.025	0.791 ± 0.251	0.665 ± 0.020	0.520 ± 0.001
Grape seed oil	8.136 ± 0.277	3.468 ± 0.072	7.099 ± 0.190	0.598 ± 0.001	0.414± 0.012	0.655 ± 0.021
Olive oil	21.136 ± 0.454	7.243 ± 0.219	18.951 ± 0.159	0.754 ± 0.084	0.602 ± 0.028	0.547 ± 0.009
Waglinol	18.027 ± 1.770	8.891 ± 0.557	12.554 ± 0.273	0.705 ± 0.028	0.787 ± 0.055	0.699 ± 0.003

Table III: Stability analysis of Formulation 6

Homogenisation time(min) mm/month		Separation	
		mm/year	
Jojoba oil	5	0.01	0.1037
	10	0.00	0.0138
	15	0.02	0.2074
Grape seed oil	5	0.00	0.0449
	10	0.00	0.0346
	15	0.00	0.0104
Olive oil	5	0.01	0.1140
	10	0.00	0.0346
	15	0.00	0.0173
Waglinol	5	0.03	0.3041
	10	0.04	0.4666
	15	0.02	0.2523

CONCLUSION

Formulation 6 with 10 minutes homogenisation time was considered the best formulation due to its smallest droplet size distribution, good stability and no separation upon one-month storage for all oils. These findings provide fundamental knowledge on the oil compositions in emulsion preparation that can be applied in developing cosmeceutical products.

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

Structural and Thermal Characterisation of *Hibiscus rosa-sinensis* Mucilage for Pharmaceutical Applications

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SUMMARY

The extraction of *Hibiscus rosa-sinensis* (HRS) leaves using water resulted in a yield of 12.46% for the HRS mucilage. It possessed a molecular weight of 2.001 x 106 ± 6.578 x 104 kDa. Its high specific viscosity of 2.84 ± 0.03 confirmed its large molecular weight. In X-ray diffraction (XRD) analysis of HRS mucilage, a broad and clear peak was seen, showing the amorphous nature of the substance. Four endotherms and one exotherm were identified in the thermal spectra of HRS mucilage. Additionally, the attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy revealed important structural and functional polysaccharide groups. HRS mucilage could be used in pharmaceutical dosage forms, such as tablet binding agents, as well as stabilisers in emulsions and suspensions, to improve drug delivery systems.

Keywords: *Hibiscus rosa-sinensis*, Mucilage, Natural substance, Polysaccharides, Characterisation

INTRODUCTION

Natural materials have been actively investigated for pharmaceutical uses due to their biocompatibility and cost viability for large-scale production owing to the availability of renewable resources. Mucilage is among the natural substances evaluated for possible pharmaceutical applications. It is extracted from plant parts including fruits, pods, seeds, flowers, and leaves (1). Mucilage is a heteropolysaccharide with a complex structure. The United States Food and Drug Administration has classified polysaccharides as generally-recognised-as-safe (GRAS). It becomes highly viscous and slimy upon contact with water. HRS mucilage is a novel source of polysaccharides that could be useful in pharmaceutical applications. This study focused on the structural and thermal characterisation of HRS mucilage by XRD, differential scanning calorimetry (DSC), and ATR-FTIR spectroscopy analysis. Additionally, its physicochemical characteristics were reported.

MATERIALS AND METHODS

HRS leaves were collected from the Malaysian Agriculture Research and Development Institute (MARDI) Serdang, Selangor, Malaysia. Dried-powdered mucilage was extracted from HRS leaves using the method described by Saidin et al. (2). Molecular weight analysis, particle size, viscosity, pH, XRD, DSC, and ATR-FTIR spectroscopy were utilised to examine the physicochemical properties, as well as structural and thermal characteristics of HRS mucilage.

RESULTS AND DISCUSSION

The percentage yield of HRS mucilage obtained through water-based extraction of HRS leaves was 12.46%. Table I summarises the physicochemical properties of HRS mucilage. HRS mucilage X-ray diffraction analysis revealed a broad and distinct peak at 2θ = 24.37 ± 0.01°, indicating its amorphous structure (Figure 1a). Most natural polysaccharides like okra gum, psyllium, cress seed mucilage, locust bean gum and xanthan gum exhibited amorphous structures, unlike flaxseed mucilage which exhibited both crystalline and amorphous structures (3).

Figure 1b depicts the DSC thermograms of HRS mucilage. The thermal spectra revealed four endotherms and one exotherm. Because the mucilage contained mostly water, the transitions between 0 and 250 °C are associated with the moisture of the extract, as well as potential volatile molecules. The insignificant endothermic peak at 233.18

Table I: Physicochemical characteristics of HRS mucilage.

Characteristics	Remarks/values
Appearance	Solid powder
Colour	Dark green
Molecular weight	2.001 x 10^6 ± 6.578 x 10^4 kDa
PDI/homogeneity and molecular weight distribution	2.36 ± 0.11
Particle size	5.772 ± 0.299 µm
Uniformity of particle size	1.485 ± 0.007
Specific viscosity	2.84 ± 0.03
pH	6.80 ± 0.01

Mean ± SD, n = 3

± 0.45 °C could be attributed to eutectic impurity or to the sensitivity of the equipment. At 261.38 ± 6.35 °C, another broad exothermic peak was observed. This peak is associated with polysaccharide decomposition or cellulose and hemicellulose depolymerisation. The broad peak indicated an amorphous characteristic, which is consistent with XRD analysis.

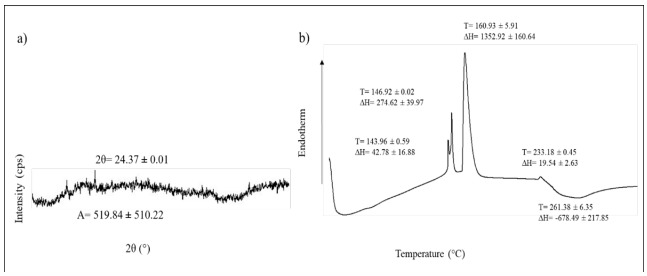

Figure 1: a) XRD diffractogram and b) DSC thermogram of HRS mucilage. A denotes area under the curve. Mean ± SF, n=3

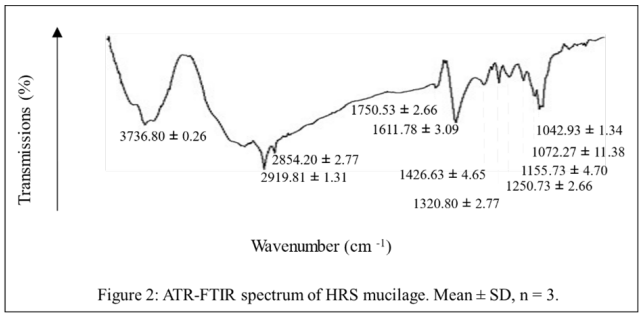

Figure 2: ATR-FTIR spectrum of HRS mucilage. Mean ± SF, n=3

The ATR-FTIR spectrum of HRS mucilage revealed the key functional and structural polysaccharide groups (Figure 2). The ATR-FTIR spectrum revealed peaks of O–H at 3736.08 ± 0.26 cm-1, alkanes at 2919.81 ± 1.31 cm^{-1} due to –CH2 and 2854.20 ± 2.77 cm^{-1} given –CH3, C=O ester at 1750.53 ± 2.66 cm^{-1}, amide at 1611.78 ± 3.09 cm^{-1}, C–O at 1320.80 ± 2.77 cm^{-1}, C–O–C or C–OH of the protein at 1042.93 ± 1.34 cm^{-1}, and C–N of aromatic amine group at 1426.63 ± 4.65 cm^{-1}. The region between 800 and 1600 cm^{-1} is known as the carbohydrate fingerprint area.

CONCLUSION

The present study showed that HRS mucilage has excellent characteristics for pharmaceutical applications. HRS mucilage could be employed in pharmaceutical dosage forms, including as tablet binding agents, as well as stabilisers in emulsions and suspensions, to improve drug delivery systems.

ACKNOWLEDGMENT

The authors wish to thank Faculty of Pharmacy, Universiti Teknologi MARA for their assistance throughout this project.

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EXTENDED ABSTRACT

Cytotoxicity of a Temperature- and pH-sensitive Poly(N-isopropylacrylamide-co-acrylic acid) Polymer and Its Monomers on BJ Cells

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SUMMARY

Preclinical safety assessment is essential in the development of new drug delivery systems before clinical translation. Therefore, the cytotoxic effect of a polymer based on copolymerised NIPAM and acrylic acid (pNIPAM-co-AAc) aimed to deliver caffeine topically was examined. The copolymer was synthesised, characterised, loaded with caffeine and subjected to an in-vitro cytotoxicity study on BJ cells. Homopolymer pNIPAM was not cytotoxic to BJ cells after 24 h of incubation (>80% cells were viable). Moreover, pNIPAM-co-AAc was cytotoxic even after 6 h exposure (<60% of cells survived). However, the cell viability was significantly enhanced when the copolymer was loaded with caffeine and the effect was more pronounced with prolonged exposure. This finding revealed the potential of caffeine to reduce the cytotoxicity of the copolymer.

Keywords: N-isoprylacrylamide, Toxicity, Caffeine, Fibroblast, Acrylic acid

INTRODUCTION

Poly(N-isopropylacrylamide) (pNIPAM) is one of the most studied synthetic polymers in biomedicine. In this study, a hydrophilic comonomer (acrylic acid, AAc) was copolymerised into NIPAM to obtain pNIPAM-co-AAc. NIPAM imparts thermal sensitivity to the particles, while AAc imparts pH sensitivity. These unique multi-responsive features can be manipulated for drug loading and release mechanisms. Caffeine was selected as a model compound due to its high stability and well-established skin permeation profiles. Our previous in-vitro permeation data showed that caffeine-loaded pNIPAM-co-AAc significantly enhanced the delivery of caffeine across the porcine epidermis (1). Moreover, the skin compatibility of topically applied pNIPAM-co-AAc was studied on ex-vivo porcine skin (2). However, there is no data on its toxicity at the cellular level. Therefore, this work examined the cytotoxicity effect of pNIPAM-co-AAc particles on human skin fibroblasts to provide a better understanding of developing pNIPAM-based particles as a topical drug delivery carrier.

MATERIALS AND METHODS

Particles of pNIPAM-co-AAc were synthesised by surfactant-free polymerisation (1). NIPAM (1 wt%) and N, N'-methylenebis-acrylamide were mixed in purified water and heated to 80°C. Then, the AAc monomer (5 wt%) was added, followed by potassium persulphate and continuously stirred under an anoxic condition. After 6 h of reaction, the crude mixture was purified by filtration and centrifugation. A homopolymer, polyNIPAM (control) was prepared likewise except in the absence of AAc. Caffeine was loaded into pNIPAM-co-AAc via post-fabrication encapsulation by allowing the freeze-dried polymer to immerse in a caffeine solution (3 wt%) at ambient temperature for 24 h. The loaded polymer was purified by centrifugation to remove free caffeine and a gel-like polymer was obtained as a final product. All polymers were screened for hydrodynamic size, thermal and pH-responsiveness, and caffeine entrapment efficiency (EE). The effects of caffeine-free pNIPAM-co-AAc and pNIPAM, caffeine-loaded pNIPAM-co-AAc and monomers [NIPAM (1 wt%) and AAc (5 wt%)] on cell viability were determined by applying treatments (100 µL dosing) separately on BJ cells for 6, 12 and 24 h before the MTT assay. All data were presented as mean ± SD and statistically analysed using SPSS version 17.0.

RESULTS AND DISCUSSION

The copolymer pNIPAM-co-AAc was successfully synthesised as monodisperse particles with the ability

to undergo a volume change in response to temperature and pH changes (Fig.1) and was moderately stable in a dispersion form (Table I). The EE of pNIPAM-co-AAc loaded with caffeine was 88.11%.

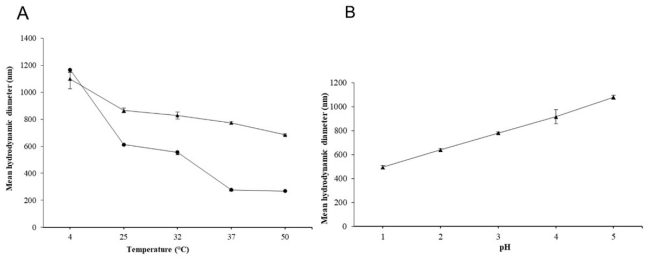

Figure 1: Mean hydrodynamic diameter of pNIPAM-co-AAc (▲) as a function of A) temperature and B) pH dispersed in purified water measured by a laser diffraction analyser (n=3 ± SD). Homopolymer, pNIPAM (●) was not tested for pH study as it did not have any significant ionisable functional groups

Table I: Size, polydispersity and zeta potential values of pNIPAM-based particles (n=3 ± SD).

Polymer	Mean hydrodynamic size (nm)	Polydispersity index	Zeta potential (mV)
pNIPAM-co-AAc	825.467 ± 7.481	0.113 ± 0.059	-31.6 ± 0.2
pNIPAM (control)	613.300 ± 19.446	0.094 ± 0.075	-27.8 ± 1.0

The cytotoxicity potential of the pNIPAM-co-AAc and its free monomers was studied in contact with the BJ cell line by MTT assay during 6, 12 and 24 h in culture, which is essential to demonstrate the biocompatibility of the materials (Fig.2A). BJ cells consistently showed decreased viability after 6 h of exposure (<50% of cells survived) to the NIPAM monomer and were more sensitive to the AAc monomer with only 12% of the cells were viable. The toxicity effect exhibited by the monomers could be due to their small molecular size and the presence of free reactive constituents, which might induce interactions with the cell components. Furthermore, the higher magnitude of toxicity exhibited by AAc (5 wt%) than NIPAM (1 wt%) could be due to its higher concentration. The concentrations were based on the amount of monomers utilised during the pNIPAM-co-AAc synthesis.

More than 80% of BJ cells survived after being exposed to pNIPAM polymer. The cellular sensitivity to pNIPAM varied with time ($p<0.05$). Furthermore, the viability of cells was lowered to less than 60% when exposed to pNIPAM-co-AAc, with no significant difference in the toxicity level at all exposure times. The phenomenon could be due to the treated cells being incubated at 37°C (above the polymer's volume phase transition temperature), which may lead to aggregation and affect cell viability.

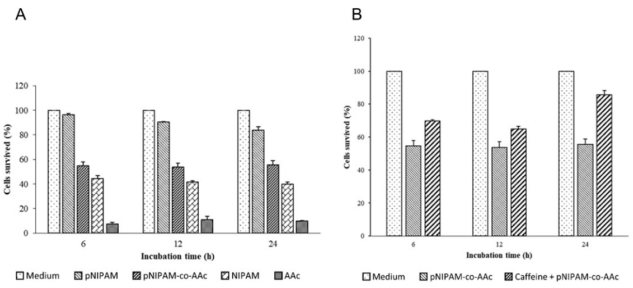

Figure 2: Cell viability of BJ cells at 6, 12 and 24 h exposed to A) onovers (NIPAM, AAc) and caffeine-free pNIPAM and pNIPAM-co-AAc caffeine-loaded pNIPAM-co-AAc. M: cells treated with medium onlt (control) (n=3 ± SD).

The BJ cells were further exposed to caffeine-loaded pNIPAM-co-AAc. Interestingly, the cell viability increased from 70% (6 h) to 86% (24 h), which suggested the ability of caffeine to reduce the cytotoxicity level of the polymer following prolonged exposure duration (Fig.2B) ($p<0.001$). The presence of caffeine might reverse the cytotoxic effect of pNIPAM-co-AAc, as caffeine was previously shown to possess a cytoprotective effect on cells by inhibiting cell apoptosis (3).

CONCLUSION

Particles of pNIPAM-co-AAc were cytotoxic to BJ cells with no significant variation across the exposure duration. However, the toxicity decreased significantly in the presence of caffeine, suggesting its cytoprotective effect on cells. Despite the cytotoxic effect shown by the monomers, pNIPAM-co-AAc might have the potential to be a candidate for topical drug delivery systems.

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EXTENDED ABSTRACT

Effect of Tannic Acid on the Functional Properties of Gelatine Films

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SUMMARY

Hydrophilic matrix systems are favoured in thin film formulations with an extended mode of drug release due to their low manufacturing cost and toxicity. The present work was designed to evaluate the feasibility of bovine gelatine crosslinked by tannic acid (GelTA) as a film matrix for oromucosal drug delivery. The effect of tannic acid concentrations on the mechanical and functional properties of GelTA films was evaluated. The crosslinking of gelatine with tannic acid generally enhanced the tensile strength (7.4–11.8 N) while reducing the elongation percentage (15.7–44%) of GelTA films. Furthermore, the films were significantly resistant to dissolution despite their rapid and massive degrees of water uptake in favour of extended delivery systems.

Keywords: Gelatine, Tannic acid, Crosslinking, Tensile strength, Film

INTRODUCTION

Gelatine has been considered one of the most promising biopolymers. Despite its biodegradability, biocompatibility and low antigenicity, gelatine exhibits poor mechanical and water-resistant properties under physiological conditions, limiting its use in drug delivery systems. Crosslinking is one of the strategies proposed to improve gelatine's limitations. However, chemical crosslinkers such as glutaraldehyde have raised toxicity concerns, resulting in using natural polyphenols as an alternative (1). Tannic acid is a polyphenolic biomolecule, non-toxic and water-soluble. Phenolic hydroxyl and carboxyl groups could interact with gelatine chains via covalent and hydrogen bonding to generate crosslinked networks (2). The crosslinking of gelatine with tannic acid (GelTA) strategy has been applied and studied in tissue engineering, surgical adhesives and food packaging. However, little data is available on the feasibility of this improved material as a film matrix for extended delivery systems. In this study, GelTA films were prepared to assess the effects of tannic acid concentrations on film properties aimed at buccal applications.

MATERIALS AND METHODS

Films were prepared by solvent evaporation (3). A 5 wt% gelatine solution was prepared by adding gelatine (bovine skin, Bloom number 225, type B) into a glycerine-water mixture (20 wt% based on gelatine dry weight), heated to 50°C and mixed until uniform. Afterwards, oxidised tannic acid (up to 10 wt% based on gelatine) was added to the gelatine and adjusted to pH 8. The resulting film-forming solution (25 mL) was cast on Teflon moulds (140 mm) and left to dry at room temperature for 48 h. A control film was prepared likewise, except without tannic acid and pH adjustment. The films were labelled as BG-TAX, where X represents the tannic acid content. Following conditioning (at room temperature and 58 ± 2% relative humidity for 14 days), films were characterized: mass (analytical balance, Sartorius Talent ML503), thickness (digital micrometre, Digimatic Indicator IDC series 543), surface pH (digital pH meter, Delta 320), dissolution time (USP-II dissolution tester; ELECTROLAB TDT-08L), swelling index, tensile strength and elongation at break (texture analyser, TA.XT. plus). Data were subjected to statistical analysis (SPSS, version 21) and the results were expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

The mass and thickness of the films consistently increased ($p > 0.05$), but the surface pH values were reduced as the tannic acid concentration increased ($p < 0.05$) (Table I). Furthermore, GelTA films (> 50 min) took a much longer time to completely dissolve compared with their non-crosslinked counterpart (~6 min). The aqueous solubility-resistant effect is dependent on the tannic acid concentration.

The swelling of polymeric film is essential for its

Table I: Mass (n = 5 ± SD), thickness (n = 5 ± SD), surface pH (n = 3 ± SD) and dissolution time (n = 3 ± SD) of GelTA films. BGTA0 (non-crosslinked) serves as control.

Films	Mass (mg)	Thickness (µm)	Surface pH	Dissolution time (min)
BG-TA0 (control)	16.52 ± 1.08	62.80 ± 7.10	7.47 ± 0.21	6.0 ± 1.0
BG-TA0.5	17.56 ± 1.89	68.07 ± 7.76	7.89 ± 0.07	50.0 ± 5.0
BG-TA2	18.42 ± 1.00	72.13 ± 3.60	7.54 ± 0.02	ND
BG-TA5	17.50 ± 1.21	72.13 ± 5.05	7.35 ± 0.06	ND
BG-TA10	19.92 ± 1.64	73.93 ± 6.27	7.40 ± 0.02	ND

ND film fragments were visibly observed in the dissolution vessel at 8 h

adhesion to biological surfaces and drug release from the film matrices. The non-crosslinked film (control) gained the maximum weight within 5 min, followed by a rapid weight loss due to gelatine dissolution (Fig.1A). GelTA films generally showed a rapid and notable swelling in the first 5 min, followed by a slower swelling until equilibrium. Films with 5 and 10 wt% tannic acid exhibited significantly lower swelling than the control film (Fig. 1B).

