ORIGINAL ARTICLE

Effects of *Alpinia galanga*, *Hibiscus sabdariffa* and *Moringa oleifera* on the Lifespan of *Caenorhabditis elegans* as an *in Vivo* Model for Anti-aging Properties

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ABSTRACT

Introduction: Many plants in Malaysia purportedly have antioxidant and anti-aging effects. Verifying in vitro anti-oxidant results in vivo would conventionally require use of animal models such as rats. In this study, the nematode *Caenorhabditis elegans* is used as an *in vivo* model as measurement of the changes to its lifespan is an indicator of anti-aging effects. **Methods:** In vitro antioxidant analyses were conducted on *Alpinia galanga*, *Hibiscus sabdariffa* and *Moringa oleifera* extracts, whereby radical scavenging activities was determined through DPPH assay whilst amount through Total Phenolic Content and Total Flavonoid Content quantifications. For the *in vivo* studies, the extracts were administered to *C. elegans* and their lifespans were observed. **Results:** DPPH assay indicated that aqueous extract of *H. sabdariffa* showed the highest radical scavenging activity with IC_{50} of 327.0 ± 0.1 µg/ml, whilst hydroethanolic extract of *H. sabdariffa* showed highest total phenolic content with 12.4 ± 0.9 mg GAE/g equivalent. Total flavonoid content was highest in ethanolic extract of *M. oleifera* with 4.2 ± 0.5 mg QE/g equivalent. The ethanolic extract of *A. galanga* at 1000 µg/ml and 100 µg/ml increased the mean lifespan of *C. elegans* by 22.7 % and 19.3 % respectively, whilst hydroethanolic extract of *H. sabdariffa* also increased it by 19.9% at 1000 µg/ml. **Conclusions:** In vitro assays need to be complemented by an *in vivo* model as a combination of several mechanisms accounts for the effects of extracts in ameliorating damages caused by aging, hence *C. elegans* can be a viable surrogate to higher animals.

Keywords: Lifespan; *C. elegans*; Natural products; *Hibiscus sabdariffa*; *Alpinia galanga*

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INTRODUCTION

Malaysia has a growing aging population with the percentage aged 65 and over increasing from 7.0% in 2021 to 7.3% in 2022, and the 60 years and over group is predicted to rise by 2030 to 15.3% (1). Geriatric conditions range from acuity impairment to serious health problems such as osteoarthritis, dementia, diabetes and cardiovascular diseases (2). As a consequence, many products can be found in the market that have purported anti-aging properties, targeting consumers who desire a more healthy aging process and who wish to delay the onset of debilitating conditions for as long as possible. The main constituents of many of these products are obtained from sources such as plants and marine organisms, as natural products are perceived by consumers as being safer than chemically synthesized drugs.

Natural products contain phenolic compounds which have been touted to have the potential to reduce aging-related diseases including neurodegenerative disorders, metabolic syndrome and cancer (3,4). Flavonoids, a major subgroup of the phenolic compounds, have also been reported to alleviate symptoms of diseases associated with increased incidence in old age such as Alzheimer's disease (5) and complications from accumulation of advanced glycation end products (6). The mechanism by which the compounds are postulated to exert their effects is through modulation of signalling pathways involved in inflammation, oxidative damage, autophagy and apoptosis (7-9). As the effects of the compounds are attributed to their antioxidant properties, methods such as free radical scavenging activities against 2,2-diphenyl-1-picrylhydrazyl, total phenolic content and total flavonoid content analyses are utilised as the in vitro means to indicate the potential of the natural products to be used as an anti-aging supplement (10,11). Although there is an array of techniques that can determine the anti-oxidative properties of natural products, there is a lack of methods that can support the data obtained before proceeding to in vivo studies in higher animals. Thus, there is a gap in the information between those gathered through chemical analyses and biological assays, and those obtained from animal studies.

Caenorhabditis elegans is a nematode that is widely used as model organisms in many biological investigations, partly due to it being the first genome from a multicellular organism to be sequenced, with homologs of about two-thirds of the genes responsible for human diseases being found to be present (12). *C. elegans* is the most broadly used and studied model organism in aging research. Its capacity to establish genetically identical populations quickly through clonal self-reproduction and its short lifespan allow rapid measurement of changes in longevity (13). Moreover, it shares important aging related pathways with mammals thus allowing a more complex picture of biological processes to be built compared to other animal models (14). Lifespan is the most common measurement of aging process; besides being a straightforward measurement which can be accomplished by recording alive versus dead individuals, it also measures overall function of the life support system (15).

