

ORIGINAL ARTICLE

Phytochemical Investigation and Antimicrobial Activity of *Muntingia calabura* L. Against Selected Pathogens

Nur Dayana Hassan Cheong, Muhamad Mustaqim Amran, Hartini Yusof

Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Introduction: As the overuse of antibiotics increases, antimicrobial resistance is becoming a significant issue, making bacteria resistant to antibiotics and rendering illnesses more challenging to treat. The declining efficacy of synthetic antibiotics in curing infectious diseases is a global concern that is growing. Thus, studies for alternatives from natural plants are in high demand. **Method:** This study was done to assess the phytochemical compounds and antimicrobial activity of methanolic *Muntingia calabura* L. leaf methanolic extract against *Staphylococcus aureus*, *Salmonella typhimurium*, and *Shigella sonnei* using the disc diffusion and broth dilution methods. **Results:** The methanolic extract of *M. calabura* L. leaves was the most successful when tested against *S. aureus* with a mean inhibition zone of 14.33 mm, followed by *S. sonnei* with 10.66 mm, while no inhibitory effect was noted on *S. typhimurium*. When tested for minimal inhibitory concentration (MIC), readings of 7.81 mg/ml and 62.50 mg/ml were recorded for *S. aureus* and *S. sonnei*, respectively. The minimum bactericidal concentration (MBC) test revealed that the methanolic extract of *M. calabura* L. leaves completely inhibited *S. aureus* and *S. sonnei* at 15.63 mg/ml and 250.00 mg/ml, respectively. The effectiveness of *M. calabura* L. leaves in inhibiting the tested organisms demonstrates the extract's potency as a remedy. **Conclusion:** The findings of this study offer a foundation for the potential use of *M. calabura* L. leaves in developing pharmaceuticals for a variety of illnesses caused by the microorganisms studied.

Malaysian Journal of Medicine and Health Sciences (2022) 18(SUPP15): 301-307. doi:10.47836/mjmh18.s15.42

Keywords: Antimicrobial activity, *Muntingia calabura* L., leaf extract, methanolic, phytochemical compounds

Corresponding Author:

Hartini Yusof, MSc
Email: hartinieyusof@gmail.com
Tel: +603-32584431

INTRODUCTION

In today's world which is filled with advanced technologies, scientists and researchers are searching for more alternatives to fulfill the needs of human beings in every aspect. Plants are one of the many sources that play an important role in our daily lives. Every part of the plant, including the leaves, roots, fruits, and others can be utilized in many different ways. More importantly, medicinal plants, are even considered commodities (1). They have been used as the main ingredient to produce medication options for various types of diseases since the olden days. Nowadays, various drugs have been derived from natural plants as they are full of potential and advantages (2). Additionally, studies on plant medicines are gaining more interest worldwide due to the inherent properties of plants such as antioxidants and antibacterial activities as well as low toxicity levels. Due to its great potential and affordability, plants are becoming the choice source for antimicrobial agents

instead of synthetic drugs that are often more costly (3).

Antimicrobial resistance is worsening day by day as the excessive use of antimicrobial drugs is getting out of hand. Not only does this increase the resistance of bacteria to the readily available drugs, but at the same time, it simultaneously causes the infections to be more challenging to treat. Centering on this matter, the fundamental concern is that more bacteria are starting to develop new strains and become more rebellious or resistant to the antibiotics in use. In other words, the pharmaceuticals dispensed to patients are not affecting the bacteria at all and the conditions of the infected patients are not improving. As claimed in several studies, infectious diseases are still ranked as one of the most common causes of death globally (4–6). Particularly in low-income countries especially, infectious and parasitic diseases are among the most dominant causes of death (5) mainly due to the lack of resources for treatments and prevention. Furthermore, the World Health Organisation (WHO) stated that two infectious diseases – lower respiratory infections and diarrheal diseases were ranked in the top ten causes of death worldwide. WHO also stated that there might be a huge reservoir of potential tuberculosis cases in the future

because about one-third of the world's population are carriers of a latent *M. tuberculosis* infection. Thus, this calls for the urgent need to generate new technologies and expertise to combat human microbial pathogens (7).