Tensile strength and elongation at break reflect the strength and flexibility of films (4). The tensile strength and elongation percentage values of GelTA films ranged from 5.3 N to 11.8 N and 15.7% to 44%, respectively (Fig. 2). An increase in tannic acid concentration consistently increased tensile strength but significantly reduced elongation at break ($p < 0.05$). The formation of covalent bonds between gelatine functional groups and tannic acid hydroxyl moieties results in the restriction of polymer chain mobility (reducing elongation at break) while promoting rigidity (enhancing tensile strength).

CONCLUSION

GelTA films have potential values to be used as a film matrix for buccal applications as they exhibit substantial aqueous medium uptake capacity, extended dissolution time and sufficient mechanical properties. However, further studies are necessary to evaluate the effects of other formulation parameters on the films' functional properties, stability, and biocompatibility.

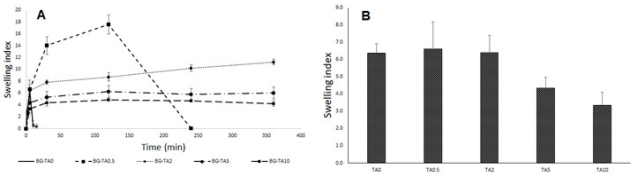

Figure 1: Swelling index of GelTA films in simulated salivary fluid, pH 6.8 at 37°C, (CA) measured over 6 h and (B) based on the tannic acid concentration measured 5 min post-contact with the fluid (n = 4 ± SD). BGTAD serves as control

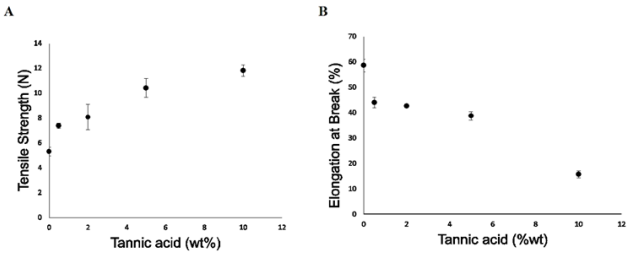

Fig. 2: **(A)** Tensile strength (*N*) and **(B)** elongation at break (%) of GelTA films in various tannic acid concentrations (up to 10 wt%; *n* = 3 ± SD).

Figure 2: Tannic strength (N) and (B) elongation at break (%) of GelTA films in various tannic acid concentration (up to 10 wt%; n = 3 ± SD)

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EXTENDED ABSTRACT

Development of a Composite Film Containing Zeolite or Activated Charcoal as Adsorbents

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SUMMARY

Conventional wound dressings such as gauze and cotton bandages absorb a large portion of the moisture of the wound causing the wound's surface to dry up, leading to slow wound healing and pain upon dressing removal. The limitations of conventional wound dressings have prompted the development of alternative polymer-based wound dressings. A polymer composite film made from sodium carboxymethyl cellulose (SCMC) and konjac glucomannan (KGM) with the addition of Zeolites (SKZ) or activated charcoal (SKAC) can potentially form an optimal film formation for the purpose of wound healing with antimicrobial effects. The studies show that mixing AC and Zeolite with SCMC increases film thickness due to a greater solid content. The AC had no significant effect on the tensile strength of the film; however, the addition of Zeolite increased the film's tensile strength. SZ films have shown that they can expand very well, but the films quickly reached their maximum limit and began to break apart. SKAC films have shown that they can extend well and keep their shape for a longer period of time without breaking.

Keywords: Sodium carboxymethyl cellulose, Konjac glucomannan, Zeolite, Wound dressing, Composite film

INTRODUCTION

SCMC is a natural polymer that has good film forming capabilities. It is low cost and can absorb large amounts of water which helps to provide wound hydration stimulation of cellular proliferation and tissue debridement. KGM is a non-starch polysaccharide that demonstrates enhanced thickening, stabilising, and gel-forming characteristics which can be very beneficial for film formation. Zeolite is a crystalline aluminosilicate comprising of an ordered array of micro pores. Its microporous structure allows it to selectively absorb various inorganic and organic compounds. Activated charcoal (AC) is a charcoal that have been treated with high temperature to become more porous. AC imparts antimicrobial qualities via adsorption mechanisms by adsorbing bacteria and microorganism. In this study, a composite film based on SCMC and KGM were incorporated with zeolites (SKZ) or activated charcoal (SKAC) and SCMC (S) film and SCMC/KGM (SK) films as control.

MATERIALS AND METHODS

Films were prepared by film casting method and evaluated for their physicochemical properties and antimicrobial capability. Different formulations were prepared based on w/w, SKZ: 1.5% SCMC, 1% KGM, and 2% zeolite. SZ: 2.5% SCMC and 2% zeolite. SKAC: 1% SCMC, 1.5% KGM and 2% AC. Each formula was weighted and left to hydrate under continuous stirring for 30 minutes at 1000 rpm. About 35 g of each mixture was poured into Petri dishes and left to dry in an oven for 24 hours at 40°C. The films were cut into 3 cm x 5 cm rectangles and clamped onto the texture analyser to evaluate their tensile strength. An electronic digital calliper was used to measure the thickness. Swelling study was conducted by submerging the films in PBS, pH 7.4 at different time intervals to swell. Initial and post swelling weights were recoded. The surface and cross section morphology were observed using scanning electron microscope (SEM). Antimicrobial studies were conducted by conducting agar diffusion test against E.coli and S.aureus strains. All tests were conducted in triplicate.

RESULTS AND DISCUSSION

During film preparation, the SKZ formulation had resulted in an inconsistent, brittle, and separated films. Suspected to be due to the interaction between zeolite and KGM. Improved characteristics were obtained after removing KGM. Hence, films solely comprising SCMC/zeolite (SZ) were produced. SKAC films were successfully produced. Therefore, SZ, SKAC, and their respective controls: control (S) and control (SK) films

Table I: Tensile strength of SZ, SKAC, and control films

Tensile strength (N)	Type of film			
	SZ	Control (S)	SKAC	Control (SK)
Average	126.58	87.84	112.55	110.8
S.D	14.99	4.91	9.7	10.46

Table II: Thickness measurement of SZ, SKAC, and control films

Thickness measurement (mm)	Type of film			
	SZ	Control (S)	SKAC	Control (SK)
Average	0.22	0.08	0.26	0.1

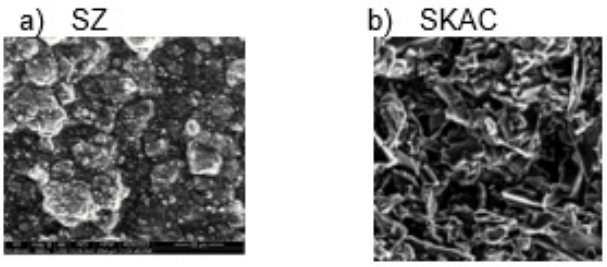

Figure 1: SEM images of a) SZ and b) SKAC under 500x magnification

were subjected to the physicochemical tests.

SZ film presented the highest average tensile strength compared to control. The presence of more solid particles and the formation of more compact structures may be the cause of the increase in tensile strength following the presence of the zeolite. The presence of KGM in both SKAC and SK films enhanced the tensile strength. However, the difference due to presence of AC was not significant.

In terms of thickness, Both SZ and SKAC films were thicker than their respective controls. Mainly due to the increase of solid content when zeolite and AC were added which contributes to the film's composition. Suitable swelling ratio for film dressings lays between 100-900%, optimal film formulation should absorb the exudate without creating a free-flowing gel due of the excessive swelling (2). Average reading for SZ: 2 mins= 373%, 5 mins=627%, 10mins=718%. Average reading for SKAC: 10 mins= 436%, 30 mins= 889%, 60 mins= 1074%. SZ films has exhibited excellent swelling capability. However, the films rapidly reached its maximum swelling capacity at 10 minutes and started to break off and turn into free-flowing gel. SKAC films had successfully exhibit excellent swelling capabilities and can maintain its shape for more than 60 minutes without breaking off.

For antimicrobial studies, all tested samples did not exhibit any antimicrobial properties as no zone of inhibition was observed. This was expected since zeolite did not possess bactericidal effects and need to be combined with another antimicrobial agent. In earlier research, X zeolite were ion exchanged with biocidal cations of copper and zinc, and these treatments were found to demonstrate excellent antimicrobial activity (3).

For the SKAC films, the concentration of AC used is low (2%). Previous studies with positive antimicrobial results had incorporated about 85-98% of AC in their dressing (4). Results suggested the need for the incorporation of antimicrobial agents to obtain antimicrobial effects or an increase in AC concentration thus require formulation optimisation.

CONCLUSION

The study successfully formulated new films by combining two adsorbent elements, SZ and SKAC. The results suggest that SKAC increases film thickness but not tensile strength, while the opposite is observed for SZ. SZ films have good swelling properties but liquidifies quickly. On the other hand, SKAC films have excellent swelling capabilities and can maintain shape for more than an hour without breaking off. There is still room for improvement in both films' integrity when they swell and their antimicrobial properties.

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EXTENDED ABSTRACT

Physicochemical Properties and Recent Modifications of Sago (*Metroxylon sagu*) Starch for Pharmaceutical and Food Applications: A Scoping Review

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SUMMARY

This study utilized a scoping review that employed the PCC (Population/Participants, Concept, and Context) framework. The researcher identified 21 articles for the physicochemical properties and 48 articles for recent modifications of sago starch extraction and modifications for pharmaceutical and food applications. The physicochemical, proximate, nutritional, phytochemical, and antioxidant properties were studied. Besides, the extraction procedures and chemical structures were modified, boosting their functionality and utility. The research has shown efforts to improve the quality and functional properties of native sago starch using physical, chemical, enzymatic techniques like crosslinking and green technology in various industrial applications, including food and non-food.

Keywords: Physicochemical properties, *Metroxylon sagu*, Functionality, Scoping Review, Sago Starch

INTRODUCTION

Sago starch has gained appeal for food and non-food applications in recent years. For the food industry, this material can be processed into a functional food for its valuable content of nutrients and functional properties. To produce a variety of functional qualities, sago starch was changed using a variety of physical and chemical processes such as microwave-heated, ultrasound-heated extraction, and hot extrusion technology. Many studies have been published on sago starch's physicochemical, functional properties, and modifications. This study aims to piece together the existing knowledge and to go over some key points connected to sago starch research, precisely the physicochemical, proximate, nutritional, phytochemical, antioxidant, and functional properties and modifications of starch extraction and its recent modifications for pharmaceutical and food applications.

MATERIALS AND METHODS

The study is a qualitative research approach, specifically Scoping Review. It aims to describe existing literature and other sources of information, commonly including findings from various study designs and methods. This search allows the researcher to give insights into how prior researchers looked at it. The Preferred Reporting Items for Systematic Reviews (PRISMA) Statement was extended to Scoping Reviews – the PRISMA-ScR. The researcher utilized online research websites from the World Wide Web to gather specific studies following the PRISMA-ScR. The keywords such as physicochemical, proximate and nutritional, phytochemical, antioxidant, and functional properties and modifications of starch extraction and its recent modifications for pharmaceutical and food applications were used during the search. The following databases were used to acquire relevant data and studies: PubMed, MedlinePlus (accessed through the University's EBSCOhost), and Google Scholar.

RESULTS AND DISCUSSION

This study summarized the published articles on Sago starch, focusing on its properties, proximate and nutritional, phytochemical, antioxidant, functional, and recent modifications in the extraction for two of its major applications, pharmaceutical and food. This Scoping Review covers twenty-one (21) articles from 2000 to 2022 for the study of the physicochemical properties and forty-eight (48) articles for recent modifications of Sago (Metroxylon sagu) starch extraction and modifications for pharmaceutical and food applications published from 2018 to 2022.

For physicochemical properties, 8 were from Malaysia (38.10%), 5 from Indonesia (23.81%), and 8 from

other Asian countries (38.09%). In the study of starch modifications, 27 articles (56.25%) were from Malaysia,15 from Indonesia (31.25%) and 6 (12.5%) from other Asian countries.

For physicochemical properties, results revealed diverse values that are due to different sources, cultivation processes, maturity, and genetic and environmental factors. The recent modifications of Sago starch extraction in improving its functionalities include microwave heating, ultrasound-assisted extraction, oxidation, compressing machine, fractionation, and hot extrusion technology, etc. Several studies showed efforts to modify native sago starch extraction to yield more starch. Moreover, some recent modifications of sago starch for pharmaceutical and food applications were gamma irradiation and annealing, microwave heat treatment, invitro fermentability hydroxypropylation, acetylation, phosphorylation, dual modification (phosphorylation/acetylation), etc.

Pursuing the quest for underutilized starch sources might lead to several potential applications of these starches in business. A fundamental understanding of its characteristics is essential to use the starch efficiently, and data on sago starch must continually be searched. Its physicochemical characteristics can be modified to increase its usability and value for commercial applications. More in-depth research on sago starch is suggested to resolve inconsistencies in certain physicochemical properties.

CONCLUSION

Despite the well-studied properties, there is still much to study on sago, particularly its modifications. A good source to answer food security and explore its value in commercial applications. The future of the sago palm appears to be secure. More research investigations are needed to uncover sago starch's promise with its broad yet unique qualities.

ACKNOWLEDGMENTS

The University of the Immaculate Conception, Davao City, Philippines, supports this work.

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EXTENDED ABSTRACT

Ex vivo Skin Permeation and Accumulation Studies on Crisaborole Nanoemulsion Cream for the Treatment of Atopic Dermatitis

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SUMMARY

The permeability of crisaborole was investigated via ex vivo Franz diffusion chambers with dorsal skin of ICR mice. Positive control (2% w/w in USP ointment, PC), 1.5% and 2.0% w/w crisaborole nanoemulsion (CN) were tested and crisaborole accumulation in skin layers was measured using the tape stripping method. The cumulative drug permeation (Qt) for PC and CN2.0% were significantly higher compared to CN1.5% at 95% confidence level. The apparent permeability coefficients (Kp) showed no significant differences in all formulations ($p<0.05$). CN1.5% and CN2.0% showed significantly higher accumulations in stratum corneum compared to PC ($p<0.05$). There was no significant difference observed in crisaborole accumulation between all formulations in epidermis.

Keywords: Atopic dermatitis, Crisaborole, Nanoemulsion, Drug permeation, Drug accumulation

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory disease identified by intense pruritus and inflamed eczematous lesion which caused by genetic mutation and environmental factor [1]. Current medication for AD includes topical steroids, immunosuppressant, H1 receptor inhibitor, phosphodiesterase-4 (PDE4) inhibitor and monoclonal antibody [2]. In the presence of allergen, PDE4 increased in AD patients where it regulates the production of inflammatory mediator via degradation of cyclic adenosine monophosphate (cAMP). Crisaborole is a PDE4 inhibitor with tetrahedral geometry which mimics cAMP and occupy binding site at PDE4 [3]. Accumulation of cAMP activates protein kinase A (PKA), thus inhibit pro-inflammatory and T cell cytokinase. Nanoemulsion was a suitable carrier to deliver lipophilic drugs such as crisaborole which exhibit higher solubilisation capacity, greater kinetic stability, and paracellular and transcellular transport capability [4]. The efficacy of crisaborole nanoemulsion was examined using Franz diffusion cell and the accumulations of crisaborole in stratum corneum and epidermis were further examined using tape stripping method.

MATERIALS AND METHODS

Nanoemulsion consists of vitamin E (Sime Darby, Malaysia), PEG-40 hydrogenated castor oil, Tween 80 and carboxymethyl cellulose (CMC) and crisaborole (Sigma Aldrich) was developed by using self-emulsification method. Incised mice skins (ICR, 2x2 cm) were shaved and cleaned using phoshate buffer (pH 7.4) prior mounting in between the donor and receptor chamber of Franz diffusion cell (Permagear, USA). Samples (n=3) were collected from the receptor chamber at 1 hour interval for 8 hours. The samples were analyzed using high performance liquid chromatography (HPLC, Waters, USA), and crisaborole concentrations were plotted over time. Tape stripping was performed using cellophane tape on previously used mice skin. Stratum corneum layer were collected with 15 strips and 20 strips for epidermis layer. Samples were suspended in a mixture of phosphate buffer (pH 7.4) and dimethyl sulfoxide (DMSO, Sigma Aldrich) (30:70) for 60 min. Samples was subjected to HPLC analysis for determination of crisaborole concentrations.

RESULTS AND DISCUSION

As for statistical analysis, we performed one-way ANOVA throughout the data at 95% confidence level. In PC, crisaborole were mixed in USP ointment as vehicle to replicate common method of topical drug delivery. While in nanoemulsion groups, crisaborole were incorporated in oil phase to increase solubility. In transdermal permeation study (Fig. 1), the cumulative

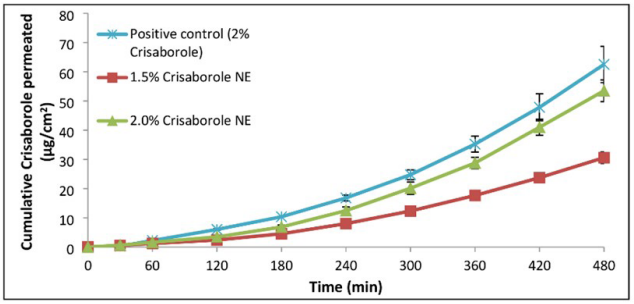

Figure 1: Transdermal profiles of CN1.5% and CN2.0% as compared to PC

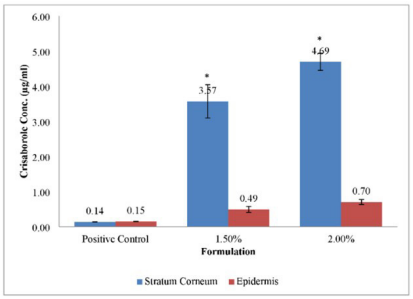

Figure 1: Concentration of crisaborole in skin layer with CN 1.5% and CN 2% cream as compared to PC

drug permeation (Qt) for PC (62.52±6.19 µg/cm^2) and CN2.0% (53.50±3.68 µg/cm^2) were significantly higher as compared to CN1.5% (30.61±1.97 µg/cm^2, where P<0.05). These results showed CN2.0% allow similar permeation profile compared to USP ointment. There was no significant difference between PC and CN2.0% due to similar concentrations of crisaborole (p<0.05).

These patterns were translated in permeation flux (J), where both PC and CN2.0% were significantly higher compared to CN1.5% indicating higher crisaborole concentration passed through the skin layer (Table I). However, there are no significant differences in the apparent permeability coefficients (Kp) between PC and both CN1.5% and CN2.0% (0.06±0.00 cm·h-1, 0.05±0.00 cm·h-1 and 0.07±0.01 cm·h-1,p<0.05). This showed that nanoemulsion and USP ointment permits similar rates of permeation through the bilayer lipid.

The advantages of nanoemulsion were demonstrated in tape striping study (Fig. 2), where the accumulation of crisaborole in stratum corneum showed that CN1.5% (3.57±0.47 µg/ml) and CN2.0% (4.69±0.24µg/ml) were significantly higher compared to PC (0.14±0.00 µg/ml, p<0.05). Due to addition of CMC, nanoemulsion was able to rehydrate the corneocytes, increases its moisture and maintaining its function. Besides that, nanoemulsions encapsulated crisaborole and sustained release over longer time period, thus increases its therapeutic effects. Meanwhile, in the epidermis layer, there were no significant differences in crisaborole accumulation between PC, CN1.5% and CN2.0%, respectively (p<0.05). Once crisaborole reached the epidermis layer, it inhibited the activity of PDE4 enzymes by mimicking cAMP resulting an increase of cAMP concentrations, thus triggering downregulation of inflammatory responses in the skin.

Table I: Flux and permeability coefficient of crisaborole nanoemulsions as compared to PC

	Flux (J)	Permeability Coefficient (Kp)
Positive Control (2% Crisaborole)	0.158±0.015	0.063±0.006
CN1.5%	0.078±0.006	0.052±0.004
CN2.0%	0.134±0.009	0.067±0.005

CONCLUSION

Crisaborole nanoemulsion formulation exhibits moisturizing effects and sustained-release properties. Altough the permeation profiles of nanoemulsion formulation were similar to PC, it has higher accumulation of crisaborole in the stratum corneum resulting in prolong anti-inflammatory effects on AD patient's skin.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Cytotoxic and Anti-angiogenic Potentials of Lactiplantibacillus spp.-derived Cell Free Supernatant against CT26 Mouse Colon Carcinoma Cells

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SUMMARY

The limitations of chemotherapy against colorectal cancer (CRC) raise the need for alternative approaches. This study examined the cytotoxic and anti-angiogenic potentials of cell free supernatant (CFS) fermented by lactic acid bacteria (LAB) (i.e., Lactiplantibacillus plantarum LAB1 and LAB12) isolated from fermented food against CRC in vitro. LAB1 was the most potent Lactiplantibacillus sp. against CT26. Immunocytostaining indicated LAB-induced (especially LAB12) downregulation of vascular endothelial growth factor (VEGF) and upregulation of thrombospondin (TSP-1). High-performance liquid chromatography (HPLC) found LAB1 to yield the highest concentration of short-chain fatty acids (SCFA). The beneficial effects of LAB against CRC were strain-dependent.