The current study was conducted to determine the effects of various extracts of plants commonly found in Malaysia, namely *Alpinia galanga*, *Hibiscus sabdariffa* and *Moringa oleifera* on the lifespan of *C. elegans*. *A. galanga*, also named Java galangal, Siamese ginger or greater galangal, is one of the member of Zingiberaceae family commonly found in Indonesia, China, Saudi Arabia, Malaysia, Egypt and Sri Lanka (16). Traditionally, *A. galanga* is used to treat eczema, bronchitis, coryza, morbili, pityriasis versicolor, otitis interna, gastritis, ulcers and cholera. Although its rhizomes are more frequently used especially in cooking, the leaves are gaining attention for health maintenance purposes. It was reported that the leaves, due to greater exposure to sunlight, have higher amounts of flavones and flavonols compared to the rhizome (17).

H. sabdariffa, also known as roselle, karkadeh, asam susur or Ribena Malaysia, is believed to have originated from West Africa and is now broadly cultivated within the tropical and sub-tropical areas in Asia, The Americas and Australia (18). Although its leaf, seed, fruit and root can be used, it is the calyx that is commonly turned into food products. The presence of phenolic compounds and anthocyanins which give its products their distinctive red colour, is thought to contribute to prevention of diseases such as cardiovascular and liver diseases (19, 20).

M. oleifera, also called murungai, drumstick tree, 'horse radish tree' or kelor, originated from India but can now be widely found in regions of Africa, Arabian Peninsula, South East Asia, the Pacific, Caribbean Islands and South America. In addition to being used traditionally in treating headaches, haemorrhoids, fevers, nose and throat inflammations, bronchitis, eye and ear infections, *M. oleifera* was also reported to have antihypertensive, diuretic, antihepatotoxic, antiurolithiatic, anti-atherosclerotic, anti-diabetic and analgesic activities. Its leaves were reported to contain glucosinolate glycosides, crypto-chlorogenic acid, isoquercetin, astragalin, β-sitosterol, quercetin and kaempferol (21, 22).

In this study, phenolics and flavonoids quantification and free radical scavenging were determined in order to assess the antioxidant capacity of the extracts. The association of this antioxidant property with the lifespan of the nematodes was elucidated.

MATERIALS AND METHODS

Preparation of extracts

All plant materials were obtained from a local herbal supplier. Ethanol 95% and distilled water were used for ethanolic and aqueous extractions respectively. For *A. galanga*, grinded leaves weighing 400 g was immersed in 1000 ml of solvent, either ethanol 95% or distilled water, and placed in a water bath for 24 hours at 45°C with regular shaking. After filtration, the remaining solid material was again extracted in a fresh 1000 ml of the solvent using the same conditions for two more times. Hence, the duration of the entire extraction process was 72 hours. The grinded *M. oleifera* leaves were extracted in the same manner. For *H. sabdariffa*, the grinded calyces were extracted with the solvents, which were 1:1 ethanol 95% to distilled water for the hydroethanolic extract, and distilled water for the aqueous extract. Calyces weighing 100g was macerated in 1000 ml of solvent and sonicated for 1 hour at room temperature. It was then left for a further 24 hours at room temperature before being filtered through Whatman no. 1 filter paper. The aqueous or hydroethanolic extracts were spray dried (Buchi Labortechnik AG, Switzerland) with the following settings; inlet temperature of 100°C, pump percentage of 5%, Q-flow of 50 and yield aspirator of 100. On the other hand, ethanolic extracts were rotary evaporated at 40°C to 45°C under reduced pressure. The extracts obtained were *A. galanga* aqueous extract (AG Aq), *A. galanga* ethanolic extract (AG Et), *H. sabdariffa* aqueous extract (HS Aq), *H. sabdariffa* 1:1 hydroethanolic extract (HS Et), *M. oleifera* aqueous extract (MO Aq) and *M. oleifera* ethanolic extract (MO Et).