Muntingia calabura (*M. calabura*) is more commonly known as 'Jamaican cherry' worldwide and 'kerukup Siam' among the locals in Malaysia (8). Multiple parts of the plant have been incorporated into treatments for different types of illnesses. As reported in Peruvian folklore, its leaves, bark, and flowers are believed to confer medicinal benefits (9). The flowers and barks are used as antibacterial agents and to reduce lower limb swelling in Peruvian traditional medicine, while the leaves, either cooked or soaked in water, are used to diminish stomach ulcers and swelling of the prostate gland, as well as to relieve headaches and cold symptoms (8,10). In addition, the leaves were also reported to have properties including anti-gout, antidiabetic, antihypertensive, antioxidant, and anti-inflammatory (11,12). *M. calabura* fruits are well-known to possess certain pharmacological activities such as anti-inflammatory properties, as well as antirheumatic, antioxidants, antidiabetic, and immunomodulatory properties (13). In Central America, its flowers are traditionally used as antispasmodic and antiseptic agents, and some compounds isolated from its roots may be used to control malignant cell growth (14). Apart from medicinal purposes, the fruits are often baked in tarts or turned into jam, and the leaf infusion is consumed as a tea-like beverage (15). Given the multiple advantages presented by the *M. calabura* plant, the purpose of this study was to assess the phytochemical compounds and antibacterial activity of *M. calabura* L. leaf extract on selected pathogens.

MATERIALS AND METHODS

Bacterial Species and Source

S. aureus (ATCC 25923), *S. typhimurium* (ATCC 12228) and *S. sonnei* (ATCC 29930) have been used in this study and they were obtained from the Microbiology Laboratory, Centre for Medical Laboratory Technology Studies, UiTM Puncak Alam.

M. calabura Leaves Sample Collection

M. calabura leaves were obtained from Kuala Kangsar, Perak in March 2018. The samples were taken to the laboratory at UiTM Puncak Alam campus for further processing.

M. calabura Leaves Processing

The leaves were left on a clean cloth to air dry at room temperature in the laboratory for two weeks. The dried leaves were then ground into small particles in a dry blender. The blended dried leaves were sieved to obtain their powder form for a sample with a greater surface area. The completed sample was kept in an airtight

container and stored in the dark. Doing this prevented the sample from being exposed to sunlight which could affect the bioactive compounds of the leaves.

Preparation of *M. calabura* Leaves Extract

With the ratio of 1:10 (w/v), 50 g of the powdered samples were measured and macerated in 500 ml of 100% methanol in a 1000 ml Schott bottle. Next, the bottle was covered with aluminum foil and left on the orbital shaker to shake for five days at room temperature. After five days, the solution was filtered twice using Whatman No. 1 filter paper. To produce the crude extract, the filtrate was concentrated using a rotary evaporator at 40°C under decreased pressure. To prevent evaporation and contamination, the methanolic extract was transferred to a Petri dish and covered with parafilm. Using an aluminium foil, the Petri dish was covered for a second time and stored at 4°C until further use.

Preparation of Concentrated *M. calabura* Leaves Extract

100 µl of 100% dimethyl sulfoxide (DMSO) and 900µl of distilled water were mixed together to produce 10% DMSO. This is because the highest suggested concentration of DMSO is 10% and using more than that might result in a false positive result. Next, 1000 mg of *M. calabura* leaves crude methanolic extract was measured and mixed with 1 ml 10% to produce an extract with a concentration of 1000 mg/ml.

Qualitative Phytochemical Screening of *M. calabura* Leaves Extract

To evaluate the presence of secondary metabolites, an aliquot of methanolic extract obtained from *M. calabura* leaves was submitted to a qualitative phytochemical examination. The presence of compounds such as flavonoids, tannins, saponins, and alkaloids was visualized via color changes and foam production in the following tests.