Keywords: Lactic acid bacteria, Colorectal cancer, Cytotoxicity, Anti-angiogenesis, Short-chain fatty acids

INTRODUCTION

CRC is the third most common cancer diagnosed worldwide and its burden is anticipated to increase by 3.2 million new cases by 2040 (1). Unfortunately, the efficacy of conventional chemotherapy and targeted therapy against CRC are often associated with side effects and compromised by cancer resistance. The limitations of current treatments against CRC raise the need for alternative approaches to preventing and/ or managing CRC. Given that the majority of CRC cases are sporadic and associated with diet, disease prevention through the consumption of probiotics may be a viable option. In fact, there is growing evidence indicating the strain-dependent usefulness of probiotic-derived bioactive metabolites against CRC. Hence, this study assessed the cytotoxic and anti-angiogenic potentials of cell free supernatant (CFS) fermented by two unique strains of lactic acid bacteria (LAB) isolated from locally fermented food against CRC in vitro.

MATERIALS AND METHODS

The LAB was identified through 16S rRNA gene sequencing and the outcomes were matched with consensus sequence from the GeneBank. The LAB was subcultured three times before centrifugation at 1,300 x g and 4° C for 15 minutes. The supernatant was filter-sterilised and screened for potential cytotoxic effects against CT26 (a mouse colon carcinoma cell line) by using the Sulforhodamine B (SRB) assay. The LAB-derived CFS was then assessed for anti-angiogenic potentials at their respective highest subtoxic concentration (IC15) by immunocytostaining CT-26 with antibodies of VEGF and TSP-1. Images were captured by a camera attached to a fluorescent microscope and analysed using the Nikon Nis-Elements BR software for intensity [FITC (VEGF) and TRITC (TSP-1)]. Subsequently, HPLC analyses were performed on the LAB-derived CFS, focusing on four major SCFA (i.e., acetic acid, butyric acid, lactic acid, and propionic acid). Statistical analysis was performed by using GraphPad Prism v.8.

RESULTS AND DISCUSSION

The LAB were identified as Lactiplantibacillus plantarum LAB1 (Accession number: JN039347) and LAB12 (Accession number: JN039358) with 99% similarity, respectively. In general, the LAB-derived CFS inhibited CT26. Relatively, LAB1-derived CFS exhibited more potent cytotoxic effects against CT26 when compared to LAB12-derived CFS. The IC50 of LAB1-derived CFS was 2.3 fold lower than that of LAB-12 derived CFS (Table 1). This is in line with probiotic mixtures (Bifidobacterium and Lactiplantibacillus spp.) which exhibited cytotoxic effect when tested against CT26 (2). As expected, paclitaxel, the positive control, was cytotoxic against CT26 due to its role in halting cell division (3).

On the other hand, LAB12-derived CFS was more anti-angiogenic when compared to LAB1-derived CFS. More specifically, LAB12-derived CFS significantly

Table I: Mean IC_{50} and IC_{15} of LAB-derived CFS against CT26

	Mean ± SD* IC_{50}	Mean ± SD* IC_{15}
LAB1-derived CFS	2.75+1.7%	0.50+0.3%
LAB12-derived CFS	6.20 ±1.6%	2.30±1.3%
MRS	13.30±2.7%	2.20+2.1%
Paclitaxel	0.24±0.1nM	0.008+0.7nM
DMSO	1.30±1.0%	0.90+0.4%

* Each data represents mean ± standard deviation (SD) of at least three independent experiments.

downregulated VEGF by 45% ($p<0.05$) [Fig1 (a)] and significantly upregulated TSP-1 expression by 44% ($p<0.05$) [Fig1 (b)]. This is an implication of the regulation of the angiogenic switch by the LAB-derived CFS which is in line with previous findings. *L. rhamnosus* GG (LGG), for instance, downregulated proangiogenic mediators VEGF and angiopoietin expressions in CRC cells (HT29 and HCT116) (4).

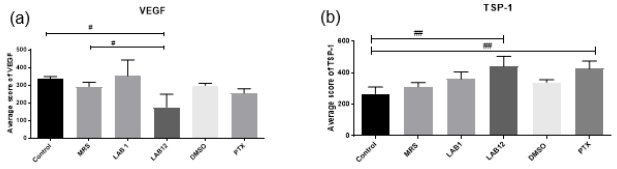

Figure 1: Effect of LAB-derived CFS against expression of (a) pro-angiogenic VEGF and (b) anti-angiogenic TSP-1 in CT26. Each bar represents mean + SD of n = 3 #p<0.05; ## p<0.01

For SCFA measurement, HPLC analyses of acetic acid (AA), butyric acid (BA), lactic acid (LA) and propionic acid (PA) in the LAB-derived CFS found LAB1-derived CFS to yield the highest concentration of all four of SCFA in relative to LAB12 (AA: 1.96±0.07 mM vs 1.86±0.0 mM, BA: 1.14±0.06 mM vs 1.08±0.02 mM, LA=1.60±0.21 mM vs 1.50±.02 mM and PA: 2.33±0.2 mM vs 2.16±0.00 mM) (Fig. 2). The potent cytotoxic effect of LAB1-derived CFS may likely be attributed to the production of SCFA given that SCFA may promote cytotoxic effects on CRC cells (5).

CONCLUSION

The present findings demonstrated the strain-dependent cytotoxic and anti-angiogenic properties of LAB-derived CFS against CRC in vitro. The LAB-derived SCFA appear to mediate their cytotoxic effects. This warrants validation in vivo studies.

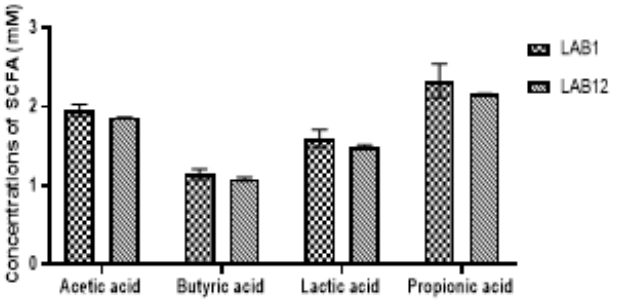

Figure 2: HPLC analyses of LAB1 and LAB12- CFS. Each bar represents mean + SD of n > 2.

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

Effects of d-α-tocopherol Supplementation on Serum and Hepatic Lipid Profiles of High Fat Diet-Fed C57BL/6J Mice

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SUMMARY

This study aimed at evaluating the beneficial effects of d-α-tocopherol against high-fat diet (HFD)-induced metabolic dyslipidaemia. C57BL/6J mice (n=11/group) were subjected to either normal chow, HFD or HFD supplemented with 2.85mg/mL d-α-tocopherol for 20 weeks. As anticipated, HFD-fed mice were dyslipidaemic and presented with significantly higher ($p<0.05$) adipose tissue content when compared to the normal chow-fed counterparts. The HFD-induced dyslipidaemia was improved by d-α-tocopherol, likely through the significantly reduced ($p<0.01$) serum total cholesterol (TC) despite the increased ($p<0.05$) serum triacylglycerol (TAG) which could be associated with the requirement of TAG-rich lipoproteins for lipid and vitamin E transport in the circulation.

Keywords: d-α-tocopherol, vitamin E, high-fat diet, hypercholesterolaemia, cholesterol

INTRODUCTION

Overnutrition has been recognised as the main aetiology of metabolic syndrome (e.g., obesity and dyslipidaemia) that predisposes affected individuals to various serious complications and cardiovascular-related mortality. The development of metabolic disorders is commonly induced by constant intake of a high-fat diet (HFD) and partly mediated by increased oxidative stress (1). It is known that excessive dietary nutrients could increase hepatic mitochondrial reactive oxygen species (ROS) production, which could in turn give rise to oxidative stress. Although antioxidant therapy using lipid-soluble vitamin E has been implied as being beneficial against these metabolic disorders, previous findings of its effects against chronic HFD feeding remain inconsistent. Whilst some studies reported that vitamin E supplementation could improve atherogenic dyslipidaemia (including improvement of hypertriglyceridaemia) (2), other studies reported otherwise (1). As such, this study was undertaken to validate the lipid modulatory potentials of d-α-tocopherol, the most biologically active vitamin E, in chronic HFD-fed mice.

MATERIALS AND METHODS

This in vivo study was approved by the Committee on Animal Research and Ethics of the UiTM (reference number: 283/2019). Briefly, seven-week-old, male C57BL/6J mice (JAX stock #000664, Jackson Laboratory, USA) were randomly divided into three groups (n=11/group) and subjected to feeding with either normal chow (15% lipid) (Altromin, Lage, Germany), HFD (35% lipid; 60% energy from fat) (Altromin, Lage, Germany) or HFD gavaged with 2.85mg/mL d-α-tocopherol (NOW FOODS, Bloomingdale, USA) for 20 weeks. The body weight of each mouse was monitored weekly. The mice were eventually anaesthetised, and terminal blood was collected for assessment of serum lipid profiles using colorimetric assay kits (Bioassay Systems, Hayward, CA, USA and Elabscience, Wuhan, China). The adipose tissues were also collected and weighed. The livers were harvested for hepatic lipid profiling using commercial assay kits (Elabscience, Wuhan, China).

RESULTS AND DISCUSSION

As expected, the HFD-fed mice were presented with significantly higher ($p<0.05$) body weights (week-8 onwards), adipose tissue and adipose tissue/ body weight ratio when compared to the chow-fed mice (Figure 1A-C).

The HFD-fed mice were also presented with dyslipidaemia when compared to the chow control (Figure 2A–D). The HFD-induced hypercholesterolaemia was improved by d-α-tocopherol supplementation, likely through significant ($p<0.01$) reduction of serum total cholesterol (TC) (Figure 2A), a risk factor of cardiovascular mortality (3). Serum triacylglycerol (TAG) was, however, increased ($p<0.05$) in d-α-tocopherol supplemented HFD-fed mice when compared to the HFD control group (Figure

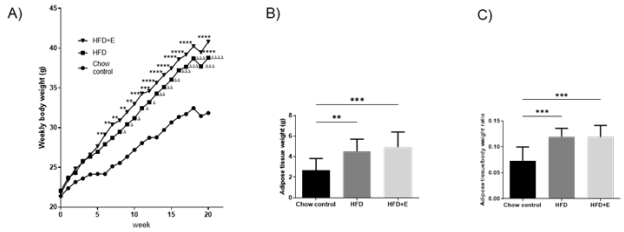

Figure 1: Effects of supplementation of d-α-tocopherol on body and adipose tissue weights of HFD-fed mice. A) Each point represents the mean body weight of 10≤n≤11. (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 when compared to chow control, Δp<0.05; ΔΔp<0.01 when compared to HFD); For B) adipose tissue weight and C) adipose tissue/body weight ratio, each bar represents mean ± SD of 10≤n≤11. *p<0.05, **p<0.01 and ***p<0.001

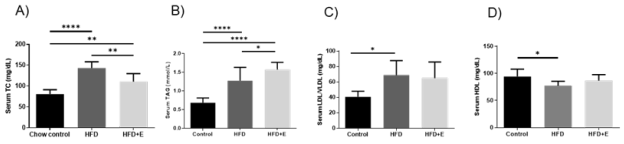

Figure 2: Effects of supplementation of d-α-tocopherol on serum lipid profiles of HFD-fed mice. For A) total cholesterol (TC); B) triacylglycerol (TAG); C) low-density lipoprotein/ very low-density lipoprotein (LDL/VLDL); D) high-density lipoprotein (HDL), each bar represents mean ± SD of 10≤n≤11. *p<0.05; **p<0.01 and ***p<0.001.

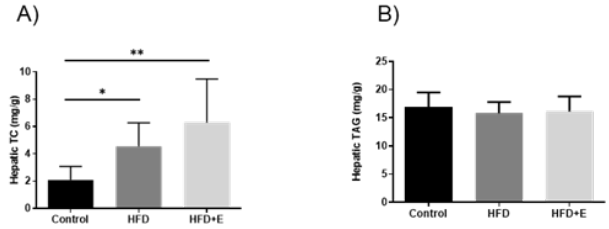

Figure 3: Effects of supplementation of d-α-tocopherol on hepatic lipid profiles of HFD-fed mice. For A) total cholesterol (TC) and B) triacylglycerol (TAG), each bar represents mean ± SD of 10≤n≤11. *p<0.05 and **p<0.01.

2B). The d-α-tocopherol supplementation also did not improve the hepatic lipid profiles of HFD-fed mice (Figure 3 A–B). The variation as seen between the serum and hepatic lipid profiles could be attributed to the lipoprotein metabolism. It is known that lipoprotein is required for the transport of cholesterol, TAG and vitamin E in the circulation (4). As such, the absorbed dietary α-tocopherol would be packaged into chylomicrons (large TAG-rich lipoprotein) in the enterocytes, secreted into the lymphatic circulation and subsequently entered the blood circulation before being delivered to the liver. The elevated serum TAG as observed in d-α-tocopherol supplemented HFD-fed mice could be associated with the increased chylomicrons in the circulation (5). When the chylomicrons are catabolised, α-tocopherol would then be carried by other lipoproteins like HDL, which might have contributed to its modest but insignificant increment in d-α-tocopherol supplemented HFD-fed mice. In the liver, α-tocopherol would have protected hepatic lipid from catabolism, thus did not result in lowering of hepatic TC and TAG. At the same time, the abundant α-tocopherol transfer proteins in the liver would simultaneously promote secretion of α-tocopherol into the circulation, which would in turn carried by LDL and VLDL, hence did not lead to lowering of serum LDL/ VLDL.

CONCLUSION

The present findings of 20-week d-α-tocopherol supplementation appear to be beneficial against HFD-induced hypercholesterolaemia. Its blood cholesterol-lowering effects could be useful for the primary prevention of heart diseases. This warrants further investigations into its prolonged use (> 20 weeks) and underlying mechanisms.

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EXTENDED ABSTRACT

Cardioprotective Effects of Ethanolic Extract of *Cedrus deodara* Roxb against Isoproterenol Induced Myocardial Infarction in Rats

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SUMMARY

Myocardial infarction (MI) is an ischaemic heart disease. Herbal drugs were some treatment options, and the current study explored the cardioprotective effects of Cedrus deodara ethanolic extract (EECD). 100 and 200 mg/kg of EECD were treated in rats (Sprague Dawley) for 30 days and induced with MI by isoproterenol 85mg/kg on 29th and 30th day. Invasive blood pressure (IBP) and electrocardiogram (ECG) showed the improvement ($p<0.001$) and serum cardiac markers (aspartate aminotransferase, lactate dehydrogenase, creatinine kinase, and alanine aminotransferase) were significantly ($p<0.001$) reduced after the treatment with EECD. This study indicated the cardioprotection by EECD in MI conditions.

Keywords: Myocardial infarction, *Cedrus deodara*, Blood pressure, Electrocardiogram, Cardiac biomarkers.

INTRODUCTION

MI is the reflective condition of the heart disease of ischemia and occurs when ischemia surpasses the myocardium's threshold level. Bestowing to the World Health Organization, deaths due to cardiovascular disease (CVD) are reported as 17.9 million each year. MI is reported to cause mortality in many cases after 28 days of detection including deaths before reaching emergency department [1]. These highlights need early recognition and need to be prevented. Drugs with antioxidants or radical sequestration properties might be beneficial for treating oxidative stress linked to CVD with MI. Medicinal plants articulate diverse pharmacotherapeutic effects in CVDs and on exploration in cardiotoxic models. The present study determined the cardioprotection of ethanolic extract of *Cedrus deodara* (EECD) on isoproterenol (ISO) arbitrated MI in rats.

MATERIALS AND METHODS

The fresh heartwood of *Cedrus deodara* was collected and authenticated by Prof. Madhava Chetty, SV University, Tirupati, India. The dried material was powdered and extracted with absolute ethanol, concentrated under a vacuum evaporator and refrigerated [2]. A preliminary phytochemical study was performed. For the pharmacological study, four groups of male SD rats (aged 8 weeks) were used. Ethics permission was obtained, Ref: I/IAEC/LCP/009/2012/WR/24. Group I as control (only vehicle treated at equal volume of drug dispersed) and normal saline was administered; Group II with ISO (85mg/kg, s.c) was administered with 24 hrs intervals on 29th and 30th day. Pre-treatment of EECD were performed on group III and IV at 100 and 200 mg/kg individually for 30 days as well as injected with ISO (85mg/kg, s.c on 29th and 30th day). Invasive blood pressure (IBP) and electrocardiogram (ECG) were analysed with PowerLab [3]. The antioxidants and blood biomarkers were studied, which indicated the protective effect of EECD on MI-induced rats.

RESULTS AND DISCUSSION

The percentage yield was noted as 4.25 (106.25g) of the original material (2.5kg). The preliminary phytochemical test identified the flavonoids, alkaloidal, glycosidic compounds with proteins, saponins and tannins. In the pharmacological study, ISO administration increased heart weight significantly ($p<0.001$). Abnormal heart attributes to augmented fluid content, oedematous intramuscular gaps and amplified protein content [4]. Pre-treatment of EECD (100mg/kg and 200mg/kg) decreased ($p<0.001$) the weight of heart, indicative of myocardial protection against infiltration. The diagnostic for MI, such as creatinine kinase MB, lactate

Table I: Biochemical parameters on treatment with EECD

Groups	LDH	CK-MB	ALT	AST	IBP (mmHg) (Mean Arterial pressure)	HR (bpm)	ST SEGMENT (sec)
	IU/L						
Control	135.2±6.57	196.2±5.19	43.80±5.86	74.99±7.16	119.0±3.36	461.3±18.25	0.10±0.00
ISO	220.6±7.76	305.3±8.90	98.19±8.03	127.7±5.96	89.40±3.98	380.4±18.42	0.35±0.06
ISO+ ECD 100	181.1±1.16*	261.3±6.67*	81.43±2.70*	105.3±1.77*	101.4±2.97*	441.0±15.11*	0.20±0.01*
ISO+ ECD 200	151.5±1.94**	199.4±4.98***	56.26±1.38***	85.32±3.02***	115.2±3.89***	471.8±15.31**	0.12±0.00***

*p<0.05, **p<0.01, ***p<0.001 indicates the comparison of drug treatment with the ISO-induced negative control substituted on one-way ANOVA statistics with Graph pad prism software (Version 7).

dehydrogenase (LDH), Aspartate transaminase (AST) and alanine transaminase (ALT) exudes from injured myocardium to the bloodstream upon rupture. The plasma membrane integrity and damage are reflective of serum marker enzyme elevation [5]. In the current evaluation, ISO-challenged rats exposed the significant escalation ($p<0.001$) in marker enzymes due to ISO-induced necrotic myocardial damage and plasma membrane exudation. EECD (100mg/kg and 200mg/kg) pre-treatment regulated ($p<0.001$) the marker enzymes in serum (Table 1). Invasive blood pressure (IBP) gives the accurate measurement of BP in powerLab gives an accurate measurement and standard methods for preclinical study of drug effects. ISO induction fluctuated and reduced the mean arterial pressure (MAP) and treatment with EECD improved. ISO induction in rats also displayed a "P" wave diminution with QRS complex, R–R interval diminution, leading to higher heart rate (Table I). These variations might be due to the loss of cell membrane integrity in the incapacitated myocardium. EECD (100mg/kg and 200mg/kg) pre-treatment in ISO-induced rats abrupted the pathological modifications in the ECG, indicating the reminiscent myocardial membrane protection.

CONCLUSION

In conclusion, the present study revealed the pharmacotherapeutic effect of *Cedrus deodara*, which maintained the cardiac dynamics with improved antioxidant enzyme levels with haemodynamics and cardiac biomarkers. These findings is a precise sustenance to recognise the beneficial pharmacological effects of *C. deodara* on cardioprotection.

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EXTENDED ABSTRACT

Natural Antioxidants in Medicinal Plants: For Better or for Worse?

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SUMMARY

Oxidative stress is one of the major contributors to the aetiology of chronic disorders. The modern lifestyle was seen to contribute significantly to the development of oxidative stress. The use of medicinal plants with antioxidant properties has been exploited to treat or prevent several human pathologies, of which oxidative stress seems to be one of the causes. These natural antioxidants, particularly polyphenols, have a variety of biological effects, including those that are anti-inflammatory, anti-ageing, anti-diabetic, anti-atherosclerotic, and anti-cancer. However, beneficial, and detrimental effects of antioxidant molecules used to reduce oxidative stress in several human conditions are apparent, especially in pre-clinical studies. Hence, natural antioxidants can also act as pro-oxidants.

Keywords: Chronic disorders, Medicinal plants, Polyphenols, Antioxidants, Pro-oxidants

INTRODUCTION

Traditional medicine was defined as all knowledge and practices, whether explicable or not, used in the diagnosis, prevention, and elimination of physical, mental, or social imbalance and solely relying on hands-on experience and observation passed down orally or in writing from generation to generation (1). Therefore, any plant with compounds that can be utilised therapeutically or as building blocks for producing effective pharmaceuticals is considered a medicinal plant. Most studies have reported that the therapeutic potential of these medicinal plants was due to the bioactive compounds present in the plants, which act as antioxidants and have different structures capable of health-promoting effects (3). However, there were some contradicting studies on the beneficial effects of these phytochemicals.