DPPH radical scavenging activity

The antioxidant capacity of the extracts was determined through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities, using a previously reported method with modifications (23). Briefly, DPPH powder weighing 0.008 g was dissolved in 100 ml ethanol. A stock solution 200 µM was acquired and diluted for experimental use to 50 µM. Stock solution of each plant extract was prepared at 10mg/ml while ascorbic acid was prepared at 1mg/ml. The extracts were diluted in series to concentrations of 2000µg/ml, 1000µg/ml, 500µg/ml, 250µg/ml and 125µg/ml respectively. Ascorbic acid was prepared with concentrations ranging from 6.25µg/ml to 200µg/ml. Later, 50 µl of the sample was added to 950 µl of DPPH in ethanol solution,

vortexed for 10 seconds and incubated in the oven for 30 minutes at 37°C before being measured at 517 nm using a UV-Vis spectrophotometer (Multiskan Spectrum, Thermo Scientific, Finland). The triplicate experiments were conducted in the dark because DPPH is light susceptible. The percentage inhibition of radicals was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \qquad \text{Equation 1}$$

where Acontrol is the absorbance of DPPH solution without extract and Asample is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant from plant extract required to decrease the initial DPPH concentration by 50%.

Total Phenolic Content (TPC)

Total phenolic content (TPC) of the extracts was determined using Folin-Ciocalteu method with slight alteration (24). Briefly, 400 µl extract was added to 2 ml Folin-Ciocalteu reagent, vortexed and left for 5 minutes at room temperature. Then 1.6 ml of 7.5% of $NaCO_3$ was added to the mixture, vortexed and allowed to stand at room temperature for 1 hour in the dark before reading the absorbance at 765nm using UV-Vis spectrophotometer. The concentration of extracts were calculated from the standard calibration curve of gallic acid and the total phenolic content was expressed as mg of gallic acid equivalent per gram of extract. All samples were analysed in triplicate.

Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) of the extracts was quantified using the colorimetric assay with some modifications, including using quercetin as standard (25). Briefly, 150 µl of extract previously dissolved in the extracting solvent was mixed with 150 µl of 2% (w/v) $AlCl_3$ in 96-well plates. The absorbance was measured using UV-Vis spectrophotometer at 435 nm after 15 minutes of incubation at room temperature. The concentration of extracts was calculated from the standard calibration curve of quercetin and the total flavonoid content was expressed as mg of quercetin equivalent per gram of extract. All samples were analysed in triplicate.

***Caenorhabditis elegans* lifespan study**

Nematode Growth Medium (NGM) agar plates were prepared by mixing 3 g NaCl, 17 g microbiology grade agar, 2.5 g peptone (Merck, Darmstadt, Germany) and 975 ml distilled water, before autoclaving at 121°C for 20 minutes (Hirayama, Japan). The agar was cooled down to 55°C before the addition of 1 ml of 1M $CaCl_2$, 1 ml of 5 mg/ml cholesterol (BD-Difco, MD, USA) in ethanol, 1 ml of 1M $MgSO_4$ and 25 ml of 1 M potassium phosphate buffer aseptically. Fluorodeoxyuridine (FUdR) (Thermo Fisher, Utah, USA) with concentration of 50 µM was added to the NGM agar to prevent progeny development. For the test plates, extracts dissolved in ethanol were used as stock solutions and mixed with the NGM agar to obtain the final concentrations of 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml. The agar was poured into 35 mm petri plates, left to dry in the laminar flow hood (IsocideTM, ESCO, Singapore) before storing in an air-tight container for two days at room temperature. Non-pathogenic E.coli strain OP50 (Caenorhabditis Genetics Centre, University of Minnesota, MN, USA) was used as the food source for the nematodes and 100 µl of OP50 was seeded onto each NGM plate. The seeded plates were left in the laminar flow hood for 1 hour to dry the OP50 suspension (26).