Test for flavonoids

2 ml of the extract was mixed with a few drops of 1% ammonia (NH₃) solution in a test tube. A yellow coloration as an indication of flavonoids was observed (16).

Test for tannins

In a 5 ml test tube, 1 ml of extract and three drops of 5% iron III chloride were mixed together. The greenish-black precipitated produced indicates the presence of tannins (17).

Test for saponins

2 ml extract was diluted with 5 ml distilled water and was shaken vigorously to obtain stable and persistent foam. The formation of the foam layer indicates the presence of saponins (17).

Test for alkaloids

1 ml of extract, 2 ml of chloroform, and a few drops of Wagner's reagent were mixed in a 5 ml tube. The development of reddish-brown precipitate shows the presence of alkaloids (17).

Antimicrobial Susceptibility Test (AST)

The AST and other antibacterial tests were performed in triplicate to decrease inadequacy in the results and enhance the output's dependability. The disc diffusion method was chosen as the preferred approach since it requires no additional equipment and allows for a wide range of discs to be tested.

Disc Diffusion Method

20 µl of 1000 mg/ml *M. calabura* leaves extract was allowed to soak into sterile discs. The drying process of the discs was done in an oven at 40 °C for 10 minutes. The positive controls used were ampicillin 10µg (AMP10) and gentamycin 30µg (CN30) while the negative control was 10% DMSO. For each of the microorganisms tested, a single colony was inoculated in 5 ml Tryptic Soy Broth (TSB) and incubated for two to three hours. The tested bacteria species (*S. aureus*, *S. typhimurium*, and *S. sonnei*) were streaked onto Mueller Hinton (MH) agar using the Kirby Bauer method of lawn streaking and each species was streaked with a new sterile cotton swab. The *M. calabura* leaves extract, as well as positive and negative control discs, were placed onto each streaked MH agar plate with even spacings. The tests were repeated three times and the zone of inhibitions was measured using a ruler in millimeter (mm) after 24 hours of incubation at 37°C. The results for each plate were recorded.

Minimum Inhibitory Concentration (MIC)

A broth dilution susceptibility test was applied to determine the MIC values of this study. Only the organisms that are sensitive to the extract in AST were tested in MIC. For each organism, three to five colonies were collected and inoculated into 5 ml of MH broth with a concentration of 1000 mg/ml to 1.95 mg/ml, before being incubated for 24 hours at 37 °C. 12 tubes were prepared and labeled one to 12. 1 ml of *M. calabura* methanolic extract was pipetted into tube one while 500 µL MH broth was pipetted into tubes two to 12. Two-fold serial dilution was performed by pipetting 500 µL of extract from tube one into tube two. Then, the suspension from tube two was transferred into tube three and the process was repeated until tube ten. Next, 100 µL of the diluted extracts from each tube was pipetted into (1-10) wells of the labeled microtiter plate. 20 µL of bacterial inoculum was pipetted into each well. For the negative control, 120 µL of MH broth was pipetted into well 11. For the positive control, 100 µL of MH broth and 20 µL of bacterial inoculum were pipetted into well 12. The microtiter plate was covered with aluminum foil and left to incubate for 24 hours at 37°C. For all organisms tested, the tests were conducted in triplicate

to ensure the validity, reliability, and level of precision of the results obtained. The presence of turbidity and "pellet" at the bottom of the well indicated bacterial growth. The growth of both *S. aureus* and *S. sonnei* were compared with the positive and negative controls and the results obtained were recorded.

Minimum Bactericidal Concentration (MBC)

To evaluate the MBC values, the contents from the wells of the MIC test were cultured onto the MH agar plate, including the positive and negative controls. The streaked MH agar plates were then incubated for 24 hours at 37°C. The lowest concentrations that showed no organism growth were determined as the MBC values. This test was also done in triplicate for each organism and the results were recorded.