These medicinal plants are i) plants or plant parts used medicinally in galenical preparations (e.g. decoctions, infusions, tincture, etc.); ii) plants used for extraction of pure substances either for direct medicinal use or for the hemisynthesis of medicinal compounds (e.g. hemisynthesis of sex hormones from diosgenin obtained from Dioscorea yams); iii) food, spice, and aromatic plants used medicinally (e.g., ginger, turmeric, lemon grass); iv) microscopic plants, e.g., fungi, actinomycetes, used for isolation of drugs; and v) fibre plants like cotton, flax, and jute, for surgical dressings (2). These medicinal plants are essential for disease prevention, and their use is suitable for all existing prevention strategies.

An antioxidant is any substance that significantly delays or prevents the oxidation of a substrate in an organism. Therefore, the use of antioxidant supplementations to avoid acute and chronic illnesses has greatly expanded. Epidemiology and observation studies have shown that taking antioxidant supplements has a positive impact. In addition, many randomised controlled trials supported the same effect of antioxidant supplementation in disease prevention and management.

Foods and medicinal plants, such as vegetables, fruits, cereals, legumes, mushrooms, beverages, flowers, spices and traditional medicinal herbs, are rich sources of natural antioxidants. These natural antioxidants have various biological effects, including anti-inflammatory, anti-ageing, anti-atherosclerotic, and anti-cancer (4). Polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamin E and C) make up the majority of these naturally occurring antioxidants derived from plant sources (5). Therefore, exploring potential antioxidant sources and promoting their use in functional foods, pharmaceuticals, and food additives depend on the efficient extraction and accurate assessment of antioxidants from foods and medicinal plants.

CHRONIC DISEASES: RELATIONSHIPS BETWEEN OXIDATIVE STRESS AND INFLAMMATION

Chronic diseases persist a year or longer and necessitate continuing medical care, restricting daily activities or

both. The most common types of chronic illness are cancer, heart disease, stroke, diabetes, and arthritis. Insulin resistance, type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), and obesity-related chronic inflammatory factors are among the diseases that chronic inflammation plays a role in the aetiology of these diseases. Chronic inflammatory disorders are pathogenically influenced by oxidative stress (2). The inflammatory process, which results in the synthesis and release of proinflammatory cytokines, is triggered by various inflammatory stimuli, including excessive ROS/RNS produced during oxidative metabolism and some natural or artificial compounds. TNF-α production and NF-κB/AP-1 activation is crucial in the inflammatory process leading to many chronic illnesses.

NATURAL ANTIOXIDANTS ON OXIDATIVE STRESS

The overproduction of ROS may result in tissue damage leading to inflammation. The composition of the functional groups in phytochemicals present in medicinal plants determines their antioxidant action. The antioxidant activity depends on the structure of functional groups for their ability to scavenge radicals and metal ion chelation. Reduction of ROS production via inhibition of ROS-producing enzymes, ROS scavenging, overexpression of antioxidant defences, or protection of antioxidant defences. These antioxidants may decrease the catalytic activity of enzymes responsible for the production of ROS and prevent oxidative damage to the macromolecules such as lipids, DNA and proteins (3).
However, in aerobic conditions, for example, polyphenols, produce superoxide radicals, which dismutate to H2O2, then interact with reduced metal ions and superoxide to produce ROS. Tocopherols can also act as prooxidants when transition metals like Cu(I) are present, depending on the matrix environment in which it is present.

NATURAL ANTIOXIDANTS ON INFLAMMATION

These antioxidants affect enzymatic and signalling systems involved in the inflammatory processes, such as tyrosine and serine-threonine protein kinases. These enzymes have been linked to cell activation activities such as T cell proliferation, B lymphocyte activation, or cytokine production by stimulated monocytes. These natural antioxidants are inhibitors of α-glucuronidase and lysozyme released from neutrophils. In addition, they inhibit arachidonic acid (AA) release from cell membranes, suppressing prostaglandin biosynthesis and consequently preventing inflammation. These antioxidants can activate phase-II antioxidant detoxification enzymes, such as MAPK, PKC, and Nrf2, and inhibit proinflammatory enzymes like COX-2, LOX, and iNOS, also suppress NF-κB and AP-1 signalling pathways. They could regulate several inflammatory mediators, including cytokines, excitatory amino acids, peptides and metabolites from the AA pathway.

In the presence of transition metals, antioxidants are prone to act as prooxidants which initiate and/or amplify inflammation via the upregulation of several different genes involved in the inflammatory response, such as those that code for pro-inflammatory cytokines and adhesion molecules. This is due to the ability of these pro-oxidants to generate excessive ROS and further promote the inflammatory process, and contribute to damaging the body's own cells and tissues.

PERSPECTIVE ON NATURAL ANTIOXIDANTS

However, there are gaps of knowledge in the roles of antioxidants. Firstly, what are the correct antioxidants doses? There is proof that more is not always better and can be even worse. Almost all purported natural antioxidants lack the traditional dose-response and may have opposing effects when doses are increased. Secondly, the metabolism of gut microbes by these natural antioxidants and their pro- or anti-inflammatory effects on gut microflora are poorly understood. Antioxidants can be absorbed intact or converted into entirely other molecules is likewise poorly understood. A molecule that potentially mediates its anti-inflammatory effect can initially be a weakly soluble compound metabolised into a more soluble metabolite in the gut (3).

Next, these natural antioxidants, especially polyphenols, may be absorbed as complexes of two or more compounds, and complexes may have very different pharmacological effects than isolated compounds alone. Thus, the balance between the beneficial and detrimental effects of the antioxidants may depend on the type, dosage, and matrix of the antioxidants. DNA, proteins, and lipids can be damaged by pro-oxidant activity in normal cells, which results in cell death and trigger the production of cytokines that cause inflammation (3).

Natural antioxidants may be helpful as adjuvant therapy for the prevention and treatment of inflammation-related disorders in animal and cell models. However, many clinical studies have not established the relevance of these experimental investigations for extrapolation to humans (conducted double-blindly) that instigate deep discussions about whether these natural antioxidants are for better or for worse. Again, there are about three factors that influence the function of an antioxidant transforming it into a prooxidant; i.e. the presence of metal ions, the concentration of the antioxidant in matrix environments and its redox potential.

CONCLUSION

These natural antioxidants may have health benefits or adverse health effects, depending on the dosage the mechanisms involved in the protective effect of natural antioxidants under challenging circumstances, i.e. as antioxidants or pro-oxidants are yet to be understood

and discussed extensively. Hence, the therapeutic potential of antioxidants from medicinal plants remains untapped against disorders caused by inflammation and whether it continues to be for better or for worse.

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EXTENDED ABSTRACT

Phytochemical Constituents and Anti-Dermatophyte Activity of *Pandanus amaryllifolius* (Roxb.) Leaf Extracts

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SUMMARY

Recently, phytochemical herbal medicines have attracted more attention and are in great demand due to their effectiveness. Pandan leaves (Pandanus amaryllifolius (PA)) are well known as a source of natural flavouring and herbal remedies in Asian countries. However, scientific evidence regarding the health effects of PA is limited. This study revealed that PA extracts are rich in coumarins, phenols, flavonoids, and terpenoids that demonstrate moderate fungicidal effects. These findings could serve as a foothold for the development of topical herbal treatments for dermatophytosis.

Keywords: Pandanus amaryllifolius, Phytochemistry, Dermatophytosis, Antifungal, Topical application

INTRODUCTION

The emergence of dermatophyte infections has increased globally (20–25% of the world population are affected), and recurring infections occur at a rate of 22.2% within 3 years, which raises the issue of the effectiveness of antifungal medications on the market (1). In the search for new therapeutic solutions to address an increasing number of relapse incidences and treatment failures of dermatophytosis, natural plant extracts have garnered attention as a rich source of antimicrobial compounds. Studies prove that PA leaf extracts contain numerous bioactive compounds that are potent against a number of bacteria (2–4). Researchers in the Philippines have isolated four different alkaloids with antibacterial properties in the PA leaf extracts (2). Nevertheless, there is a paucity of information regarding the anti-dermatophyte properties of this plant. Hence, this study aimed to determine the phytochemical constituents of PA extracts and evaluate their ability to eradicate dermatophytes.

MATERIALS AND METHODS

Leaves of PA collected in Kuala Lipis, Pahang were identified by a botanist at Universiti Putra Malaysia (Voucher number: KM0003/22). The leaf extracts were prepared by consecutive maceration in three organic solvents of increasing polarity (i.e., hexane (PA-Hex), methanol (PA-Met), and distilled water (PA-Aqua)) in a 1:10 ratio, according to Taha H. et al. (2). The resulting crude extracts were tested for phytochemical screening, total phenolic content (TPC) and total flavonoid content (TFC) as described by Patacsil M. et al. (4). Furthermore, in-vitro antimicrobial studies were carried out using the broth microdilution method in accordance with the Guidelines of the Clinical and Laboratory Standards Institute M38-A2 protocol (5). Clotrimazole (Hi-media, India) was used as positive control. Minimum inhibitory concentrations (MICs) were visually determined using inverted reading mirrors and defined as the lowest drug concentration that causes 100% growth inhibition compared to the growth control. The minimum fungicidal concentrations (MFCs) were determined by inoculating 10 µL from each treatment well onto Sabouraud Dextrose Agar (Hi-media, India) plates. The plates were then incubated for 72 hours at 30°C. The lowest concentration with no visible growth on the SDA plates was defined as the MFC. The MICindex (MFC/MIC) of each extract was calculated against each test strain. Analysis of variance (ANOVA) with Duncan's post-hoc tests were used to analyse and compare the group data, with $p<0.05$ as the limit of significance.

RESULTS AND DISCUSSION

The percentage of crude extracts obtained through maceration of PA leaves with three different solvents (hexane, methanol, and distilled water) is as shown in Fig. 1A. The total percentage of extracts yielded through this method was 26.78%. Among the solvents used, PA-Met resulted in the highest extraction yield (17.08%),

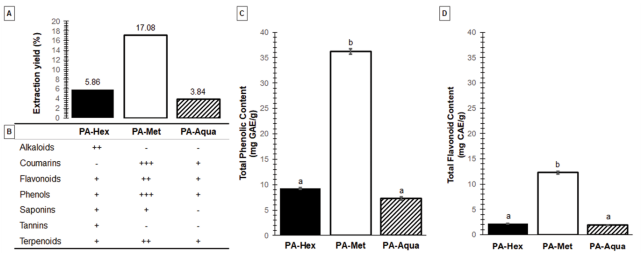

Figure 1: Phytochemical constituents of PA leaf extracts. (A) percentage of extraction yield; (B) phytochemical screening; +++ highly present; ++ moderately present; + slightly present; - absent; (C) total phenolic content (TPC); (D) total flavonoid content (TFC). The bars with different letters are significantly different from each other at p<0.05.

followed by PA-Hex (5.86%) and PA-Aqua (3.84%).

The phytochemical test of PA extracts revealed the existence of phenols, flavonoids, and terpenoids (Fig. 1B). Saponins were absent in the PA-Aqua, coumarins were absent in the PA-Hex, while alkaloids and tannins were only present in the PA-Hex. The highest values of TPC and TFC were observed in PA-Met (36.22±0.49 mg GAE/g; 12.29±0.25 mg CAE/g) followed by PA-Hex (9.23±0.17 mg GAE/g; 2.21±0.03 mg CAE/g) and PA-Aqua (7.26±0.33 mg GAE/g; 1.88±0.01 mg CAE/g).

This finding is in line with a study done by Suwannakul S. et al. who found that an ethanol extract of PA has an abundance of phenols that possess antibacterial effects against oral pathogens (4). In this present study, PA-Hex and PA-Met, which possess numerous bioactive compounds, especially phenolics, exhibited moderate antimicrobial activities against the tested strains, with MIC ranging from 128–512 µg/ml and MFC ranging from 128–1024 µg/ml as shown in Table I. Indeed, clotrimazole is significantly more potent (16–32 folds) as compared to other extracts against tested strains. However, PA-Hex and PA-Met exhibited fungicidal activity (MICindex ≤2) whilst clotrimazole demonstrated fungistatic activity (MICindex >2). Clotrimazole is an imidazole that is remarked as a fungistatic agent that interferes with ergosterol biosynthesis (an essential component of the fungal cytoplasmic membrane) by targeting the enzyme lanosterol 14-α-demethylase.

CONCLUSION

PA-Hex and PA-Met possess bioactive phytochemicals that contribute to moderate antifungal properties. This warrants further research toward the isolation and identification of their therapeutically active compounds for topical antifungal application to pinpoint these findings.

Table I: *In vitro* anti-dermatophyte activities of PA leaf extracts

Extracts/ Drug	*Trichophyton mentagrophytes* ATCC-9533			*Trichophyton rubrum* ATCC-28188		
	MIC (µg/ml)	MFC (µg/ml)	MIC_{index}	MIC (µg/ml)	MFC (µg/ml)	MIC_{index}
PA-Hex	256^{b}	512^{b}	2	128^{b}	128^{b}	1
PA-Met	512^{b}	1024^{b}	2	256^{b}	512^{c}	2
PA-Aqua	Na	Na	Nd	2048^{c}	Na	Nd
Clotrimazole	16^{a}	64^{a}	4	8^{a}	64^{a}	8

Note: Na – no activity at concentrations < 2048 µg/ml. Nd – not determined. "$^{a-c}$" Values within a column with different superscripts are significantly different from each other at $p<0.05$.

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EXTENDED ABSTRACT

Microbial Biotransformation of Synthetic Hormone Drugs and Bioactivities of its Transformed Products

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SUMMARY

Type 2 Diabetes Mellitus (T2DM) is often characterised by hyperglycemia, and the two inhibitors of thymidine phosphorylase and α-glucosidase are proven to help prevent T2DM. Steroids are widely known for their numerous medicinal uses, including potent α-glucosidase inhibitors. Microbial transformation is an effective method for creating new steroidal medicines from microorganisms, including yeasts, bacteria, and plant cells. It has been demonstrated that the approach may be used effectively to generate steroid-active pharmaceutical ingredients (APIs) and essential intermediates. This method has a naturally high yield outcome, is environment friendly and is a less time-consuming process that can synthesise steroid drugs more specifically. Estriol and 17-trenbolone were chosen since microbial transformation research on them is still ongoing.

Keywords: Biotransformation, α-Glucosidase, Thymidine phosphorylase, Estriol, 17β-Trenbolone

INTRODUCTION

Type 2 diabetes mellitus is a metabolic disease characterized by hyperglycemia. A-Glucosidases re membrane-bound enzymes that help the absorption of glucose in the small intestine. Thus, inhibition of α-glucosidase can significantly decrease postprandial hyperglycemia after a mixed carbohydrate diet and can be a key strategy in the control of type 2 diabetes mellitus (1). The efficiency of thymidine phosphorylase inhibitor in blocking the angiogenic factor of TP (2) and α-glucosidase inhibitor, which postpones the body's conversion of carbohydrates to sugar, have been proven to be able to prevent T2DM. Steroidal compounds exhibited various levels of activity against the enzymes such as tyrosinase and α-glucosidase in comparison to the standard inhibitors. Due to their therapeutic advantages, steroids are second only to antibiotics in market demand. However, research conducted in the 1950s attempted to produce the synthetic steroid molecule using the biotransformation approach because the compound has been created chemically. This is the source of both the increasing potency of steroids and the concept of their use as a drug (3). Using the wide variety of fungi and selected media conditions has the potential to produce many new substrates with higher yields of desirable products, which can later use as drugs to treat various diseases or used s a valuable starting material ("lead") for the drug discovery process. The less hazardous substrates are reaction-, enantiomer-, and region-specific- (4).

MATERIALS AND METHODS

Cultures of *Trichothecium roseum*, Beauveria bassiana, Aspergillus niger and Penicillium verrucosum were grown on Potato Dextrose Agar (PDA) and preserved at 4°C. The broth media was prepared by mixing 10g glucose, 5g NaCl, 5g KH2PO4 and 10.0mL glycerol into 1.0mL distilled water (5). The transformations and purity of the compound were analysed by silica-coated TLC plates (PF254). The crude material was then fractionated using RP-UHPLC (Thermo Fisher Scientific) equipped with Synergi 4 µm Hydro-RP 80 A column using acetonitrile/water. The bioactivity of the biotransformed metabolites against thymidine phosphorylase and α-glucosidase will be tested, and the binding mechanism of the potential inhibitors against thymidine phosphorylase and α-glucosidase will be studied by using saturation transfer difference (STD) NMR experiments through Bruker Avance III 600-NMR Spectrometer, and water-ligand observed via gradient spectroscopy (waterLOGSY) experiments. The IC50 will be presented as mean ± S.E.M (standard error of the

mean).

RESULTS AND DISCUSSION

All four fungi of *Trichothecium roseum, Beauveria bassiana, Aspergillus niger* and *Penicillium verrucosum* were tested against estriol, while only *Trichothecium roseum* and Beauveria bassiana was tested against 17β-trenbolone. The time-course analysis was also carried out to check the optimum number of days for the fungal growth. All tested fungi had the best growth on the 12-Day. The transformation formed was studied using TLC plates placed inside different ratios of hexane and ethyl acetate mixture. Only two out of four tested fungi were given positive results. For the 17β-trenbolone, only *T. roseum* tested positive results, while *B. bassiana* showed negative results on TLC plates.

Crude extract of microbial transformation of estriol with *Trichothecium roseum* was then further tested using RP-HPLC. Based on Figure 1, four new transformed metabolites were detected by RP-UHPLC after being compared with negative control media and reaction flask media (Figure 1). The new metabolites all show high concentrations, the highest at 2000 mAU and at least at 500 mAU.

Meanwhile, the transformation of 17β-trenbolone using *Trichothecium roseum* sp. also shows four new transformed metabolites (Figure 2). For the concentration of this transformation, most are at 500 mAU, while the rest is less than 250 mAU. The new metabolites formed will further be separated using semi-preparative RP-HPLC and will further be investigated using STD-NMR and waterLOGSY experiments.

CONCLUSION

The study showed that the microbial transformation

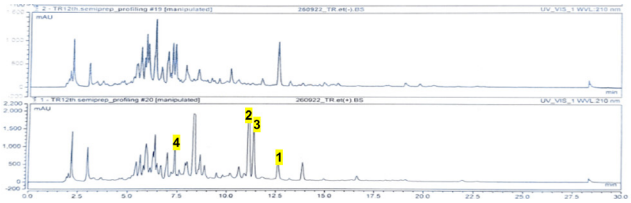

Figure 1: HPLC chromatography of reaction flask of estriol using *Trichothecium roseum* for biotransformation

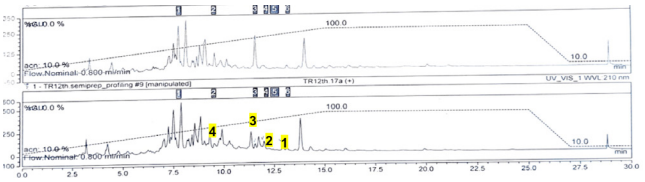

Figure 2: HPLC chromatography of negative control and reaction flask of 17β-trenbolone by using *Trichothecium roseum* for biotransformation

of synthetic steroids, estriol and 17β-trenbolone could mostly be possible using *Trichothecium roseum* with peaks higher than 500 mAU for estriol biotransformation. This shows that the diverse species of fungi give many possible potential transformed metabolites for different substrates.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Optimisation of High-Performance Thin-Layer Chromatography-Based Antioxidant Profiling of *Caulerpa lentillifera* Extracts

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SUMMARY

This study optimised a high-performance thin-layer chromatography (HPTLC) method that could facilitate the visualisation and quantification of antioxidative phytochemical compounds in Caulerpa lentillifera. This method involved the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and ferric chloride (FeCl3) as derivatising agents. It was found that the incubation period for DPPH• was consistent with 60 minutes in the dark, while the plate that was derivatised with FeCl3 needed to be neutralised using NH_4OH and then dried at 100°C for 5 minutes. The antioxidant activity of the seaweed extracts can now be analysed using Videoscan and quantified as gallic acid equivalents (GAE).

Keywords: *Caulerpa lentillifera*, high-performance thin layer chromatography, liquid-liquid extraction, 2,2-diphenyl-1-picrylhydrazyl, ferric chloride

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects mainly the elderly with dementia (1). The treatment options for AD are limited and do not cure the root cause of the disease. This raises the need for alternative neuroprotective approaches. In this regard, natural products have emerged as a viable option, given their multitudinous bioactive compounds. *Caulerpa lentillifera* J. Agardh is a naturally edible green seaweed that has increasingly gained popularity due to its high nutritional quality (2). In order to uncover the neuroprotective potential of *C. lentillifera* against neuroinflammation, the present study was undertaken to optimise a simple HPTLC method that would allow the visualisation and quantification of phytochemical compounds that could potentially contribute to its antioxidant activity.