Caenorhabditis elegans (*C. elegans*) wild-type Bristol N2 was obtained from Caenorhabditis Genetics Centre (University of Minnesota, MN, USA). The nematodes, previously maintained on blank NGM agar plates at 20 °C in a refrigerated incubator (Binder, USA) and synchronized to stage L3/L4, were transferred to the test plates containing the extracts. The nematodes were then observed every day under a stereomicroscope (Nikon, Japan) and were considered dead if the nematodes show loss of spontaneous movement or did not respond to the touch of the nematode picker made from a 2.5 cm 32 gauge platinum wire (Bio-Rad Laboratory, Hercules, USA) mounted into the tip of a glass pasteur pipette. The survival of the nematodes was scored through elimination of the dead ones. The whole process was repeated for all concentrations of the extracts and the experiments were run in triplicate. The positive control was mixed tocotrienols (Toco) containing α-, γ- and δ-tocotrienols at 12.3%, 20.6% and 5.3% respectively, (Carotech Bhd, Ipoh, Perak) and the negative controls were blank NGM and ethanol 1%.

Statistical Analysis

Data were analyzed by using one-way ANOVA and when the results showed statistically significant difference ($p<0.05$), Tukey's Honestly Significant Difference (HSD) test was used for pairwise comparison of means at $p< 0.05$. The lifespan of *C. elegans* as number of days was plotted and presented as Kaplan-Meier curve by using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA) and Log-Rank test was conducted. Mean lifespan of *C. elegans* was presented as mean ± standard deviation (SD) of triplicate.

RESULTS

DPPH radical scavenging activity

The aqueous extract of *H. sabdariffa* showed the highest radical scavenging activity amongst the plant extracts, with IC_{50} of 327.0 ± 0.1 µg/ml while aqueous extract of *M. oleifera* showed the lowest with IC_{50} of 1816.8 ± 0.2 µg/ml (Table I). The ranking order from highest to lowest activity for the extracts was aqueous extract of

Table I : DPPH assay IC_{50} values of extracts and ascorbic acid

Test compounds	IC_{50} values, µg/ml
Ascorbic acid	43.0 ± 0.2
A. galanga (Ethanolic extract)	531.5 ± 0.1*
A. galanga (Aqueous extract)	1473.8 ± 0.1*
H. sabdariffa (Aqueous extract)	327.0 ± 0.1*
H. sabdariffa (Hydroethanolic extract)	514.5 ± 0.1*
M. oleifera (Ethanolic extract)	1591.7 ± 0.1*
M. oleifera (Aqueous extract)	1816.8 ± 0.2*

(Mean ± SD, n=3, *p < 0.05 to ascorbic acid positive control)

H. sabdariffa > hydroethanolic extract of *H. sabdariffa* > ethanolic extract of *A. galanga* > aqueous extract of *A. galanga* > ethanolic extract of *M. oleifera* > aqueous extract of *M. oleifera*.

Total Phenolic Content (TPC)

The plant extract with highest total phenolic content was hydroethanolic extract of *H. sabdariffa* with 12.4 ± 0.9 mg GAE/g, and the lowest was aqueous extract of *A. galanga* with 4.0 ± 0.2 mg GAE/g (Table II). The ranking order from highest to lowest of total phenolic content values for all the plant extracts was hydroethanolic extract of *H. sabdariffa* > aqueous extract of *H. sabdariffa* > ethanolic extract of *A. galanga* > aqueous extract of *M. oleifera* > ethanolic extract of *M. oleifera* > aqueous extract of *A. galanga*.

Total Flavonoid Content

The plant extract with highest total flavonoid content was ethanolic extract of *M. oleifera* with 4.2 ± 0.5 mg QE/g and the lowest was aqueous extract of *H. sabdariffa* with 0.4 ± 0.1 mg QE/g. The ranking order from highest to lowest of total flavonoid content values for all the plant extracts was ethanolic extract of *M. oleifera* > hydroethanolic extract of *H. sabdariffa* > ethanolic extract of *A. galanga* > aqueous extract of *A. galanga* > aqueous extract of *M. oleifera* > aqueous extract of *H. sabdariffa*.

Effects of plant extracts on the lifespan of *C. elegans*

The mean lifespan of the blank NGM and ethanol 1% treated *C. elegans* as negative controls in this study was 17.6 ± 0.2 and 17.5 ± 0.4 days respectively (Table III). Ethanol 1% was also tested as a control in this study as researchers previously postulated that ethanol at low concentrations may enhance the nematodes mean lifespan (27). From the results obtained in this study, the difference between ethanol and blank control was just 0.6%, therefore ethanol in the concentrations used in the extracts for the procedures would not significantly affect the lifespan of the nematodes. For the tocotrienols treated nematodes, the mean lifespan ranged from 19.6 ± 0.7 to 21.6 ± 0.4 days depending on the concentration. Mixed tocotrienols was used as the positive control as its antioxidant properties and ability to ameliorate inflammatory conditions that could be related to the aged status, have been established pre-clinically and clinically (28, 29).