Statistical Analysis

All data collected were calculated as the mean ± standard deviation with a minimum of three independent experiments. The data collected were tabulated by using One-Way Analysis of

RESULTS

The *M. calabura* leaves in powder form were soaked in methanol solvent for five days to ensure the release of all bioactive compounds present. As shown in Table I, flavonoids, saponins, alkaloids, and tannins were detected.

Table I: Phytochemical Screening of *M. calabura* L. Leaf Extract

Bioactive compound	Observation	Result
Tannins	Brownish green coloration	Positive
Flavonoids	Yellow coloration	Positive
Saponins	Stable foam production	Positive
Alkaloids	Reddish brown precipitate	Positive

As summarised in Table II, the highest inhibition zone value was shown by *S. aureus* at 14.33 mm compared to *S. sonnei* with a value of 10.66 mm, and a clear zone of inhibition was shown by *S. typhimurium* against the methanolic extract of *M. calabura* L. leaves. ANNOVA test was conducted in this research study to evaluate the comparative effects of three different tested organisms after being treated with 1000 mg/ml of *M. calabura* L. methanolic leaves extract. According to the table above, it can be summarized that the p-values of all three tested organisms were less than the standard value ($p < 0.05$). This shows that the ANNOVA test was significant enough to prove that all selected organisms were significant statistically.

Based on Table III, for *S. aureus*, wells 1-8 showed no visible growth after overnight incubation. Therefore, the MIC readings were 7.81 mg/ml (well 8) for *S. aureus* and 62.5 mg/ml (well 5) for *S. sonnei*. The results of this experiment were validated by comparing the turbidity of the positive control and the negative control. The

Table II: The Mean Inhibition Zones of *M. calabura* L. Leaf Extract Against Selected Bacterial Strains

Variables	Mean zone of inhibition (mm)	Standard deviation (SD)	Standard error mean	95% Confidence interval of the difference		df	P-value
				Lower bound	Upper bound		
<i>S. aureus</i> (ATCC 25923)	14.33	1.15	0.66	11.46	17.20	2	0.00
<i>S. typhimurium</i> (ATCC 12228)	0.00	0.00	0.00	0.00	0.00	2	0.00
<i>S. sonnei</i> (ATCC 29930)	10.66	0.58	0.33	9.23	12.10	2	0.00

Table III: Mean MIC For *S. aureus* And *S. sonnei* Against *M. calabura* Leaf Extract

Well	Concentration of dilution (mg/ml)	Result	
		<i>S. aureus</i> ATCC 25923	<i>S. sonnei</i> ATCC 29930
1	1000	C	C
2	500	C	C
3	250	C	C
4	125	C	C
5	62.50	C	C
6	31.25	C	T
7	15.63	C	T
8	7.81	C	T
9	3.91	T	T
10	1.95	T	T
11	Negative control (120 µl MHB)	C	C
12	Positive control (100 µl MHB + 20 µl inoculum)	T	T

C: clear (no bacteria growth), T: turbid (bacteria growth)

positive control showed visible growth (turbid broth) while the negative control showed no visible growth (clear broth).

The mean MBC result of *M. calabura* L. leaves extract against *S. aureus* and *S. sonnei* in triplicate is shown in Table IV.

DISCUSSION

Flavonoids, saponins, alkaloids, and tannins were detected showing the suitability of the maceration technique in the present study. Buhian et al. obtained similar results when screening for phytochemical compounds in *M. calabura* leaves using ethanol as an extraction solvent (18). The air-dry method was performed as it is the most reliable method to maintain the quality

and prevent the loss of phytochemical compounds in *M. calabura* leaves. As proven by Mudau et al., it was found that the total phenolic and antioxidant contents of shade-dried (air-dried) bush tea were higher compared to the sun and oven-dried samples (19). Dried leaves were also chosen as they allowed for a more extended maintenance period than fresh leaves; the latter requires a processing time of fewer than three hours to maintain the freshness of samples (20). They also contain higher flavonoid contents than fresh samples as proven with *Moringa oleifera* by Vongsak et al. (21). Furthermore, fresh samples were not preferred as they are more prone to fragility and deterioration. Samples in powder form, which have more homogenized and smaller particles, are recommended due to their greater surface area in contact with the extraction solvent (22), ensuring the maximum output in the extraction procedure.