MATERIALS AND METHODS

DPPH•, $FeCl_3$ and gallic acid were purchased from Sigma Aldrich (USA). Solvents were acquired from Chemiz (Malaysia). Seaweed (supplied by Gamai Sdn. Bhd. from Port Dickson, Malaysia) was cleansed, freeze-dried and ground to powder before extraction using methanol, and fractionation using hexane, chloroform and ethyl acetate. Extracts and standard were sprayed onto 20x10cm normal phase Silica gel-60-F254 using the Autosampler-IV. Bands were separated with a mixture of toluene, n-hexane, ethyl acetate, acetic acid and formic acid in the Automated-Multiple-Development-Chamber-2 (migration distance=70mm). Images were recorded by the TLC-Visualiser-Documentation-System. Chromatogram was derivatised by 0.2% (w/v) DPPH and 2% (w/v) neutralised FeCl3, respectively. Parameters that were optimised, including sample extraction, mobile phase percentage and derivatisation condition for DPPH• and $FeCl_3$ reagents.

RESULTS AND DISCUSSION

In terms of sample preparation, this study subjected the seaweed to a freeze-drying process before extraction, as freeze-dried seaweed was found to preserve the antioxidant compounds better when compared to oven-

dried seaweed (3). In chromatogram separation, the best separation was produced using a mixture of 52% toluene, 26% ethyl acetate and 22% formic acid as a mobile phase with a migration distance of 70 mm (Fig. 1). This study found that toluene and formic acid had better and more consistent separation selectivity than n-hexane and acetic acid on both extract and gallic acid. This HPTLC method involved the use of DPPH• and FeCl3 as derivatising agents based on their ability to scavenge non-biological stable free radicals and chelate iron ions, respectively. DPPH• is a stable purple-coloured free radical characterised by the delocalisation of its unpaired electron over the entire molecule. When reduced by antioxidants in the sample, its extensive conjugation would be disrupted, resulting in its yellow reduced form (4). On the other hand, colour formation on reaction is an identification test commonly used to determine the presence of simple phenolic compounds. In the presence of $FeCl_3$, compounds containing hydrogen-bonded phenolic groups would appear dark purple, whereas flavonoids with a catechol group would be green (5). The present study found incubation period for DPPH• was consistent with 60 minutes in the dark, while the plate derivatised with $FeCl_3$ needed to be neutralised using NH_4OH and then dried at 100°C for 5 minutes (Fig. 2). The standard stock solution of gallic acid (100 µg/mL) in methanol was applied in the range of 0.1 µg/band to 3.0 µg/band to develop a standard linear curve (Fig. 3). The antioxidant activity of the individual band of the seaweed extracts can now be analysed using Videoscan and quantified as gallic acid equivalents (GAE).

CONCLUSION

Altogether, the derivatisation of DPPH• and $FeCl_3$ assay on HPTLC is a straightforward strategy for antioxidant analysis. All parameters, including sample extraction, mobile phase percentage and derivatisation condition for DPPH• and $FeCl_3$, were successfully optimised for visualisation and quantification of the antioxidative phytochemical compounds in *C. lentillifera* extracts.

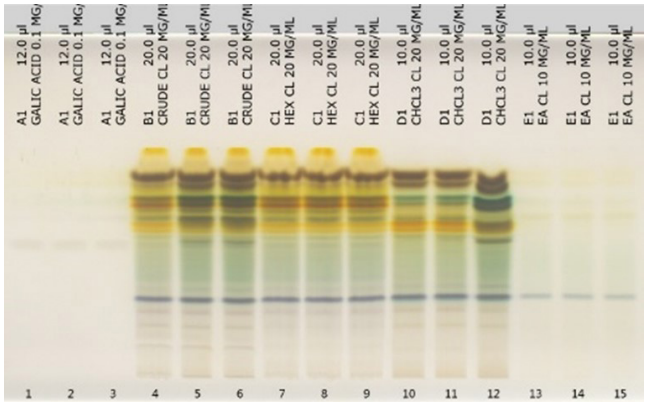

Figure 1: HPTLC chromatogram of *C. lentillifera* extracts and standard, Track 1-3 gallic acid, Track 4-6 methanol extract, Track 7-9 hexane extract, Track 10-12 chloroform extract, Track 13-15 ethyl acetate extract

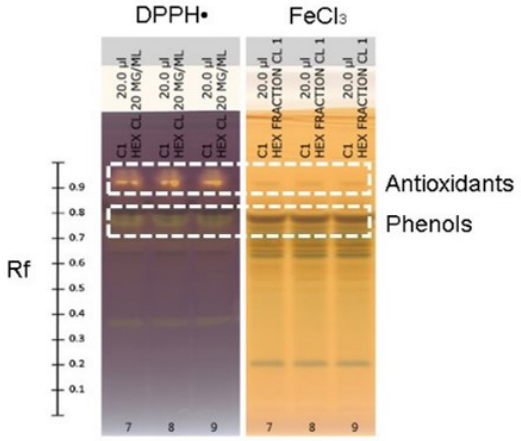

Figure 2: Hexane extract chromatogram derivatised with DPPH• and $FeCl_3$

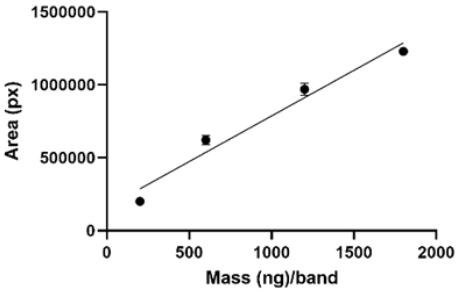

Figure 3: Linear gallic acid standard curve

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EXTENDED ABSTRACT

Health Benefits of Pre-processed *Kappaphycus alvarezii*: Extraction, Phytochemical Analysis and Antioxidant Properties

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SUMMARY

Kappaphycus alvarezii (*K. alvarezii*) is an edible and highly nutritious seaweed that has received attention for its role in preventing numerous health conditions. This study aims to explore the phytochemicals of *K. alvarezii* extracts that may be associated with their antioxidant properties. The standard qualitative phytochemical test was performed to identify the presence of metabolites, whilst the DPPH assay was used to assess the antioxidant activity of the different polarities of extracts. *K. alvarezii*'s phytochemical screening revealed the presence of triterpenoids, glycosides, sterols, alkaloids, and phenolics. The methanolic extract showed the greatest antioxidant activity, presumably due to the presence of high phenolic content in the extract.

Keywords: *Kappaphycus alvarezii,* phytochemical screening, antioxidant activity.

INTRODUCTION

Red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta) are the three classifications of seaweed that are characterized primarily by their photosynthetic pigments. *Kappaphycus alvarezii* (*K. alvarezii*), a class of Rhodophyceae, a red algae species, is an important raw material for the industrial manufacture of agar, carrageenan, and alginates (1). Recent pharmaceutical and nutraceutical research has centered on antioxidant, anticancer, antiviral, antibacterial, and other pharmacological potentials. *K. alvarezii* contains polysaccharides, phytosterols, soluble fibre, catechins, flavanols, and polyunsaturated fatty acids, which may be accountable for several pharmacological actions. Thus, this study aimed to assess the phytochemical composition and antioxidant properties of the various polarity of *K. alvarezii* extracts. This work is specifically carried out for the pre-processed local *K. alvarezii*, which undergoes preliminary processing (cleaning, bleaching and grinding) by the Malaysian Agricultural Research and Development Institute (MARDI) and is commercially used for foods and nutraceuticals industries.

MATERIALS AND METHODS

Pre-processed *K. alvarezii* powder was obtained from Food Science and Technology Research Center, MARDI. Extraction was accomplished using a 500 g sample of the lyophilized *K. alvarezii* powder in hexane, methanol and water, consecutively with frequent stirring. The extract was filtered and concentrated through a rotatory evaporator. The extracts were screened for alkaloids, saponin, terpenoids, glycosides, sterols and phenolics according to the standard method (2). Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay adapted from Ling et al. (2015) with a slight modification. An aliquot of extracts with concentrations ranging from 0.25 mg/mL, 0.125 mg/mL, 0.063 mg/mL, 0.031 mg/mL, and 0.016 mg/mL was prepared (3). Ascorbic acid at the same concentrations was used as a control. The mixture was shaken to homogenize and then allowed to react in the dark. After 30 minutes, the absorbance of the reaction mixture was recorded at 518 nm and the percentage of inhibition was calculated. The antioxidant activity is reported in terms of the number of

Figure 1: Dried *K. alvarezii*

equivalents of ascorbic acid.

RESULTS AND DISCUSSION

The hexane extract of *K. alvarezii* showed the presence of alkaloids, triterpenoids, and sterol, whereas the methanol extract indicated the presence of alkaloids, glycosides, sterol, and phenolics. The aqueous extraction yielded only glycosides and phenolics, while saponin was not found in any of the three extracts (Table I). Most of the relatively polar constituents were dominated in the methanolic extract as a result of consecutive extraction (4).

Table I: Qualitative phytochemical analysis of *K. alvarezii* extracts

Phytochemicals	Hexane extract	Methanol extract	Aqueous extract
Alkaloids	+	+	-
Saponin	-	-	-
Terpenoids	+	-	-
Glycosides	-	+	+
Sterols	+	+	-
Phenolics	-	+	+

+ = detected; **-** = not detected

Among the three *K. alvarezii* extracts, the methanol extract exhibited the highest antioxidant activity compared to others (Figure 2). In contrast to ascorbic acid, the percentage of inhibition at given concentrations (0.016-0.25 mg/mL) of the extracts is relatively low. The highest concentration (0.25 mg/mL) gave a percentage of inhibition of 4.1 %, 21.3 % and 10.4 % for hexane, methanol, and aqueous extracts, respectively, whereas ascorbic acid showed 87.4% inhibition. This observation is consistent with the findings by Lantah et al., (2017), who found that crude extracts of seaweed exhibited lower inhibition activity than pure ascorbic acid (5). This could be due to the presence of other phytochemicals other than those in the phenolics group, which do not work synergistically to enhance the activity. Studies have shown that secondary metabolites such as phenolic and flavonoids are potent free radical scavengers, which may account for their antioxidant properties. Hence, the extracts possess the ability to serve as a natural antioxidant and are valuable as an alternative therapy for general health.

CONCLUSION

The study revealed that the locally available *K. alvarezii* extracts at different polarities were rich in various phytochemicals, including alkaloids, triterpenoids, glycosides, sterols, and phenolics. The highest antioxidant activity of *K. alvarezii* was found in the methanolic extract as compared to others. This conclusion is supported by the fact that the methanolic extract contains more phytochemicals, particularly phenolics, than other extracts. Therefore, *K. alvarezii* has the potential to be used as an alternative medicine and as an ingredient in foods, medications as well as dietary supplements.

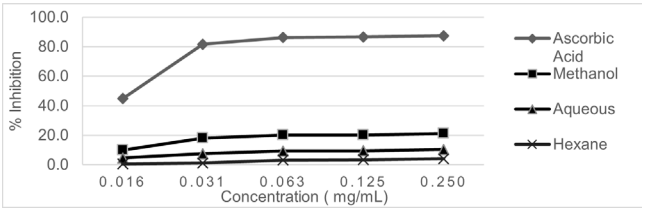

Figure 2: Percentage of inhibition of *K. alvarezii* extracts

ACKNOWLEDGMENTS

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EXTENDED ABSTRACT

Antimalarial and Hepatoprotective Effects of Methanol Extract of *Goniothalamus lanceolatus* Miq. Root in Plasmodium Berghei-Infected Mice

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SUMMARY

Malaria is one of the deadliest infectious diseases in tropical countries, with pregnant women and children under the age of five being the most vulnerable populations. There is a pressing need for new antimalarial agents due to the emergence and resurgence of drug-resistance parasites. This study investigated the antimalarial and hepatoprotective effects of methanol extract of *Goniothalamus lanceolatus* root against *Plasmodium berghei*-infected mice using 4-day suppressive test. The results indicate that the extract prolonged the survival time of infected mice and significantly ($p<0.05$) suppressed parasitaemia in a dose-dependent manner. At the dose of 300 mg/kg, the extract demonstrated significant ($p<0.05$) chemosuppression activity at 70%, and significantly ($p<0.05$) reduced serum aspartate transaminase (AST) level with a corresponding improvement in hepatocellular structure as compared to the vehicle control group.

Keywords: *Goniothalamus lanceolatus*, Plasmodium berghei, Antimalarial activity, Hepatoprotective, Malaria

INTRODUCTION

Malaria continues to plague the world's population and the upsurge in the global burden of drug resistance parasites has intensified the need to discover new antimalarial agents (1). Natural products played a significant role in malaria chemotherapy, of which quinine and artemisinin are the two most valuable antimalarial agents from medicinal plants. *Goniothalamus lanceolatus* Miq. is member of the Annonaceae family and native to the rainforest jungle of Sarawak, Malaysia. Different parts of the plant are used by the native communities to treat various ailments such as fever, colds, and skin diseases (2). We have recently reported the in vitro antiplasmodial activity of *G. lanceolatus* crude extracts with the root methanol extract identified as a promising antiplasmodial starting point (3). Since only the tip of the iceberg has been evaluated, this study aims to further elucidate the antimalarial and hepatoprotective effects of methanol extract of *G. lanceolatus* root in malaria-infected mice model.

MATERIALS AND METHODS

Plasmodium berghei (NK65) was obtained from MR4 and maintained by serial passage in ICR mice on weekly basis. Infected ICR mice were randomly divided into five groups of six mice each. Groups I, II, and III received oral treatment of 30 mg/kg bwt, 100 mg/kg bwt, and 300 mg/kg bwt of root methanol extract. Group IV received 5 mg/kg bwt of chloroquine while Group V was given 0.2 mL of the vehicle (Tween 60). All animal care and procedures were approved by the UiTM Animal Research and Ethics Committee (103/2015). Liver function test, and histopathological analysis of the liver were performed to evaluate the hepatoprotective effects of the extract against malarial infection. A semiquantitative scoring system was used to evaluate the degree of splenic injury and expressed as hepatic histopathological scores (HHS). All data were

analysed using GraphPad Prism version 6.0. Values for measurements were presented as mean ± SD.

RESULTS AND DISCUSSION

The extract prolonged the survival time of infected mice and significantly ($p<0.05$) suppressed parasitaemia in a dose-dependent manner (Table I). At the dose of 300 mg/kg, the extract demonstrated significant ($p<0.05$) chemosuppression activity (70%) when compared to the vehicle control group. This result reflects the ability of the extract to inhibit parasite replication and extend the survival of P. berghei-infected mice.

The liver is among the major organ directly affected by malarial parasites (4). Malaria-induced liver injury is characterized by hepatocyte necrosis, vascular congestion, and cellular inflammation with clusters of malarial pigment (5). Significant perturbation to the parenchymal tissues of the liver may lead to hepatocellular membrane damage and leakage of liver enzymes which is further manifested by elevated serum levels of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) (4). However, administration of root methanol extract at the dose of 300 mg/kg significantly ($p<0.05$) reduced serum aspartate transaminase (AST) level with a corresponding improvement in hepatocellular structure as compared to the vehicle control group (Table II and Figure 1). Mice treated with the extract exhibited moderately disorganized hepatocellular structure with mild inflammation and less malarial pigments. These observations were further supported by a significantly ($p<0.05$) reduced value of HHS in mice treated with 300 mg/kg of the extract . These results are indicative of the hepatoprotective activity of the extract that could be linked to the putative active compounds present in the extract that further contribute to its ability to suppress the multiplication of malarial parasites and ameliorate hepatic injury during malarial infection.

Table I: Percentage of chemosuppression and mean survival time (MST) of *P. berghei*-infected mice treated with *G. lanceolatus* root methanol extract (30, 100, 300 mg/kg)

Treatment	Dose (mg/kg)	% Chemosuppression	MST (day)
Root methanol extract	30	17.2	18.0±1.7
	100	38.2	18.3±0.3
	300	70.0*	18.7±0.3*
Chloroquine	5	59.9*	18.7±0.0*
Vehicle control	0.2 ml	26.7	13.0±0.7

Values are expressed as mean ± SD; *$P < 0.05$ when compared to vehicle control mice.

Table II: Effects of *G. lanceolatus* root methanol extract (30, 100, 300 mg/kg) on liver enzymes.

Test (IU/L)	Root methanol extract (mg/kg)			Chloro-quine	Vehicle control
	30	100	300		
ALT	74.62 ±9.84	69.92 ±9.38	69.54 ±19.98	59.34 ±6.15	95.43 ±5.44
AST	241.20 ±8.97	240.10 ±11.07	150.20 ±12.03*	105.40 ±26.83*	261.70 ±18.35
ALP	17.25 ±2.67	11.74 ±0.17	11.16 ±1.33	15.59 ±1.98	21.69 ±2.51

Values are expressed as mean ± SD. *$P < 0.05$ when compared to vehicle control mice.

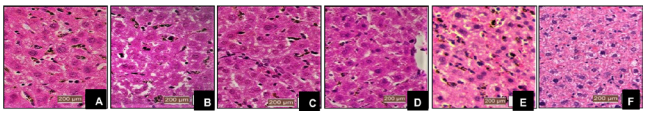

Figure 1: Representative photomicrographs of hepatic tissues (H&E stain) with 400x magnifications of (A) root methanol extract (30 mg/kg) treated mice; (B) root methanol extract (100 mg/kg) treated mice; (C) root methanol extract (300 mg/kg) treated mice; (D) chloroquine-treated mice; (E) vehicle-treated mice; (F) uninfected control mice.

CONCLUSION

The present findings indicate that methanol extract of *G. lanceolatus* root exhibits active antimalarial activity in a dose-dependent manner and is able to alleviate Plasmodium-induced liver damage due to malarial infection. However, further bioassay-guided fractionation will be necessary to decipher the pharmacological basis of its antiplasmodial activity.

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EXTENDED ABSTRACT

Biotransformation of Antibiotic using Tropical Versus Psychrotolerant Fungi as Biocatalyst

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SUMMARY

The antibiotics overuse and misuse lead to the development of drug resistance in humans, animals, and harmful microorganisms, rendering them ineffective against the diseases. The microbial transformation of antibiotics ciprofloxacin was performed using *Beauveria bassiana*, and R3-2 SP 17 (psychrotolerant fungi) with the goal of generating new, modified, and enhanced antibiotics. The HPLC profile of the resulted extracts was compared and analysed with the starting material, positive and negative controls. The appearance of a new peak in the HPLC profile of resulted extracts indicated the presence of more polar biotransformed metabolites, which might be the hydroxylated derivatives of ciprofloxacin which may be enhanced antibiotics.

Keywords: Biotransformation, Antibiotics, Ciprofloxacin, Beauveria bassiana, Psychrotolerant fungi

INTRODUCTION

Ciprofloxacin is an antibiotic that belongs to a class of medication known as second-generation fluoroquinolone and is one of the most significantly used drugs in this class. Ciprofloxacin was initially used successfully to treat infections, and resistance was extremely rare (1). However, its widespread usage in human medicine has led to the emergence of resistant strains (1). Nevertheless, no studies have been done on the transformation of ciprofloxacin by psychrotolerant fungi. Therefore, in continuation of our previous research on biotransformation (2-3) for the first time, we report here the biotransformation of ciprofloxacin using tropical fungi, *Beauveria bassiana*, and psychrotolerant fungi, R3-2 SP 17 as biocatalyst.

MATERIALS AND METHODS

The fermentation medium was prepared, and extraction was done according to the protocol reported in the literature (4). The HPLC analysis was conducted on the resulted extracts to indicate the existence of a biotransformed product based on peaks in the chromatogram. The sample analysis using HPLC was accomplished with a flow rate of 1 mL/minute, a column temperature of 30°C, and an injection volume of 10 µl. The detection wavelengths were set to 254nm and 280nm.

All resulted extracts' HPLC profiles were analysed and compared to those of negative and positive controls. The positive and negative control results have been used as references for all the extracts to distinguish the biotransformation metabolite by comparing the peaks in the control and experimental chromatogram profiles.

RESULTS AND DISCUSSION

Ciprofloxacin, which served as the starting material, had been fermented with *B. bassiana* and R3-2 SP 17 for 5, 9, and 14 days. At present, there is no research reported on the biotransformation of ciprofloxacin with B. bassiana. However, there was research that used *B. bassiana* as a biocatalyst for the biotransformation of first-generation quinolones, which is cinoxacin.