The ethanolic extract of *A. galanga* at the highest concentration tested of 1000 µg/ml increased the mean lifespan of *C. elegans* by 22.7% compared to blank NGM and by 23.4% compared to ethanol 1%. Hydroethanolic extract of *H. sabdariffa* also increased the mean lifespan compared to the blank NGM nematodes by 19.9% and by 20.6% compared to ethanol 1%. The lifespan of the *C. elegans* treated with the ethanolic extract of *A. galanga* was 21.6 ± 0.1 days and treated with hydroethanolic extract of *H. sabdariffa* was 21.1 ± 0.3 days, in comparison to the positive control treated with tocotrienols which was 21.6 ± 0.4 days. On the other hand, the aqueous extracts of both *A. galanga* and *H. sabdariffa* did not prolong the lifespan of the nematodes, nor did the ethanolic and aqueous extracts of *M. oleifera* (Fig. 1).

At the concentration of 100 µg/ml, the ethanolic extract of *A. galanga* increased the lifespan by 19.9 % and 20.0 % compared to blank NGM and ethanol 1% respectively, whilst the other extracts did not elicit a similar effect in the nematodes (Fig. 2). At the lower concentrations of 10 µg/ml and 1 µg/ml, the extracts did not increase the lifespan of the *C. elegans* significantly (Fig. 3-4).

DISCUSSION

Ethanolic extract of *A. galanga*, hydroethanolic extract of *H. sabdariffa* and aqueous extract of *M. oleifera* increased the lifespan of *C. elegans* in a dose-dependent

Table II : Total phenolic content (TPC) and total flavonoid content (TFC) of extracts

Test compounds	TPC, mg GAE/g	TFC, mg QE/g
A. galanga (Aqueous extract)	4.0 ± 0.2	1.4 ± 0.1
A. galanga (Ethanolic extract)	5.0 ± 0.9	1.6 ± 0.4
H. sabdariffa (Aqueous extract)	5.7 ± 0.6	0.4 ± 0.1
H. sabdariffa (Hydroethanolic extract)	12.4 ± 0.9	3.5 ± 0.6
M. oleifera (Aqueous extract)	4.8 ± 0.8	1.0 ± 0.2
M. oleifera (Ethanolic extract)	4.8 ± 0.5	4.2 ± 0.5

(Mean ± SD, n=3)

Table III : Mean lifespan of *C. elegans*

Test compounds	Days
Blank NGM	17.6 ± 0.2
Ethanol 1%	17.5 ± 0.4
1000 µg/ml	
A. galanga (Aqueous extract)	17.9 ± 0.4
A. galanga (Ethanolic extract)	21.6 ± 0.[1*]#
H. sabdariffa (Aqueous extract)	17.0 ± 1.1
H. sabdariffa (Hydroethanolic extract)	21.1 ± 0.3[*#]
M. oleifera (Aqueous extract)	18.2 ± 0.6
M. oleifera (Ethanolic extract)	17.8 ± 0.9
Tocotrienols	21.6 ± 0.4[*#]
100 µg/ml	
A. galanga (Aqueous extract)	17.8 ± 1.7
A. galanga (Ethanolic extract)	21.0 ± 0.5[*#]
H. sabdariffa (Aqueous extract)	16.7 ± 2.5
H. sabdariffa (Hydroethanolic extract)	18.8 ± 0.9
M. oleifera (Aqueous extract)	17.3 ± 0.3
M. oleifera (Ethanolic extract)	17.5 ± 1.2
Tocotrienols	21.1 ± 0.5[*#]
10 µg/ml	
A. galanga (Aqueous extract)	18.1 ± 1.8
A. galanga (Ethanolic extract)	20.8 ± 0.1
H. sabdariffa (Aqueous extract)	17.8 ± 0.6
H. sabdariffa (Hydroethanolic extract)	17.5 ± 2.4
M. oleifera (Aqueous extract)	16.6 ± 0.5
M. oleifera (Ethanolic extract)	16.9 ± 1.0
Tocotrienols	21.1 ± 0.6[*#]
1 µg/ml	
A. galanga (Aqueous extract)	17.0 ± 1.4
A. galanga (Ethanolic extract)	17.1 ± 2.9
H. sabdariffa (Aqueous extract)	16.6 ± 0.2
H. sabdariffa (Hydroethanolic extract)	17.4 ± 0.7
M. oleifera (Aqueous extract)	16.1 ± 1.5
M. oleifera (Ethanolic extract)	17.3 ± 0.7
Tocotrienols	19.6 ± 0.7