Based on the solvent miscibility, methanol is one of the solvents that have a high polarity index among other types of extraction solvents, thus making it the solvent of choice in the present study. The study done by Surjowardojo et al. showed that methanol produced the highest percentage of rendement as an extract (23). According to a previous study on *M. calabura* leaves, it was found that only methanol extract exhibited inhibitory effects on *S. aureus* 25923 and *S. aureus* 33591 with the MIC/MBC values of 1250 and 2500 µg/ml, respectively. Thus, methanol can be considered the most efficient solvent of choice for the extraction of *M. calabura* leaves compared to chloroform and aqueous solvents (24). It is important to note the index of the polarity of the solvents in use as this affects the output of extraction in terms of quantity, the bioactive compounds, and the antimicrobial performance of the plant extract (25). The maceration technique was used with an extract to solvent ratio of 1:10 (w/v) as it is the most suitable method to break down the plant cell walls for the maximum release of phytochemicals

Table IV: Mean MBC For *S. aureus* and *S. sonnei* Against *M. calabura* Leaf Extract

Bacteria	MBC Dilution (mg/mL)							
	1 (1000)	2 (500)	3 (250)	4 (125)	5 (62.50)	6 (31.25)	7 (15.63)	8 (7.81)
<i>S. aureus</i> ATCC 25923	-	-	-	-	-	-	-	/
<i>S. sonnei</i> ATCC 29930	-	-	-	/	/	/	/	/

Growth (/) = indicates growth of organism and No growth (-) = indicates organism inhibited/killed

to be completed with ease (22). The extract was stored in the chiller until further use as suggested in a study conducted by Sujarwodojo et al., the zones of inhibition produced were larger compared to the extracts kept at room temperature (26).

It was observed that tannins, flavonoids, saponins, and alkaloids were the compounds present in the methanolic extract. The results obtained are supported by a previous study done by Singh et al. which revealed the availability of flavonoids and alkaloids in the leaves of *M. calabura* even though they showed more significant amounts in the extract of *M. calabura* flowers (27). Sibi et al. also reported the presence of flavonoids and tannins during the phytochemical studies of *M. calabura* leaves (28). The antimicrobial activities of tannins are owed to the inhibition of extracellular microbial enzymes, oxidative phosphorylation, and disruption of cellular membrane permeability (29), making it toxic to bacteria. Flavonoids are also an excellent antimicrobial compound as they exhibit bacterial membrane disruptions, inhibition of cell envelope synthesis, and nucleic acid synthesis among the many mechanisms of action to disintegrate microorganisms (30). Saponins are also effective in the killing of microorganisms by means of cell wall degradation, followed by disruption of the cytoplasmic membrane that eventually leads to cell contents leakage (31). Alkaloids are found in the leaves of *M. calabura* just as reported by Sari et al. with the ethanolic extract (32). This compound is known to be cytotoxic and has a wide range of physiological effects (18). Phytochemical screening of the *M. calabura* leaves was done in this current research despite previously reported results because as mentioned by Mudau et al. and Kumar et al., there is a significant relationship between soil composition, climate conditions, and photoperiod and phytochemicals, which explains why the phytochemical content of a given plant varies from one location to another, or even within the same nation (33,34).

The methanolic extract of *M. calabura* leaves was significantly effective against the two organisms but not against *S. typhimurium*. Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation (35). The test is done in triplicate to ensure the reliability and accuracy of the results obtained. The MIC test showed a reading of 7.81 mg/ml for *S. aureus* while 62.50 mg/ml was obtained for *S. sonnei*. Similar to a study done by Fernando et al., the highest antimicrobial activity was found against *S. aureus* and *B. cereus* (36). Following that, the MBC test results presented that the methanolic *M. calabura* leaves extract successfully inhibited *S. aureus* at the concentration of 15.63 mg/ml and for *S. sonnei*, the MBC result was higher at 250.00 mg/ml. This indicates that the inhibition of *S. aureus* requires a lower concentration of extract compared to *S. sonnei*. A previous study done by Zakaria et al. also

showed similar results where the methanolic extract of *M. calabura* leaves was also found to be most effective against *S. aureus*, even though their study did not include antibiotic sensitivity testing (24).