HPLC profiles for all resulted extracts were analysed and compared with HPLC profiles of negative and positive controls (Fig 1). The positive and negative control results have been used as references for all the extracts to identify the biotransformed metabolites by comparing the peaks

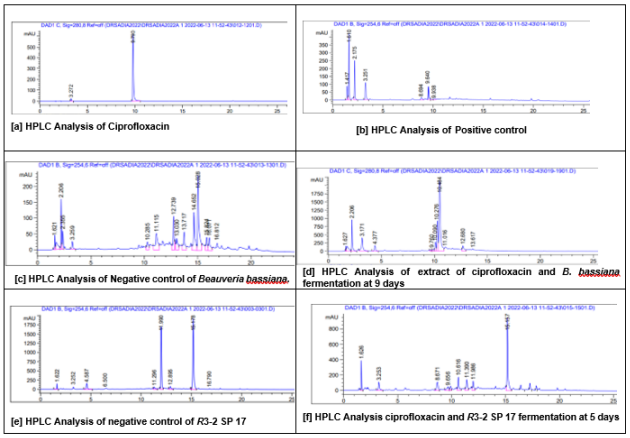

Figure 1: HPLC Analysis for Ciprofloxacin's fermentation with *Beauveria bassiana*'s and R3-2 SP 17

in the control and experimental chromatogram profiles. The HPLC profiles of the resulted product extracts showed peaks with a retention duration of 5 minutes. The majority of the peaks also reappeared in the HPLC profiles of the positive and negative controls, indicating that the peak corresponds to the media's components and fungal metabolites (Table I).

CONCLUSION

Based on HPLC profiles we can conclude that 9-day fermentation for B. bassiana, while 5 days for R3-2 SP 17 can be attempt in future for large scale fermentation to isolate and identify biotransformed metabolites of Ciprofloxacin. Further this study's findings will be useful in discovering a new drug candidate which might be useful to the pharmaceuticals.

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Table I: Biotransformation of the ciprofloxacin by *Beauveria bassiana* & *R3-2 SP 17*

Extracts recorded at 280 nm	Major peak retention time (min)	Minor peak retention time (min)
Starting material (Ciprofloxacin)	9.8	3.2
Positive Control (Media + Ciprofloxacin)	-	8.7, 9.6, 9.9, and 10.4
Negative control (Media + *B. bassiana*)	11.2,12.8, 15.02	10.4, 10.5, 13.30, 13.7, 14.6, 15.8, 16.0
Extraction on Day 5	10.5	10.08 and 11.018
Extraction on Day 9	10.5	10.0, 11.0, 12.6, and 13.6
Extraction on Day 14	-	-
Starting material (Ciprofloxacin)	9.	3.2
Negative control (Media with *R*3-2 SP 17)	11.9, 15.1	6.5, 8.6, 10.08, 12.4, 16.8, and 25.7
Extraction on Day 5	10.4	5.9, 8.9, 9.8, 10.6, and 11.4
Extraction on Day 9	11.4	8.9, 9.9, 10.4, and 10.6
Extraction on Day 14	12.4	9.9, and 11.4

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EXTENDED ABSTRACT

In-vitro Antioxidant Activities of *Ficus carica*: Systematic Review and Meta-analysis

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SUMMARY

Antioxidant has become important in the treatment of disorders induced by oxidative stress. Plants, like *F. carica* was associated with antioxidant activity. Systematic review and meta-analysis were employed to evaluate the potency of antioxidant activity of *F. carica*. From the 470 studies only six studies met the prescribed inclusion criteria. The findings of the six included studies showed that *F. carica* had notable antioxidant levels based on the IC50 results. In the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method, using ascorbic acid, a notable free radical scavenging activity of *F. carica* was observed. Meanwhile, the antioxidant of *F. carica* was also high using the Ferric reducing antioxidant power (FRAP) method with Butylated hydroxytoluene (BHT). The graph in the forest plot revealed that *F. carica* exhibit antioxidant action but not as potent as ascorbic acid and BHT.

Keywords: *Ficus carica*, ascorbic acid, butylated hydroxytoluene, 1,1-diphenyl-2-picrylhydrazyl assay, ferric reducing antioxidant power assay

INTRODUCTION

Oxidative stress plays a significant role in the progression of diseases such as cancer, diabetes, hypertension, and all other metabolic disorders (1). Oxidative damages produced by free radicals exacerbated and hastened the progression of most health concerns. Due to these concerns, bioactive chemicals in plants, such as antioxidants, have garnered favorable attention in recent years as these bioactive compounds manifested cytoprotective activities against certain diseases. Among these notable bioactive chemicals are polyphenols. These polyphenols in the form of metabolites present in specific plants (such as the Ficus species) are known to possess antioxidant characteristics. They are recognized to manifest redox activities, which include the ability to absorb and neutralize free radicals. Several studies have demonstrated the biological benefits of polyphenols present in Ficus plants, which made *F. carica* among the most extensively researched Ficus plant species due to its antioxidant properties. *F. carica* is a deciduous tree belonging to the Moraceae family with an edible fruit with high commercial value (2). The antioxidant characteristics in-vitro of the Ficus plant have been shown to inhibit the proliferation of cancer cells (3). Hence the presentation of this research activity to review all recently-concluded studies and known published reports concerning the antioxidant activity of *F. carica* in in-vitro methods, utilizing the meta-analysis procedure to determine the inherent potency of antioxidant activity against other commonly known antioxidants such as ascorbic acid and butylated hydroxytoluene.

MATERIALS AND METHODS

This study utilized a systematic review and meta-analysis. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) used to acquire relevant data and studies from the online database. The systematic searches include combinations of keywords such as *F. carica*, antioxidant activity, in-vitro studies, DPPH and FRAP, and standard control, such as ascorbic acid and butylated hydroxytoluene. The Office of Health Assessment Tool (OHAT) implemented quality assessment for bias risk. Inclusion criteria for data extraction contain details such as the study's title, author, date published, place of study, and study design used in evaluating the antioxidant activity of F carica. A flow diagram based on the PRISMA design constructed to demonstrate the process of selecting and eliminating research until all criteria met. Forest plots was typically use to display graphical data from meta-analysis, from which each study shows its effect size and the corresponding 95 percent confidence interval.

RESULTS AND DISCUSSION

Study selection started with 470 studies from various electronic databases. Studies further screened for eligibility. The study criteria list was based on the Point of focus-Intervention-Comparator-Outcome (PICO) elements of the review question. Inclusion requirements

of the study include *F. carica* plant, antioxidant activity, in-vitro studies, DPPH, FRAP, ascorbic acid, and BHT. The exclusion criteria included studies that were found to have no comparator, or the comparator is different from the criteria (N=10), the results of the studies were not expressed in mean and standard deviation (N=13), and studies that do not use methods like DPPH and FRAP (N=8). Finally, six studies were selected which include the study of Javaid et al. (2021; Int J Agric Biol) (4), Ahmed at al. (2013; Int J Bioassays) (5), Begum et al (2020; Pure Appl Biol), SwaroopaRani et al. (2021; Int J Res Anal Rev), Loizzo et al. (2014; J Sci Food Agric), Martos et al. (2015; Nat Prod Commun) (Figure 1).

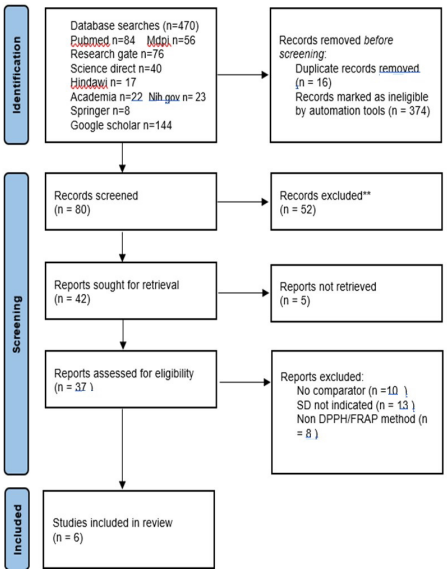
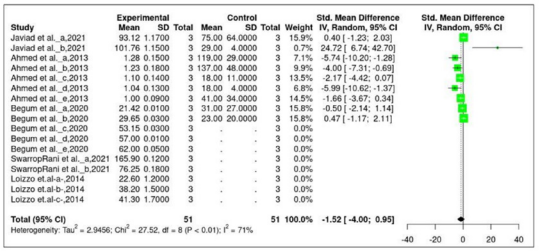


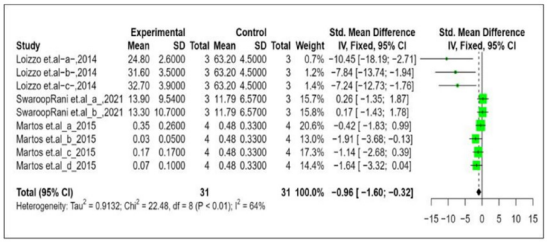
Figure 1: Flow chart of the study

The six included studies of *F. carica* use different plant parts such as the leaves, stem bark, and fruit from cultivars available in their region. The intervention employed in studies 1,2,3 and 4 was DPPH radical scavenging activity assay. While studies 5 and 6 use the ferric-reducing antioxidant assay. Comparators were ascorbic acid, which made use of the DPPH assay, whereas butylated hydroxytoluene used the FRAP method assay. The outcome observed in the six studies was the antioxidant activity of the different plant parts *F. carica*.

The forest plot of the five studies containing 17 data sets of antioxidant activity of *F. carica* was compared to ascorbic acid using the DPPH method. The study of Begum et al. (6) also revealed that the *F. carica* plant extract used does not show significant potency of antioxidant activity. Moreover, the thin box in the forest plots represents the weights related to the very small sample sizes of the included studies. In some studies, a narrow confidence interval of whiskers emerged, indicating more reliable data. The result of the forest plot of *F. carica* using the ferric reducing antioxidant power method and butylated hydroxytoluene as a comparator implies that there were no significant differences in the potency of the antioxidant activity between *F. carica*



Forest Plot of *F. carica* using DPPH Method and Ascorbic Acid as Control



Forest Plot of *F. carica* using FRAP Method and BHT as Control

Figure 2: Forest plot of *F. carica*

plant extract and butylated hydroxytoluene. The overall effect size of *F. carica* plant extract is associated with significantly low potency on the free radical scavenging activity relative to ascorbic acid. Moreover, the ferric-reducing antioxidant power of *F. carica* plant extract is not comparable with the standard control, butylated hydroxytoluene

CONCLUSION

Based on the analysis conveyed in the study, the systematic review of the six included studies revealed the antioxidant activity of *F. carica*. However, in the meta-analysis, the forest plot of F.carica using the DPPH method and compared to ascorbic acid revealed that the plant does not exhibit a potent antioxidant activity when compared to the standard drug. Moreover, when compared with BHT using the FRAP method, it was found that the potency of antioxidants is not comparable with the standard drug.

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EXTENDED ABSTRACT

Loop-mediated Isothermal Amplification (LAMP): A Potential Alternative for Nucleic Acid Amplification Method in COVID-19 Detection

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SUMMARY

Reverse Transcription Loop-Mediated Isothermal Amplification is a method of amplifying genetic material, specifically RNA, using isothermal condition. This study aims to develop and optimize a new LAMP primer set targeting the conserved region of the SARS-CoV-2 nucleocapsid gene. The designed primer set was subjected to colorimetric RT-LAMP-based assay to allow rapid visual detection of the virus. The results showed improvement in the sensitivity of the assay when tested on the sample with a high viral load (Ct value <25). Further analysis of the RT-LAMP product on 2% gel electrophoresis validated the RT-LAMP-based assay results.

Keyword: SARS-CoV-2, RT-LAMP, RT-PCR, COVID-19, Molecular Diagnosis

INTRODUCTION

The global pandemic of Coronavirus disease 2019 (COVID-19) was caused by the emergence of the highly contagious severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), making early diagnosis a crucial part in the strategies to combat the spread of the disease. The current widely accepted method, reverse transcription quantitative real-time PCR (RT-qPCR), may be hindered by the need for sophisticated instruments and highly trained personnel (1). Reverse transcription loop-mediated isothermal amplification (RT-LAMP) presents as a potential alternative diagnostic tool to identify infected individuals in a timely and cost-effective manner. Due to its ability to amplify genetic material at a constant temperature without thermal cycler, and generate faster results, RT-LAMP could potentially offer a larger diagnostic capacity than RT-PCR, making it a better choice for monitoring the pandemic (2,3).

MATERIALS AND METHODS

A new set of primer specific for the nucleocapsid (N) gene sequence was used in RT-LAMP assay to amplify and detect the presence of SARS-CoV-2. Extracted RNA samples from 6 positive and 6 negative-verified samples were used to validate the clinical performance of the assay, with real-time RT-PCR assay as the standard reference method. Briefly, RT-LAMP reaction master mix was prepared in a total volume of 25µL, including 2µL of extracted RNA samples and incubated at 65oC for 35 minutes in GFL-70 Blast Dry Oven. At the end of incubation, results were analysed based on colorimetric changes and gel electrophoresis. Positive amplification reaction was considered only if the reaction mixture turns from pink to yellow and showed ladder banding pattern on gel separation analysis. Using the online statistical calculator MedCalc and SPSS version 20.0 software (SPSS IBM Corp., Armonk, NY, USA), the clinical sensitivity, specificity, and predictive values of the developed assay were calculated.

RESULTS AND DISCUSSION

Results from RT-qPCR analysis showed varying degrees of viral load amongst the 6 confirmed positive samples based on their cycle threshold (Ct) value readings. A Ct value under 38 was considered as positive sample, while a Ct value of 0 or over 38 Ct was considered a negative sample. One positive sample recorded a low Ct value in the range of 25 to 30, while the other remaining 5 positive samples showed higher Ct value in the range of 31 to 35. Low Ct values indicate for samples with high viral loads, while high Ct values indicate samples

with medium to low viral loads. All 6 negative samples showed no Ct value, indicating that no amplification reaction took place.

By comparing the RT-qPCR and RT-LAMP assay results, the colorimetric RT-LAMP assay was more suitable for the detection of SARS-CoV-2 among individuals with high viral load (Ct value <30). However, for individuals with moderate or low viral load (Ct value >30), the colorimetric result may be inconsistent and may yield false negative results. Further incubation for at least 40 minutes is recommended when testing samples with moderate and low viral load in order to increase the sensitivity of the assay to 100%. Despite this, the specificity of the assay remained high at 100% for both 35 and 40 minutes of incubation time, as all negative-verified samples remained undetected by colorimetric RT-LAMP assay. Hence, the sensitivity and specificity of the assay were improved to 100% after a total of 40 minutes incubation.

Table I: Evaluation of colorimetric RT-LAMP assay performance based on comparison with RT-PCR

		Ct	RT-LAMP			
			Positive (35 min)	Positive (40 min)	Negative (35 min)	Negative (40 min)
RT-qPCR	Positive	25-30	1/1	1/1	-	-
		31-35	2/5	5/5	-	-
	Negative	Negative	-	-	6/6	6/6

Further validation on 2% gel electrophoresis separation showed the presence of ladder-banding pattern in all positive-verified samples. The ladder-banding pattern is the unique pattern for LAMP amplified products when performing gel electrophoresis analysis, thus verifying the presence and amplification of the desired target sequence of SARS-CoV-2.

CONCLUSION

The present study shows successful detection of SARS-CoV-2 based on colorimetric RT-LAMP assay. Besides SARS-CoV-2, this assay has been previously applied in detection of other viral pathogens, including influenza, dengue, Ebola, and Zika virus. Its simplicity, low cost, and rapid turnaround time make it a promising alternative to RT-PCR-based tests.

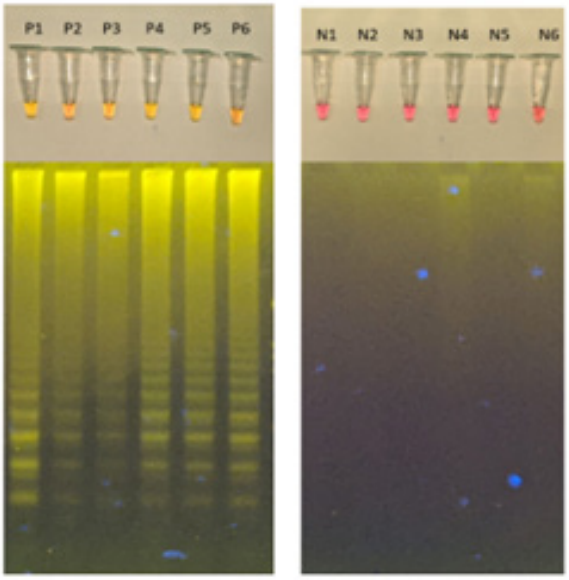

Figure 1: Evaluation of colorimetric RT-LAMP assay using N primer on real clinical sample based on colorimetric and gel electrophoresis analysis. P1-P6: positive COVID-19 sample; N1-N6: negative COVID-19 sample.

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

A Systematic Review Protocol for Qualitative Appraisal of Non-Pharmacological Interventions against Gut Dysbiosis and Increased Intestinal Permeability in Older Adults

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SUMMARY

This protocol is designed to appraise non-pharmacological interventions (diet and physical exercise) against gut dysbiosis and increased intestinal permeability in older adults. A literature search will be performed through online databases by using predefined keywords. Only studies on older adults (>60 years old), gut microbiota and/or intestinal permeability and non-pharmacological interventions will be selected. Previous findings will be qualitatively appraised and correlated to other health parameters. Shortlisted studies will be assessed for risks of bias. This protocol is expected to uncover positive effects of non-pharmacological approaches in gut microbiota composition and intestinal permeability in older adults.

Keywords: Older adults, dysbiosis, gut microbiota, intestinal permeability, non-pharmacological interventions

INTRODUCTION

The gut microbiota composition of older individuals differs significantly from young and middle-aged adults, with symptoms of gut dysbiosis characterised by a decrease in beneficial bacteria [(1). It is believed that gut dysbiosis would increase the permeability of the mucosal barrier, allowing bacteria and the products they produce to enter the circulatory system. Gut dysbiosis causes an increased risk of cancer (i.e., myeloma, stomach, liver and colorectal) and age-related neurodegenerative diseases (i.e., Alzheimer's disease and stroke) in the elderly. Nevertheless, recent findings support the reversal of gut dysbiosis and increased gut permeability through diet and exercise. However, previous reviews of these findings emphasised a specific intervention over multi-domain interventions; only a few have reported the correlation with gut microbiota or the intestinal epithelial barrier in the elderly. As such, this protocol (CRD42022369261) is designed for qualitative appraisal of non-pharmacological interventions against gut dysbiosis and increased intestinal permeability in older adults.

MATERIALS AND METHODS

A literature search will be performed through PubMed, Scopus, ScienceDirect and the Cochrane Library using keywords which included "frail", "frailty", "elderly"," ageing", "older adult", "gut microbiota", "gut microbiome", "leaky gut", "dysbiosis", "intestinal epithelial barrier". "intestinal permeability"," diet intervention", "exercise", "supplements", "fiber", "protein", "bacteriotheraphy" and "fat". Study selection will be based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (2). Duplicated search outcomes will be eliminated, and relevant publications will be identified through titles and abstracts. Shortlisted studies will be screened based on inclusion and exclusion criteria. The remaining studies will be qualitatively appraised for primary outcomes and correlated to secondary outcomes. Shortlisted studies will also be evaluated for risks of bias using the Cochrane Collaboration's Risk of Bias 2 for crossover and parallel intervention studies (3) and NIH Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group for pre-post intervention studies.

RESULTS AND DISCUSSION

For a literature search, this protocol will incorporate search a strategy that uses medical subject headings (MeSH terms) and free text strings with search terms merged by using Boolean operators. Fig. 1 depicts the overview of the systematic review protocol. The rationale for selecting the four online databases is based on their comprehensive collection of peer-reviewed journals in scientific and biomedical research from a wide range of international publishers. The present literature search should detect studies that fulfil the inclusion and exclusion criteria of the study selection, regardless of the year of publication (Table I). Eligibility of each study and data extraction will be independently assessed and performed by four authors (HIHH, SML, CFN and KR) using a standardised, electronic extraction form. Discrepancies will be resolved through discussion and consensus. The primary outcomes of this systematic review include differential gut microbiota composition and/or intestinal permeability in older adults, whereas the secondary outcomes include changes in metabolites, short-chain fatty acid and inflammatory markers.

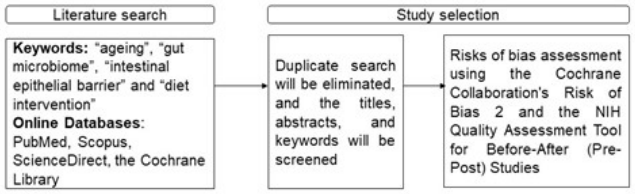

Figure 1: Overview of the protocol

The shortlisted studies will be evaluated for risks of bias independently by four authors (HIHH, SML, CFN and KR) using the Cochrane Collaboration's Risk of Bias 2 which is specific for crossover and parallel intervention studies and the NIH Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group which is suited for pre-post intervention studies. The former will be graded as 'Low', 'High', or 'Some concerns' whereas the latter will be considered as good when total quality scores are <9, fair when overall quality scores are between 5-8 and poor when <4. Discrepancies in assessments will be resolved through discussion and consensus. The present systematic review anticipated variations in metagenomic sequencing methods and platforms across the shortlisted studies. 16S rRNA-based methods, for instance, cannot distinguish species like shotgun metagenomics. Therefore, researchers could only analyse shifts at the genus level (4).