(Mean ± SD, N=3, p< 0.05 compared to blank NGM (*) and ethanol 1% controls(#))

manner, with the *A. galanga* and *H. sabdariffa* extracts significantly increasing the lifespan by 19-23% at the dose of 1000 µg/ml. This study has shown that higher concentrations of extract are necessary to elicit a response in the nematodes due to the impermeable cuticle of *C. elegans*. The main route of extract uptake would be through the concomitant ingestion of the extract-loaded NGM agar during the nematode feeding on OP50 bacterial lawn. A study with ethosuximide demonstrated that the *C. elegans* treated externally with 2 mg/ml of the pharmaceutical drug have a much lower internal concentration of 30 µg/ml (30).

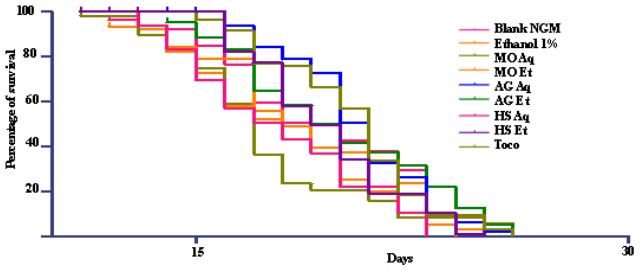

Fig. 1 : Lifespan of *C. elegans* when administered with different plant extracts at 1000 µg/ml (Mean ± SD, N=3.

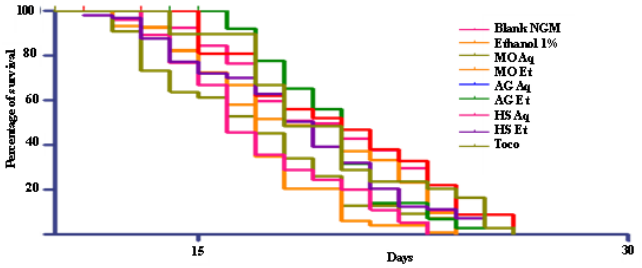

Fig. 2 : Lifespan of *C. elegans* when administered with different plant extracts at 100 µg/ml (Mean ± SD, N=3).

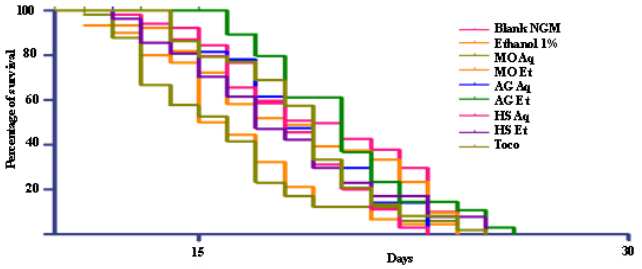

Fig. 3 : Lifespan of *C. elegans* when administered with different plant extracts at 10 µg/ml (Mean ± SD, N=3).

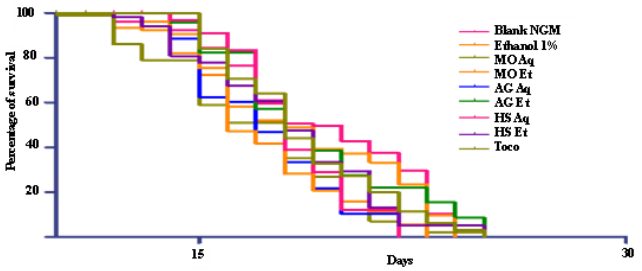

Fig. 4 : Lifespan of *C. elegans* when administered with different plant extracts at 1 µg/ml (Mean ± SD, N=3).