Due to the zero inhibition zone during AST for *S. typhimurium*, many likelihood variables have been considered. The first reason was the permeability in the cell membrane, which was caused by the substitution of amino acids in the outer membrane. This modification results in the permeability of the barrier for antibacterial molecules (37). Moreover, *S. typhimurium* not being able to be inhibited by *M. calabura* leaves extract might be due to the composition of gram-negative bacteria with more complex structures than gram-positive organisms. They are more resistant to extract molecules that are trying to penetrate the membrane which consists of lipopolysaccharide, lipoprotein, peptidoglycan, and porins proteins (38). This also explains the higher concentration of extract needed to inhibit the growth of *S. sonnei* than *S. aureus* in the MBC tests. *Staphylococci* have been reported to be one of the most commonly encountered pathogens in clinical practice. Also, *S. aureus* has been reported to be a major cause of nosocomial infections, food poisoning, and a wide range of other disorders (39). The results obtained from the tests provide evidence that the extract of *M. calabura* leaves is able to inhibit both gram-positive and negative organisms, just with different strength and concentration requirements. Therefore, *M. calabura* is a great alternate source of antibacterial agents that may be used to treat a variety of diseases with some necessary improvements involved.

CONCLUSION

This study discovered that *M. calabura* L. leaves can be utilized as a natural substance that has the potential to be used to cure certain bacterial infections. The availability of phytochemical constituents in the methanol extract of *M. calabura* leaves differed when compared to other extraction solvents. There were also significant variations in the antibacterial effects of *M. calabura* against the tested bacterial species where the extract is only effective against *S. aureus* and *S. sonnei*. However, the antimicrobial tests utilized in this research were only able to provide the most basic information to aid in reducing bacterial infections. Considering the convincing potential of the antibacterial properties of *M. calabura* L. leaves, additional research on this natural component is urgently needed to offer more solid results and evidence.

To improve the quality of results obtained from this study, it is advised that quantitative techniques such as high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) be used in the phytochemical screening of the plant extract as they produce more accurate and precise results. Other

polar solvents, such as ethanol and water, are also recommended for testing. Even if there are other studies done with these solvents, different concentrations and assay methods could be employed to explore the full potential of this plant.

ACKNOWLEDGMENT

The authors thank the Faculty of Health Sciences at the UiTM Selangor for providing the laboratory facilities and financial support.