Table I: Inclusion and exclusion criteria

Inclusion	Exclusion
• Non-pharmacology interventions (diet, physical exercise, prebiotics, and probiotics)	• Review articles, research articles without available full textbook chapters, conference papers, theses, case reports, abstract-only articles and non-peer reviewed
• Study correlated with gut microbiome and/or intestinal permeability	• Preclinical studies (*in vivo* and *in vitro* studies)
• Older adults (>60 years old)	• Non-English
	• Studies that did not report gut microbiome and/or intestinal permeability of older adults separately from other age group

* For studies with subjects of wide age range (including those <60 years old), subgroup extraction and analysis will be performed.

CONCLUSION

This protocol is expected to uncover important insights into the potential positive changes in gut microbiota composition and intestinal permeability in older adults through non-pharmacological approaches.

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

Structural Alterations in Liver Endothelium of Rats with Dexamethasone-Induced Insulin Resistance

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SUMMARY

Insulin resistance (IR) is associated with various liver pathologies such as inflammation and lipid accumulation in hepatocytes. However, the effect of insulin resistance on liver endothelium is yet to be investigated. This study aimed to analyse changes at the liver endothelium in an insulin resistance rat model. Using scanning electron microscopy, we performed the structural analysis of liver sinusoidal endothelial cells (LSECs) from insulin resistance rats that were induced with dexamethasone. The results showed that IR has caused a significant decrease in fenestration frequency and endothelial porosity. These findings highlight the important role of liver ultrastructure in the development of metabolic diseases.

Keywords: Insulin resistance, Dexamethasone, Liver endothelium, Fenestrations, Electron microscopy

INTRODUCTION

The liver plays a major role in the regulation of glucose homeostasis, which is tightly regulated by insulin. Liver sinusoidal endothelial cells (LSECs) are perforated with transcellular fenestrations that provide permeability and access to substrates between sinusoidal blood and hepatocytes. During ageing and in several liver disorders, LSECs undergo structural changes ranging from decreased fenestration number (defenestration) and/or diameter known as capillarisation (1). Insulin resistance is defined as the inability of tissues to respond to normal circulating levels of insulin and has been reported to promote non-alcoholic fatty liver disease (NAFLD) (2). However, the association between insulin resistance and fenestrations is yet to be investigated. Hence, this study aimed to analyse changes at the liver endothelium in the dexamethasone induced insulin resistance rat model. We hypothesised that LSECs ultrastructure is a contributing factor to the clinical manifestations of insulin resistance.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats were divided into two groups where the control group (n=8) received 0.9% NaCl and the treatment group (n=8) received dexamethasone injection (1 mg/kg) intraperitoneally once a day for ten days (3). Body weight and fasting blood glucose were recorded daily. At day 11, all rats were sacrificed using ketamine/xylazine followed by cardiac puncture. Terminal blood was collected for measurement of serum insulin via colorimetric assay kit. Rats were dissected and livers were perfusion-fixed and processed for electron microscopy. Fenestrations were examined using Quanta FEG450 scanning electron microscope at 15000x magnification. Ten random images per sample were taken for analysis of fenestration frequency, diameter and liver porosity using ImageJ software (4). All data were analysed using SPSS Version 23.0. The values for measurements were presented as mean ± SD.

RESULTS AND DISCUSSION

Dexamethasone has caused a significant decreasing in daily body weight starting at day 3 of treatment (378.2 ± 8.9 vs. 332.2 ± 11.6 g) and subsequently mean body weight changes compared to control (5.38 vs. -22.92%) throughout the experiment. There is no significant difference in the liver-to-body weight ratio, although the treated liver showed a higher value compared to the control. Fasting plasma insulin concentrations were significantly elevated in the treatment group compared to the control group (Figure 1A). As hyperinsulinemia occurs, the homeostatic model assessment index for insulin resistance (HOMA-IR), calculated from the fasting glucose-insulin product, was significantly increased by more than two-fold in the treatment group, indicating insulin resistance (Figure 1B). Dexamethasone has a negative effect on pancreatic beta cell function by reducing insulin receptor sensitivity, leading to

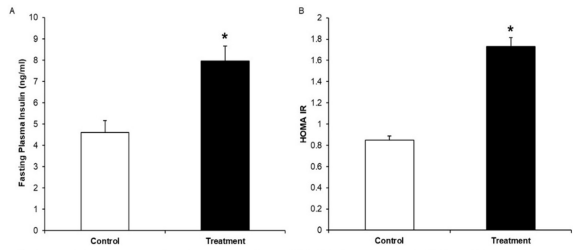

Figure 1: Insulin resistance parameters in control and treatment group. (A) Fasting plasma insulin levels (B) Homeostatic model assessment index for insulin resistance (HOMA-IR). *Significant at p<0.05.

decreased glucose uptake, tissue starvation, proteolysis and consequently weight reduction (3).

The liver endothelium observed via SEM showed a normal fenestration morphology in control rats (Figure 2A) while a marked defenestration is present in insulin resistance rats (Figure 2B). Quantification analysis of LSECs fenestrations showed a significant decrease in fenestration frequency and liver porosity, but not fenestration diameter for the insulin resistant liver (Table I). Although the mechanism of defenestration is still under investigation, the alterations observed in this animal model are probably due to the dexamethasone effect on mitochondrial energy production at the LSECs that causes a depletion of ATP that is needed to support the cytoskeleton rings for the maintenance of fenestrae patency (5).

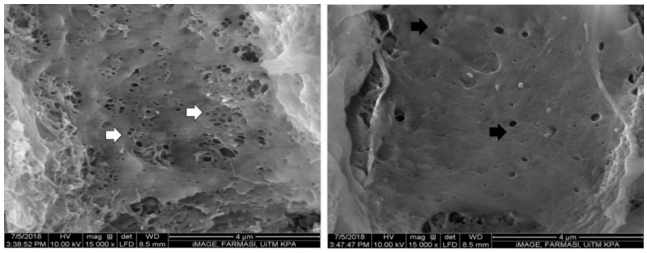

Figure 2: Representative scanning electron micrograph of liver sinusoids. (A) Control liver showing fenestrations clustered in sieve plates (white arrows). (B) Marked defenestration is observed in insulin resistance rats (black arrows). (Mag 15000X)

CONCLUSION

Insulin resistance has the ability to affect the integrity of the liver endothelium, specifically fenestration frequency and liver porosity. These findings affirm the important role of liver ultrastructure in hepatic metabolic processes and highlight the LSECs as potential therapeutic targets for metabolic diseases such as diabetes and metabolic syndrome.

Table I: Quantification analysis of LSEC fenestrations

	Control (n=6)	Treatment (n=6)
Frequency[a]	3.21 ± 1.2	2.65 ± 1.0*
Diameter	0.08 ± 0.1	0.07 ± 0.2
Porosity[b]	2.17 ± 0.7	1.77 ± 0.9*

[a]Total number of fenestrations in the total area of the field examined, [b]Area of the endothelial surface covered with fenestrations, *Significant at p<0.05.

ACKNOWLEDGEMENTS

This work is supported by Fundamental Research Grant Scheme (FRGS 5/3 (22/2016) from Ministry of Higher Education Malaysia.

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EXTENDED ABSTRACT

In Silico Identification and Prediction of MicroRNA in the 3'Untranslated Region of Long Non-Coding RNA MIR497HG: A Step towards HCC Candidate Biomarker

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SUMMARY

Aberrant expression of long non-coding RNA (lncRNA) MIR497HG is associated with the progression of hepatocellular carcinoma (HCC). It is well known that some lncRNAs produce microRNAs (miRNAs), which act as regulators of gene expression. Multiple miRNAs in human genes are known to influence the expression of HCC target genes. Employing in silico approach for miRNA analysis has become pivotal in cancer pathogenesis. This study aims to analyse the 3'untranslated region (3'UTR) of the MIR497HG region to predict novel miRNAs as regulators of the MIR497HG in HCC. The discovery of novel miRNAs from can be utilised for miRNA-based therapy for HCC.

Keywords: MicroRNA, *in silico* prediction, miRNA precursor, long non-coding RNA, MIR497HG

INTRODUCTION

Long non-coding RNAs (lncRNAs) are expressed differentially and act as tumour suppressors or oncogenes. The MIR497HG has been reported to be a tumour suppressor in hepatocellular carcinoma (HCC) (1). Advances in molecular biology have been focused on microRNA (miRNA) as biomarkers for the diagnosis and prognosis of HCC. Moreover, there is growing interest in exploring miRNA-based therapy for HCC. Experimental prediction of miRNA is laborious, costly and time-consuming. In silico method provides a reliable and rapid platform for pre-miRNA identification. The 3'UTR of MIR497HG was utilised as the focus of this study as it serves as a generation of mature miRNA. Interestingly, the 3'UTR can be targeted by a single or multiple miRNAs. Overall, the in silico approach is presented for identifying miRNA predicted in the 3'UTR of MIR497HG that may serve as potential therapeutic target for HCC.

MATERIALS AND METHODS

MIR497HG sequence was retrieved at the National Centre for Biotechnological Information (NCBI) RefSeq databases (https://www.ncbi.nlm.nih.gov/). The coding region and open reading frame (ORF) were analysed using the ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/). The ORF sequence was analysed using the UTRDB tool (http://utrdb.ba.itb.cnr.it/) to determine the 3'UTR region. The miRFold was used to analyse the pre-miRNA hairpin structure. The RNAfold 2.4.13 (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) was used to fold the secondary structure with minimum free energy (MFE). The iMiRNA-PseDPC (http://bioinformatics.hitsz.edu.cn/iMiRNA-PseDPC/server) was used to distinguish real and pseudo pre-miRNA using distance-pair composition approach. Mature miRNA sequence was identified by MatureBayes (http://mirna.imbb.forth.gr/MatureBayes.html) with the Naive Bayes Classifier (NBC).The miRBase (https://www.mirbase.org/) was utilised as a reference database to ensure that the candidate miRNA sequences are not reported as previously published mature miRNA. The sequence of miRNA candidates was searched using the Smith-Waterman local alignment algorithm (SSEARCH).

RESULTS AND DISCUSSION

A total of 44 potential pre-miRNAs originating from 3'UTR of the MIR497HG were predicted. The length of pre-miRNAs varied from 52 nucleotides (nt) to 118 nt. The pre-miRNA hairpin structures were manually checked according to a uniform system and miRNA annotation criteria; (i) consists of at least 16 nt base pairing and (ii) both arms are separated by 8-40 nt in length, loop sequence (2). To ensure appropriate hairpin stability, the minimum free energy (MFE) was set at -25 kcal/mol.

To discriminate pseudo pre-miRNAs from real pre-miRNAs, non-conserved hairpins were filtered out. The

pre-miRNAs were predicted based on the statistical calculation of the reference dataset. A total of six real pre-miRNAs were predicted. The potential pre-miRNA hairpin structures were manually checked according to the uniform system and updated miRNA annotation criteria. The hairpin structure of pre-miRNA nominees was identified (Fig. 1).

A total of six mature miRNAs were identified by the MatureBayes. The sequence of six miRNAs was further screened using the Smith-Waterman Local alignment algorithm (SSEARCH) for finding the nucleotide similarity. Two novel mature miRNAs were identified as not aligning with any known miRNAs (Table I).

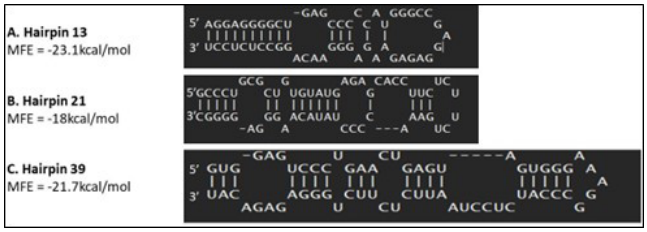

Figure 1: Hairpin structures of the candidate miRNA precursors were shown as text format (A) Hairpin 13 (B) Hairpin 21 and (C) Hairpin 39

Table I: List of predicted novel miRNAs

Hairpin	Length (nt)	Location	Mature miRNA Sequence
Sequence_13_3′-UTR MIR497HG_5p	22	5′	GGCUGAGCCCCCAUGGG-CCGAG
Sequence_39_3′-UTR MIR497HG_3p	22	3′	CUCCUAAUUCUCUUCUG-GGAGA

The novel miRNAs identified in 3′UTR of MIR497HG were located in the 3′-arm and 5′-arm of the stem-loop hairpin structure, which supports that mature miRNA can be produced from either of the two arms of the stem-loop hairpin structure and can be non-canonical (3). Annotation of miRNAs based on the in silico established criteria was to avoid wrongly predicted miRNAs. This study is important to determine whether the lncRNA has an important biological role in its cognate miRNAs. This study only provides a fundamental step-by-step approach to the identification of miRNA. Further study in laboratory experiments should be performed to verify the validity of the novel miRNAs characterised in this present study.

CONCLUSION

This study has provided insight into in silico prediction of miRNAs from the 3′UTR of MIR497HG. These miRNAs are novel and have not yet been reported. In the future, the potential miRNAs need to be further characterised and validated through experimental approaches.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

A Systematic Review and Meta-Analysis Protocol for Qualitative and Quantitative Appraisals of Faecal Markers for Intestinal Permeability and Intestinal Inflammation in Older Adults

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SUMMARY

This protocol is designed for qualitative and quantitative appraisals of faecal markers for intestinal permeability and inflammation in older adults. A literature search will be performed through four electronic databases by using pre-defined keywords. Eligible studies should report older adults of ≥60 years old and the outcomes of the faecal markers of interest. Shortlisted studies will be assessed for risks of bias before qualitative appraisal and analysis for correlation with other health parameters. Meta-analysis will be performed by using the Review Manager Software. This protocol will uncover potential faecal markers of intestinal permeability and intestinal inflammation in older adults.

Keywords: Older adults; intestinal permeability; intestinal inflammation; faecal markers; systematic review

INTRODUCTION

Although the ageing population is interpreted by some as the unstoppable success story of global life expectancy, extended lifespan has also become a cause for concern, especially with regard to the inevitable increased burden of late-life diseases. This could be attributed, in part, to increased leakiness and inflammation of the gut. In spite of the established relationship between increased intestinal permeability and intestinal inflammation in older adults, the clinical significance of increased intestinal permeability in the diagnosed health condition of older adults remains under-examined. Furthermore, previous systematic reviews focused mainly on blood inflammatory markers but not faecal samples. Faecal markers are non-invasive markers and would thus be more suited for the detection of changes in the gut when compared to blood-based samples (1). As such, this protocol (CRD42022362036) is designed for qualitative (systematic review) and quantitative (meta-analysis) appraisals of faecal markers for intestinal permeability and intestinal inflammation in older adults.

MATERIALS AND METHODS

A systematic review will be performed by using predefined keywords ("intestinal permeability", "leaky gut", "intestinal epithelial barrier", "intestinal barrier integrity", "tight junction", "zonulin", "calprotectin", "intestinal inflammation "faecal marker", "lactoferrin", "alpha-1 antitrypsin", "elderly", "ageing", "older adult" and "aged") for literature search through PubMed, Scopus, ScienceDirect and the Cochrane Library. Study selection will be based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (2). All duplicated search outcomes will be removed. The titles and abstracts of the remaining studies will be screened for their relevance to this study. Shortlisted studies will be screened based on the inclusion and exclusion criteria. Shortlisted studies will also be determined for their risks of bias using the Quality Assessment Tool of the National Institute of Health (NIH) for case-control, cohort and cross-sectional studies (3). A meta-analysis will be performed for potential faecal markers by using the Review Manager Software (The Cochrane Collaboration, 2020).

RESULTS AND DISCUSSION

Fig. 1 illustrates the overview of the protocol. This protocol will incorporate keywords, MeSH terms, free text and Boolean operators with combined terms as the

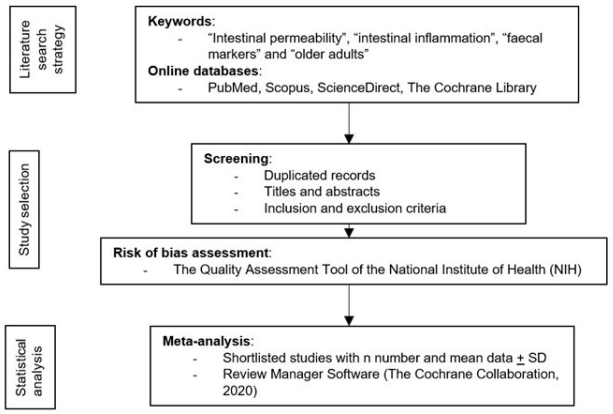

Figure 1: The overview of the present protocol for systematic review and meta-analysis

search strategy. The four online databases were chosen based on precision, significant criteria in medical journals, large range of journals, usefulness for keyword searches and citation analysis (4, 5). Literature search based on the search strategy is expected to detect studies that meet the inclusion and exclusion criteria of the study selection (Table I). Basically, eligible studies should report older adults ≥60 years old and the relevant faecal markers for intestinal permeability and intestinal inflammation in older adults.

Subsequently, the shortlisted studies will be qualitatively appraised for the primary outcome on faecal indicators of intestinal permeability and intestinal inflammation in older adults. The primary findings will also be correlated to secondary outcomes like gender, age, BMI, cognitive performance, medication, the severity of symptoms and the gut microbiota. As for the risks of bias assessment, the shortlisted studies will be evaluated independently by each author using the Quality Assessment Tool of the NIH which comprises 12-14 questions depending on the type of study. Discrepancies in assessments will be resolved through discussion and consensus among the authors. The outcomes for the risks of bias will be categorised as poor, fair, or good based on the number of favourable answers. For the meta-analysis, only shortlisted studies with n numbers and mean data + SD will be included. As for eligible studies that did not report such information, the respective corresponding author(s) and/or co-author(s) will be contacted via email. The potential limitation of this study will be the small number of reported findings on faecal markers for intestinal permeability and intestinal inflammation specifically in older adults, given that previous studies predominantly focused on blood-based markers.

Table I The inclusion and exclusion criteria for the study selection.

Inclusion	Exclusion
Elderly population ≥60 years	Review articles, grey literature and related
Studies that assessed stool markers for intestinal inflammation	*In vivo, in vitro*, preclinical studies
Studies that assessed stool markers for the intestinal permeability	Intervention study
	Non-English

CONCLUSION

Altogether, the present qualitative and quantitative appraisals of previous findings will provide important insight into potential faecal markers of intestinal permeability and inflammation in older adults, thus serving as a useful guide for future studies.

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

Ameliorative Effects of Alpha-Tocotrienol Supplementation on the Embryonic Development and Estrogen Profile in Nicotine-Treated Mice

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SUMMARY

Infertility and pregnancy failure have increased due to smoking. Nicotine is a toxic alkaloid in cigarettes that induces oxidative stress, giving rise to adverse effects on ovarian follicle growth and the number of follicles produced. Embryos retrieved from female mice were cultured in vitro until the hatched blastocyst stage development. Serum isolated for estrogen profiling was analysed using enzyme linked immunosorbent assays (ELISA). In this study, alpha-tocotrienol supplementation was able to delay the detrimental effects of oxidative stress, thus improving the development of embryos and increasing the level of estrogen in the female reproductive system.

Keywords: Alpha-tocotrienol, Nicotine, Vitamin E, Embryos, Estrogen

INTRODUCTION

Exposure to oxidative stress induced by nicotine adversely affects fertility by retarding preimplantation embryonic development, reducing the rate of embryonic cleavage and disrupting endocrine hormone levels (1]. Nicotine, one of the active components of tobacco smoke, is known to be a prooxidant and is associated with increased lipid per-oxidation due to inhibition of antioxidant enzymes (2]. This process leads to the formation of free radicals or reactive oxygen species (ROS) in the female reproductive system. Alpha-tocotrienol, one of the forms of vitamin E that has powerful antioxidant properties is reported to be able to reduce the oxidative stress reaction by scavenging free radicals in cells and cell organelles (3].Therefore, this study was designed to determine the effectiveness of alpha-tocotrienol (α-TCT) to counter the detrimental effects of nicotine in mice thus improving the development of preimplantation embryos and the level of the estrogen profile.

MATERIALS AND METHODS

All procedures were performed with approval from the Universiti Teknologi MARA Committee on Animal Research and Ethics (UiTM CARE: 287/2019). Six- to eight-week old female Balb/c mice were divided into 5 groups (Group A – E) with 6 mice per group. Group A (control) received 0.1 ml of tocopherol-stripped corn oil, Group B was given 3 mg/kg bw/day of nicotine alone, groups C-E were concurrently treated with 3 mg/kg bw/day of nicotine and 10, 20 or 30 mg/kg bw/day α-TCT for 7 consecutive days. Following the completion of the treatments, all females were super ovulated and immediately mated overnight with male mice. The normal embryos were collected 48 hours after mating and subjected to in vitro culture. The development of embryos was monitored daily until the hatched blastocyst stage. Serum from blood samples was collected for estrogen analysis using ELISA. Results were statistically analysed using analysis of variance (ANOVA) and presented as mean ± SEM.