The magnitude of lifespan extension seen in the current study is consistent with some studies testing natural products using wild-type *C. elegans*. It was previously reported that the median lifespan of *C. elegans* for the

control group was 16.8 to 21.8 days and the percentage increment upon administration of Myrtus communis L, Rubus sanctus Schreb, Hedera helix and Salvia verticillata extracts were 10-25% at concentration range of 125 µg/ml to 1000 µg/ml (31), whilst others reported increments of 20% to 22% for other plant derived active ingredients (32, 33). On the other hand, 100 µg/ml of standardized extract of Gingko biloba leaves extended the median lifespan and maximum lifespan by only one day (less than 10%) of wild-type nematodes (34). Other studies that focussed on studying the effects of extracts on the longevity of *C. elegans* which have been prior subjected to oxidative or thermal stress reported increased survival of 10% to 80% compared to nematodes which have not been pre-treated with the extracts (33, 35).

One of the proposed mechanisms of anti-aging in *C. elegans* is direct antioxidant activity in which the capability to induce activity of *C. elegans* own defense mechanisms against oxidative stress was increased (36). However, the current study has shown that strong antioxidant activities is not necessarily predictive of lifespan extension. Aqueous extract of *H. sabdariffa* which had the highest antioxidant activity was not able to increase the mean lifespan of *C. elegans*, but extracts with lower antioxidant activity as indicated by the higher IC50 value in DPPH assay such as hydroethanolic extract of *H. sabdariffa* and ethanolic extract of *A. galanga* were able to extend the lifespan.

Plant extracts contain a mixture of compounds and it is likely that anti-aging effects are triggered by multiple compounds rather than a single compound. Hence it can be suggested that the observed anti-aging outcome is due to additive and synergistic effects of many bioactive compounds found in those plant extracts. Determining the mechanism of action of plant extracts that contain a mixture of compounds require systematic molecular biology approach in combination with phytochemistry studies, since some compounds may cause responses that are unrelated to delayed aging. More recently, the extract of the endophytic fungus Paecilomyces variotii was reported to increase the lifespan of *C. elegans* through promoting the nuclear localization of the transcription factor SKN-1, with the net result of enhanced oxidative stress resistance (37). Another study also showed that SKN-1 activity was promoted which attenuated oxidative stress, this time in hyperglycaemic *C. elegans*, by the flavonoids of Apios americana Medik flowers (38). Other researchers proposed modulation of oxidative stress via the DAF-16/FoxO and SKN-1/Nrf-2 signaling pathways (35), and together with the HSF-1 pathway (39) led to lifespan extension in *C. elegans* by plant extracts.

The current study has reinforced the postulation that plant adaptogens can be identified through *C. elegans*. Adaptogens is a term coined to describe synthetic compounds or plant extracts that have the ability to enhance the body's stability against physical loads without increasing oxygen consumption (40). In particular, the adaptogenic properties of plant extracts are attributed to not only a single molecule, but to the combination of different constituents (41). Administration of anthocyanins, proanthocyanidins and chlorogenic acid obtained from the fractionation of blueberry juice failed to protect *C. elegans* against acute oxidative stress, as opposed to when whole blueberry extract was given (42).

CONCLUSION

In this study, three plants which can be found in Malaysia namely *A. galanga*, *H. sabdariffa* and *M. oleifera* were investigated to determine their effects on *C. elegans* longevity. Ethanolic extract of *A. galanga* at concentrations of 100 to 1000 µg/ml and hydroethanolic extract of *H. sabdariffa* at 1000 µg/ml extended the lifespan of the nematodes significantly, which suggest that high concentrations of extracts are needed to induce the chain of events that lead to anti-aging effects in whole organisms. It is shown here that in vitro antioxidative indicators such as quantification of antioxidative compounds and free radical quenching activity are inadequate measurements of anti-aging capability of natural products. *C. elegans* is a feasible surrogate *in vivo* technique that can provide additional information before proceeding to the more costly and protracted studies in higher animals such as mice and rats, and may be useful in the selection process of a plant candidate for development into an anti-aging nutraceutical or regulated herbal product.

ACKNOWLEDGEMENT

This work was supported by a Short-Term Research Project Grant 304/PFARMASI/6313131 from Universiti Sains Malaysia.

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