REFERENCES

- Craig SR, Glover DM. Conservation, cultivation, and commodification of medicinal plants in the greater himalayan-tibetan plateau. *Asian Med.* 2020;5(2):219–42. doi: 10.1163/157342109X568793
- Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, et al. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov.* 2021;20(3):200–16. doi: 10.1038/s41573-020-00114-z
- Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing new antimicrobial therapies: Are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?. *Pharmacognosy reviews.* 2017 Jul;11(22):57. doi: 10.4103/phrev.phrev_21_17
- Michaud CM. Global Burden of Infectious Diseases. *Encycl Microbiol.* 2009;444–54. doi: 10.1016/B978-012373944-5.00185-1
- Mathers CD, Boerma T, Fat DM, Mathers C. Global and regional causes of death. *Br Med Bull.* 2009;92:7–32. doi: 10.1093/bmb/ldp028.
- Holmes KK, Bertozzi S, Bloom BR, Jha P, Gelband H, DeMaria LM, Horton S. Major infectious diseases: key messages from disease control priorities. *Major infectious diseases.* 2017 Nov 3. doi: 10.1596/978-1-4648-0524-0_ch1
- Soares NC, Bou G, Blackburn JM. Proteomics of microbial human pathogens. *Frontiers in Microbiology.* 2016 Nov 4;7:1742. doi: 10.3389/fmicb.2016.01742. eCollection 2016.
- Mahmood ND, Nasir NL, Rofiee MS, Tohid SM, Ching SM, Teh LK, Salleh MZ, Zakaria ZA. *Muntingia calabura*: A review of its traditional uses, chemical properties, and pharmacological observations. *Pharmaceutical Biology.* 2014 Dec 1;52(12):1598–623. doi: 10.3109/13880209.2014.908397.
- Zakaria ZA, Sulaiman MR, Jais AMM, Somchit MN, Jayaraman KV, Balakhrisnan G, et al. The antinociceptive activity of *Muntingia calabura* aqueous extract and the involvement of L-arginine/nitric oxide/cyclic guanosine monophosphate pathway in its observed activity in mice. *Fundam Clin Pharmacol.* 2006;20(4):365–72. doi: 10.1111/j.1472-8206.2006.00412.x.
- Preethi K, Premasudha P, Keerthana K. Anti-inflammatory activity of *Muntingia calabura* fruits. *Pharmacogn.* 2012;4(30):51–6. doi: 10.5530/pj.2012.30.10
- Muhammad Ansori AN, Viol Dhea K, Solikhah TI. Medicinal properties of *Muntingia calabura* L.: A Review. *Res J Pharm Tech.* 2021;14(8):4508–12. doi: 10.52711/0974-360X.2021.00784
- Sarojini S, Mounika B. *Muntingia calabura* (Jamaica cherry): An overview. *PharmaTutor.* 2018;6(11):1–9. doi: 10.29161/PT.v6.i11.2018.1
- Sujono TA, Kusumowati ITD, Munawaroh R. Effects of Jamaican cherry (*Muntingia calabura* L.) fruits extract on immunoglobulin G levels and hematological profiles in mice. *Pharmacogn J.* 2021;13(2):535–41. doi: 10.5530/pj.2021.13.67
- Figueiredo RA de, Oliveira AA de, Zacharias MA, Barbosa SM, Pereira FF, Cazela GN, et al. Reproductive ecology of the exotic tree *Muntingia calabura* L. (Muntingiaceae) in southeastern Brazil. *Rev Árvore.* 2008;32(6):993–9. doi: 10.1590/S0100-67622008000600004
- Zakaria ZA, Mustapha S, Sulaiman MR, Mat Jais AM, Somchit MN, Abdullah FC. The antinociceptive action of aqueous extract from *Muntingia calabura* leaves: The role of opioid receptors. *Med Princ Pract.* 2007;16(2):130–6. doi: 10.1159/000098366
- Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pac J Trop Biomed.* 2013;3(9):705–10. doi: 10.1016/S2221-1691(13)60142-2
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. *Int Pharm Sci.* 2011;1(1):98–106.
- Buhian WPC, Rubio RO, Valle DL, Martin-Puzon JJ. Bioactive metabolite profiles and antimicrobial activity of ethanolic extracts from *Muntingia calabura* L. leaves and stems. *Asian Pac J Trop Biomed.* 2016;6(8):682–5. doi: 10.1016/j.apjtb.2016.06.006
- Mudau FN, Ngezimana W. Effect of different drying methods on chemical composition and antimicrobial activity of bush tea (*Athrixia phyllicoides*). *Int J Agric Biol.* 2014;16(5):1011–4.
- Sulaiman SF, Sajak AAB, Ooi KL, Supriatno, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. *J Food Compos Anal.* 2011;24(4–5):506–15. doi: 10.1016/j.jfca.2011.01.020
- Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Ind Crops Prod.* 2013;44:566–71. doi: 10.1016/j.indcrop.2012.09.021
- Azwanida NN. A Review on the Extraction methods