RESULTS AND DISCUSSION

The overwhelming level of ROS in the female reproductive system will lead to an oxidative stress condition that eventually contributes to an increase in embryo fragmentation (Figure 1). As a result, the arrested embryos cannot further develop into blastocyst, which will prevent the successful implantation process. In order to reduce the oxidative stress effect, supplementation of antioxidantscould potentially reduce the ROS damage in cells by improving the quality of embryos and embryonic development due to nicotine induced oxidative stress (4]. The results of this study indicated that nicotine caused embryonic retardation to develop until the blastocyst stage in nicotine-treated mice as compared to the control group (Fig. 1). When compared to the nicotine groups, groups treated concurrently with nicotine and α-TCT supplementations at different doses (10 mg/kg, 20 mg/kg and 30 mg/kg) were able

Figure 1: The photomicrographs of preimplantation of normal embryo development *in vitro* x100 magnification

to improve embryonic development until the blastocyst stage (Table I).

In the estrogen analysis, estrogen levels weresignificantly lower in the nicotine group compared to thecontrol group and significantly higher in the group concurrently treated with nicotine and α-TCT at the dosages of 20 and 30 mg/kg bw/day as compared to the nicotine group (Fig. 2).

CONCLUSION

The finding proposed that α-TCT supplementation was able to improve embryonic development and increase the level of estrogen in nicotine-treated mice at the dosage of 10, 20 and 30 mg/kg bw/day.

Table I: Effect of Various Doses of α-TCT Intervention on the Number of Embryonic Development in Nicotine Treated-Mice

GROUP	STAGES EMBRYONIC DEVELOPMENT					
	2-4 cell stage	4-8 cell stage	8-16 cell stage	Morula	Blasto-cyst	Hatched blasto-cyst
Vehicle Control	5.62 ± 0.65	4.38 ± 0.68	2.25 ± 0.25	1.25 ± 0.25	0.62 ± 0.26	0.00 ± 0.00
Nicotine	2.25 ± 0.82	1.25 ± 0.75*	0.62 ± 0.32*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Nicotine + 10mg/kg of α-TCT	6.25 ± 1.25	5.62 ± 1.58	3.75 ± 1.01	3.75 ± 1.01*	2.5 ± 0.76*	1.00 ± 0.66*
Nicotine + 20mg/kg of α-TCT	7.50 ± 1.41	6.88 ± 2.05	6.88 ± 2.05**	5.00 ± 1.45**	3.12 ± 0.55**	2.50 ± 0.76**
Nicotine + 30mg/kg of α-TCT	9.38 ± 0.65*	7.50 ± 1.41*	7.50 ± 1.41**	6.88± 2.05***	6.25 ± 2.01***	4.38 ± 0.86***

Data were expressed as mean ± SEM. Data were analyzed using ANOVA test.*p<0.05; **p<0.01; ***p<0.001 significantly different from the control group.

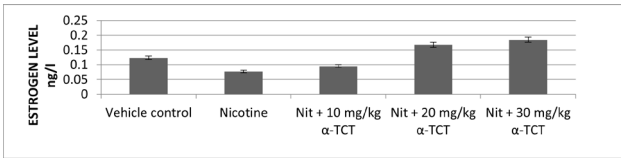

Figure 2: Data were expressed as mean ± SEM. Data were analyzed using ANOVA test. *p<0.05 different from the control group and # p<0.05 significantly different from the nicotine group

ACKNOWLEDGEMENTS

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EXTENDED ABSTRACT

Halal Pharmaceuticals Research Trends and Coverage: A Systematic Literature Review

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SUMMARY

Pharmaceuticals have been among the items that have seen significant growth in the halal business, both Malaysia and internationally, over the years. While the food industry has received most of the attention in the halal industry, there have been few systematic literature review (SLR) on halal pharmaceutical research to date. This SLR was conducted to compile pertinent information on pre-established eligibility criteria and research questions. The goal of this SLR was to examine the trends in the previous studies on halal pharmaceutical research, to assess at current practices, issues, and success factors within this sector.

Keywords: Halal Pharmaceuticals, Systematic Literature Review, Halal Research Trends

INTRODUCTION

The Arabic word Halal means "allowable, acceptable, permitted and permissible." According to the Malaysian Halal Pharmaceutical Standard MS2424:2019 halal is defined as matters that are lawful and permitted in Islam based on Shariah law and fatwas. In the same standards, Shariah law is defined as the commands of Allah concerning the conduct of the people who are accountable (mukallaf), which consist of demands (commandments and prohibitions), an option or hukm wadh'i while fatwa means a legal opinion concerning Islamic law issued by Muslim Scholar. To be considered Halal, a product must comply with Islamic Shari'ah requirements as stated in the Holy Qur'an and hadith. Halal encompasses all facets of a Muslim's life, including medicines, not just food or food-related products. This SLR will explicate the trends and areas commonly covered in previous halal pharmaceuticals publications, using a reproducible, scientific, and transparent methodology to gather relevant data that meets the predefined eligibility criteria and answers research questions.

MATERIALS AND METHODS

This SLR followed a clear methodological technique and utilized PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) as a guide to process and filter the eligible papers for review, involving identification, screening, eligibility review and inclusion decisions (1) .The databases used for the data collection were Scopus, Emerald Insight, MyCite and Google Scholar, and the literature types included were journal articles with empirical data, research papers, forums, and case studies. Book chapters, reviews, conceptual articles, announcements, descriptive articles, and any articles based on analysis of secondary sources were excluded. Additionally, only articles published in English and Malay were included, and the timeline was set to publications between 2011 and 2021 to allow adequate time period to observe the evolution of research and related publications. The keywords used during the SLR were "halal AND pharmaceutical" OR "halal medicine" and other synonyms or equivalent indication of pharmaceuticals.

RESULTS AND DISCUSSION

A total of 29 articles were downloaded from the selected databases, with 16 from Scopus, 10 from Google Scholar, 3 from MyCite and 1 from Emerald Insight (Figure 1a). In terms of year of publication, 2020 had the most papers (7), while 2011 and 2019 had no relevant publications. Malaysia (MY) had the most publications (19), followed by Indonesia (ID) with six, other countries, Iran (IR), United Arab Emirates (UAE), Jordan (JD) and United States (US) with one publication each (Figure 1b). The common research methodologies used were quantitative studies (20 publications), qualitative studies (7 publications) lab-based research (3 publications). Table I below shows the focus of previous studies found from this SLR.

The finding showed that most of the studies assessed the

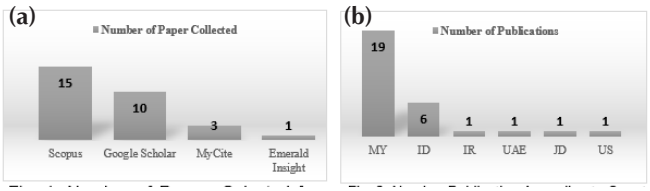

Figure 1: (a) Number of Papers Selected from the Each Databases; (b) Number Publication According to Country

Table I: Scope of Studies Covered in the publication Review

Scope of Study	Number of Publications (n= 29)
Knowledge/Awareness	7
Knowledge, Attitude & Perception (KAP)	5
Purchase intention	5
Authentication process	3
Halal certification Process	2
Ingredients Review	2
Warehousing	2
Vaccine production	1
Halal Legal Framework	1
Halal Supply chain	1
Total	**29**

knowledge or awareness of various target audiences, such as patients, students, academician, pharmacists, and doctors (2,3). Generally, the majority of the target audiences in Malaysia have good knowledge and positive attitudes and perceptions on halal pharmaceuticals, while studies in the US, Jordan, and UAE showed opposite results. Studies on customers' purchase intentions showed that customers' intention to purchase were positively influence by attitude, religiosity, knowledge of halal products, family and friends and perceived behavioral control. Subsequently, ingredients authentication studies used Duplex polymerase chain reaction (PCR), PCR and southern-hybridization on the biochip, Fourier-transform infrared spectroscopy, and chemometrics to detect porcine DNA in pharmaceutical products (4). Analysis of results also showed that success factors depend on customers' demand, awareness, trust in the halal logo, purchase intention, religiosity, and customer satisfaction. Issues identified include patients' lack of awareness of medicines' halal status, scarcity of knowledge about halal pharmaceuticals, difficulty in obtaining halal pharmaceutical certification due to ingredients selection, use of non-halal raw materials, suppliers' inability to provide documents for halal certification, and cost for adopting halal (5).

CONCLUSION

This SLR met its research objective, showing that Malaysia is leading in halal pharmaceutical research, with quantitative research being the most common methodology used, focusing on knowledge and awareness of halal pharmaceuticals among various target populations. Factors related to customers and suppliers influences the success and challenges in this sector.

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EXTENDED ABSTRACT

Potential Use of Zebrafish as Danon Disease Model: Comparative Computational Modeling and Protein-Protein Interaction Analysis on the LAMP2 Gene of Human, Mouse and Zebrafish

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SUMMARY

Danon disease is an X-linked dominant metabolic disorder, causes heart and skeletal muscle weakness, along with intellectual disability. Mutations in lysosome-associated membrane protein 2 (LAMP2) are believed to cause the disease, which can be found in the lysosome membrane. This study performed computational homology modelling to predict the protein structure of zebrafish LAMP2 to study the effect of LAMP2 gene mutations. The predicted LAMP2 protein structure was compared to human and mouse LAMP2 structures to assess the quality of the predicted protein structure, and LAMP2 protein-protein interaction analysis was conducted. The findings support the use of zebrafish as an animal model for Danon disease.

Keywords: *Danio rerio*, zebrafish, animal model, LAMP2, Danon disease

INTRODUCTION

Danon disease, a rare genetic disorder characterised by hypertrophy cardiomyopathy, skeletal myopathy, and mental retardation. It is believed to be caused by mutations in LAMP2, resulting in autophagy disruption, as LAMP2 in lysosome aids the fusion of autophagosomes to lysosomes in the final step of autophagy. Different mutations, however, have different effects on protein expression, which explains the phenotypic diversity in Danon disease [1]. Current treatments are unable to cure the disease, only preventing sudden death and heart failure. Animal models such as mice have been used in research to better characterise the disease due to its broad clinical manifestation. In this study, the zebrafish was chosen due to its easy genetic manipulation and rapid development compared to mice [2]. To assess the model's reliability, LAMP2 homology modelling in human-zebrafish and human-mouse was performed, as well as LAMP2 protein-protein interactions in humans, zebrafish, and mouse to determine the functionality of interacting genes.

MATERIALS AND METHODS

The NCBI database was searched for the amino acid sequences of human LAMP2 homologs in zebrafish and mouse, followed by the prediction of their 3D structures using the i-TASSER (https://zhanggroup.org/I-TASSER/). The 3D structures were then refined to ensure high confidence models for modelling purposes. The alignment of the isoforms between human-zebrafish and human-mouse was conducted, and the 3D structures were visualised in PyMOL v2.5.2. The stereochemical quality and overall quality factor of each 3D model were checked by using the Ramachandran plot and ERRAT program by SAVES v6.0 (https://saves.mbi.ucla.edu/) for 3D structure verification purposes. The protein-protein interaction (PPI) analysis of LAMP2 in human, zebrafish, and mouse were performed by using STRING to determine the proteins involved with the LAMP2 activity [3].

RESULTS AND DISCUSSION

The refined protein models of LAMP2A, LAMP2B, and LAMP2C of human, zebrafish and mouse were aligned and visualised using PyMOL. Figure 1 shows the visualisation of the 3D structures of zebrafish, human, and mouse. Structural similarity between the template and the predicted structure was verified with the TM-score, with a score of >0.50 indicating a model of

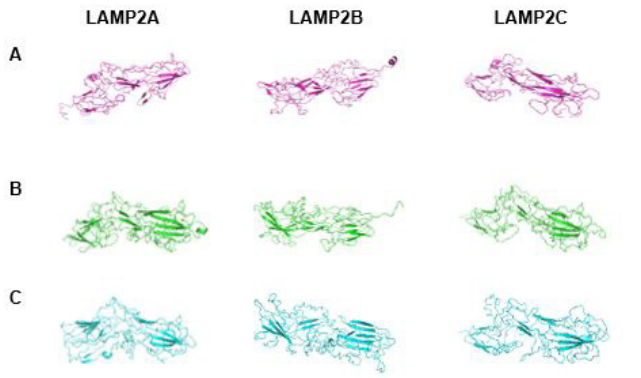

Figure 1: Zebrafish 3D models (A) Zebrafish (B) Human (C) Mouse

correct topology and <0.17 means a random similarity. Based on the results, all the scores are >0.4 on average.

The stereochemical and overall quality of each 3D model was verified by checking the Ramachandran plot and the ERRAT program. Over 90% of the amino acid residues of predicted 3D structures of human, zebrafish, and mouse LAMP2 proteins were located within the allowed region, indicating good quality models. The ERRAT score, an overall quality score for non-bonding atomic interactions with higher scores indicating higher quality and a commonly accepted range of more than 50 [4], was 64.02, 60.66, and 50.00 for the zebrafish 3D model, respectively (Table I). According to the current model available, the results can be considered to pass the accepted range.

Finally, the determination of proteins involved with the LAMP2 activity using STRING discovered ten different interacting proteins associated with LAMP2 in human, zebrafish, and mouse as shown in Figure 2. Proteins associated with autophagy lysosomal pathway, such as HSPA8, map1lc3b, and Rab7, will be investigated to understand the impact of LAMP2 variations through mutational impact prediction in future study.

Table I: TM-score, Ramachandran plot and ERRAT scores for zebrafish, human, and mouse 3D models

Isoforms	Organism	TM-score	Ramachandran plot	ERRAT
LAMP2A	Zebrafish	0.53	97.2%	64.02
	Human	0.46	96.2%	55.05
	Mouse	0.44	96.4%	67.81

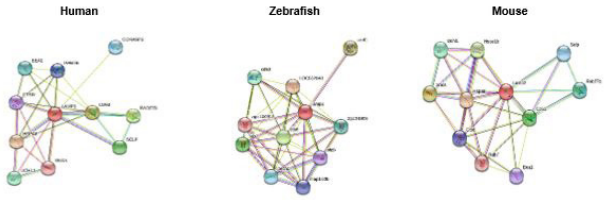

Figure 2: Protein-protein Interactions of LAMP2 in (A) human, (B) mouse, and (C) zebrafish

CONCLUSION

In the current study, the Ramachandran plot and ERRAT scores of the predicted 3D structure of the zebrafish LAMP2 protein are >90% and >50, respectively, indicating that the model is of high quality and can be used for subsequent analysis. However, model optimisation processes are recommended for further improvement.

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EXTENDED ABSTRACT

Antibacterial Activity of Fractions from Marine Endophytic Fungi Against Gram-Negative Bacteria

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SUMMARY

Marine natural products are reported to exhibit various bioactivity such as antimicrobial, anti-tuberculosis, antiviral, antiprotozoal, antioxidant, immunomodulatory, anti-diabetes, anti-inflammatory and anticancer. Biologically active metabolites obtained from marine endophytic fungi are reported to have excellent activity for the treatment of various diseases particularly against antibacterial resistance. In this study, the extracts of two marine endophytic fungi isolated from *Gracilaria coronopifolia* (CN) and *Gracilaria arcuate zanardini* (MV) were investigated for antibacterial activity against three pathogenic Gram-negative bacteria. To verify the antibacterial components of these endophytic fungal extracts, the antibacterial component was isolated by using a bio-guided fractionation assay. Both extracts exhibited promising antibacterial activity (50.5% to 82.1%) and worth to be further investigated as an antibacterial agent.

Keywords: marine, seaweed, endophytic fungi, bioassay-guided fractionation, antibacterial

INTRODUCTION

Endophytic fungi are a vast and unexploited source of potent new natural compounds for industrial and medical applications. The marine environment is a rich unique source of structural and functional metabolites, of which marine organisms are host to diverse endophytic fungi with pharmaceutical potential (1). The main biological roles exhibited by marine endophytic fungi include anticancer, antimicrobial, UVB photo-protective, antimicrobial, antioxidant, and wound healing properties (2-5). However, to date, these producers of bioactive secondary metabolites are much lower when compared to endophytic fungus from terrestrial plants (2).

MATERIALS AND METHODS

Eight endophytes from eight different seaweeds [*Gracilaria coronopifolia* (CN) and *Gracilaria arcuata zanardini* (MV)] were grown on potato dextrose agar (PDA) for 1 month before being extracted. The extraction method was carried out according to Ariffin et al. (2011). The endophytic fungi on PDA were macerated and transferred to a conical flask filled with 100% ethyl acetate and the resultant mixture was stirred overnight at room temperature. The extract was filtered through No 1 filter paper, 20–25 mm in diameter (Whatman, Maidstone, 195 UK) after which sodium sulfate (3-4 g) was added to further remove the aqueous layer within the extract. The sodium sulfate was removed by filtration before the organic phase was dried by rotary evaporation. The resulting extracts were weighed and dissolved in methanol and used to determine the antibacterial activity using bioassay guided fractions.

An analytical HPLC was carried out using a linear gradient of 10%-75% water and trifluoracetic acid [ACN/H_2O+TFA] at 37°C for 30 minutes for every cycle. Screening of antibacterial properties [against Pseudomonas aeruginosa (ATCC 9721), Escherichia coli (ATCC 10798) and Salmonella typhimurium ATCC14028] of each extract was done using polypropylene-microplate 96 wells plates. Only 90 wells were filled with the fractions (each peak in one well). The bacterial suspension density was compared with the McFarland turbidity standard and loaded into the 96 well plates containing the dried isolated fractions. The plates were then incubated overnight at 37°C and treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and the bacterial viability was assessed by measuring wavelength absorption at 540nm using a microplate reader.

RESULTS AND DISCUSSION

Out of the eight fungal extracts tested, only two that were isolated from the red seaweeds (CN and MV) exhibited antibacterial activity using bioassay-guided fractions. About 73 fractions (37 from CN & 36 from MV) showed

antibacterial activity against the three pathogens (*P.aeruginosa, E.coli* and *S.typhimurium*) (Figure 1). *P. aeruginosa* was more susceptible to fractions from both extracts (50.5% to 82.1%) when compared to *S. typhimurium* (30.1% to 54.4%) and *E. coli* (30.1% to 54.4%). However, the percentage of inhibition by endophytic fractions from MV (60.2% to 82.1%) was slightly higher against P.aeruginosa than endophytic fractions from CN e (30.1% to 59.1%).

The present study shows that both marine endophytic fungal fractions seem to have promising activity against *P. aeruginosa*. These isolated active fractions would be worth investigating as an alternative source for new antibacterial agents, particularly for *P. aeruginosa* since more than half of these strains are not susceptible to many commercial antibiotics.

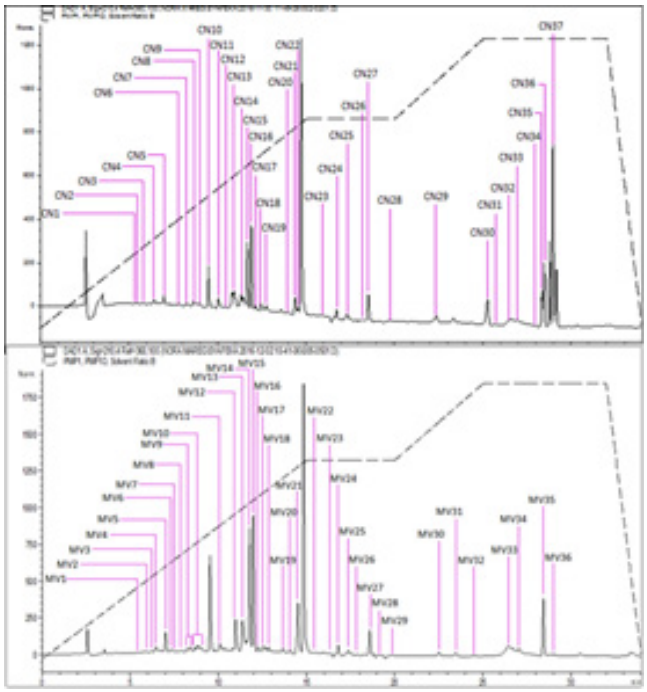

Figure 1: The total number of active peaks (indicated as CN1-CN37 & MV1-MV36) observed in the HPLC chromatogram of CN (A) and MV (B) against *P. aeruginosa E. coli* and *S. typhimurium*

CONCLUSION

The present study exhibited promising antibacterial activity against three gram-negative bacteria. A few major peaks have been identified from endophytic fraction of CN (CN16, CN21, CN22, CN37, CN35 & CN36) and MV (M13, MV14, MV21 & MV35).

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