- use in medicinal plants, principle, strength and limitation. *Med Aromat Plants*. 2015;4(196):2167-0412. doi:10.4172/2167-0412.1000196
23. Surjowardojo P, Thohari IS, Ridhowi A. Quantitative and qualitative phytochemicals analysis of *Muntingia calabura*. *J Biol Agric Healthc*. 2014;4(16):84–9.
 24. Zakaria ZA, Sufian AS, Ramasamy K, Ahmat N, Sulaiman MR, Arifah AK, et al. In vitro antimicrobial activity of *Muntingia calabura* extracts and fractions. *African J Microbiol Res*. 2010;4(4):304–8.
 25. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*. 2009;14(6):2167–80. doi: 10.3390/molecules14062167
 26. Sujarwodojo P, Imam T, Firmansyah TS, Aswah R. Antibacterial activity of *Muntingia calabura* Lam. against some selected bacteria causing mastitis. In 1st International Conference in One Health (ICOH 2017) 2017 Jul (pp. 216-220). Atlantis Press. doi: 10.2991/icoh-17.2018.42
 27. Singh R, Iye S, Prasad S, Deshmukh N, Gupta U, Zanje A, Patil S, Joshi S. Phytochemical analysis of *Muntingia calabura* extracts possessing antimicrobial and anti-fouling activities. *Int. J. Pharmacogn. Phytochem. Res*. 2017;9:826-32. doi: 10.25258/phyto.v9i6.8186
 28. Sibi G, Naveen R, Dhananjaya K, Ravikumar KR, Mallesha H. Potential use of *Muntingia calabura* L. extracts against human and plant pathogens. *Pharmacogn J*. 2012;4(34):44–7. doi: 10.5530/pj.2012.34.8
 29. Puljula E, Walton G, Woodward MJ, Karonen M. Antimicrobial Activities of Ellagitannins against *Clostridiales perfringens*, *Escherichia coli*, *Lactobacillus plantarum* and *Staphylococcus aureus*. *Molecules*. 2020;25(16):3714. doi: 10.3390/molecules25163714
 30. Górniak I, Bartoszewski R, Kryliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*. 2019 Feb;18(1):241-72. 10.1007/s11101-018-9591-z
 31. Dong S, Yang X, Zhao L, Zhang F, Hou Z, Xue P. Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria. *Industrial crops and products*. 2020 Jul 1;149:112350. doi: 10.1016/j.indcrop.2020.112350
 32. Sari SA, Ernita M, Mara MN, AR MR. Identification of Active Compounds on *Muntingia calabura* L. Leaves using Different Polarity Solvents. *Indones J Chem Sci Technol*. 2020;3(1):1–7. doi: 10.24114/ijcst.v3i1.18309
 33. Mudau HS, Mokoboki HK, Ravhuhali KE, Mkhize Z. Effect of soil type: Qualitative and quantitative analysis of phytochemicals in some browse species leaves found in Savannah biome of South Africa. *Molecules*. 2022;27(5). doi: 10.3390/molecules27051462
 34. Kumar S, Yadav A, Yadav M, Yadav JP. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm. f. *BMC research notes*. 2017 Dec;10(1):1-2. doi: 10.1186/s13104-017-2385-3
 35. Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*. 2001;48(1):5–16. doi: 10.1093/jac/dkf083
 36. Fernando O, Mogoll C. In vitro Antibacterial and Antioxidant Activity of *Muntingia calabura* Fruits Extract. *Contemp Eng Sci*. 2018;11(18):881–90. doi: 10.12988/ces.2018.8255
 37. Delcour AH. Outer membrane permeability and antibiotic resistance. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*. 2009 May 1;1794(5):808-16. doi: doi: 10.1016/j.bbapap.2008.11.005
 38. Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. *Cold Spring Harb Perspect Biol*. 2010;2(5). doi: 10.1101/cshperspect.a000414
 39. Rubin RJ, Harrington CA, Poon A, Dietrich K, Greene JA, Moiduddin A. The Economic Impact of *Staphylococcus aureus* Infection in New York City Hospitals. *Emerg Infect Dis*. 1999;5(1):9–17. doi: 10.3201/eid0501.990102.