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Antioxidant and Lifespan-Extending Effects of a Rejuvenating Thai Traditional Polyherbal Remedy (Phy-Blica-O) in *Caenorhabditis elegans*

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ABSTRACT

Despite the long historical use of polyherbal combinations in traditional medicine, these remedies have only recently gained attention. The purpose of the present study was to explore the antioxidant capacity of traditional tonifying remedies and to examine their effect on the longevity and oxidative stress resistance of *Caenorhabditis elegans*. Twenty herbal mixture extracts were made as either infusions or decoctions and tested by six different free-radical scavenging techniques. The selected formula, Phy-Blica-O (THP-R016), was used to examine the longevity of *C. elegans*. The antioxidant-related chemical markers were qualified by HPLC-ESI-MS. Among the tested extracts, Phy-Blica-O possessed remarkable free-radical scavenging activity, with IC_{50} values of 0.13, 0.2, and 0.07 mg/mL against 1,1-diphenyl-2-picrylhydrazyl free radical, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical, and the superoxide anion, respectively, and it had a peroxyl scavenging effect, with an ORAC value of 35.41 µM TE per gram of extract. Phy-Blica-O had the highest phenolic and flavonoid contents of 651.2 GAE mg/g and 79.4 CAE mg/g, respectively. Phy-Blica-O significantly extended the lifespan of *C. elegans* under normal growth conditions. It could be attributed to a minor increase in catalase and superoxide dismutase activities, as well as a decrease in lipofuscin and protein carbonyl levels in *C. elegans*. These results demonstrated the promising antioxidant capacities found in Phy-Blica-O that are partly involved in the longevity of *C. elegans*. Further analysis of Phy-Blica-O is required to gain insights into the lifespan-extending agents, thereby developing novel, traditional phytochemical interventions for age-related symptoms.

Keywords: *Caenorhabditis elegans*, Antioxidant activity, Lifespan extending effects, Traditional tonifying remedy.

Introduction

Facing rapid growth in the aging global population, chronic age-related diseases such as dementia, cancer, cardiovascular disease, and diabetes are becoming some of the most significant threats to society, creating huge medical and socioeconomic burdens.[1,2] For example, in the United States, national spending on cancer is projected to be almost $160 billion in 2020,[2] whereas the total national costs of health care for dementia, including Alzheimer's disease, are estimated at $305 billion for 2020. The prevalence of Alzheimer's disease is expected to increase to 13 million by 2050.[3] Aging is an inevitable progressive decline in tissue integrity and body functions with age. This description is based on several published theories, including the free radical theory of aging.[4,5] Despite the well-accepted notion that oxidative damage increases with age, a reduction in oxidative damage has been proven to extend the lifespan of various model organisms.[6]

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Thus, the discovery of novel compounds or products that reduce reactive oxygen species (ROS)-mediated oxidative damage could modulate the deterioration of tissue function with age. It could lead to new strategies to prevent or delay the onset of chronic age-related diseases.

Studies have revealed that 10–80% of the world population, including developing and industrialized countries, have used herbal medicines to treat diseases.[7,8] Furthermore, it is well-established that medicinal plants contain efficient antioxidant defense systems, in particular, their secondary metabolites such as tannins, phenolic acids, ascorbic acid, carotenoids, flavonoids, etc.[9] Previous studies have proven that plant-derived antioxidants, particularly resveratrol,[10] curcumin,[11] quercetin,[12] and epigallocatechin-3-gallate,[13] exhibit longevity-extending abilities and diminish ROS-mediated oxidative damages, thereby providing beneficial effects for the treatment of age-related diseases.

Polyherbal medicines (or herbal mixtures) are the backbone of traditional medical practices worldwide, and they have been used not only for the treatment of diseases but also for promoting longevity. Intensive studies have been performed on polyherbal formulations use in traditional Chinese medicine, such as Gengnianchun,[14] Jianpi-Yangwei,[15] Liangyi Gao,[16] and Liuwei Dihuang.[17] These polyherbal medicines have been used clinically to nourish the liver, kidney, spleen, stomach, etc., or treat age-related symptoms such as faintness, poor memory, dizziness, etc. They possess remarkable free radical scavenging effects and reduce oxidative stress-mediated symptoms. Furthermore, the remedies were found to extend the lifespan and delay aging-related declines in *Caenorhabditis elegans*.

Despite five tonifying agents found in The National List of Essential Medicines in Thai traditional medicine,[18] no scientific data have been reported on polyherbal tonifying formulations. These remedies, namely, Tri-Kesorn-Mas, Tri-Pi-Kad, Benjakul, Pluk-Fai-That, and Bumrung-Lohit, have been prescribed as blood tonics and used for the treatment of mental and physical fatigue. However, studies into the mechanisms of actions of these polyherbal medicines are difficult due to the composition complexity. Furthermore, it should be noted that several studies have recorded the utilization of medicinal plants and polyherbal medicines by folkloric practitioners, [19,20] but their lifespan-extending effects have never been explored.

Therefore, in the present study, we aimed to select the effective polyherbal mixture from 20 tonifying formulas prescribed and recorded by folkloric practitioners in Phatthalung and Songkhla provinces, Thailand, [21] using various *in vitro* antioxidant techniques. The biological markers responsible for the antioxidant abilities of the effective formula were proposed. Subsequently, the lifespan-extending and protective effects toward oxidative stress were evaluated using *C. elegans* models.

Materials and Methods

Plant materials and preparation of tested extracts

A total of 20 herbal formulations prescribed to promote health and longevity by traditional folk healers in Songkhla and Phatthalung provinces were used.[21] Medicinal plant samples used in this present work were authenticated by a specialized botanist, Assistant Professor Dr. Katesarin Maneenoon. Their taxonomic identities were confirmed, and the voucher specimens were deposited in the herbarium of Materia Medica within the Faculty of Traditional Thai Medicine, Prince of Songkla University, Thailand, as detailed in Table 1.

The oven-dried and finely powdered plant parts (passing through an 80-mesh screen) were weighed separately and then combined in the proper proportions, as previously described. In a similar manner to their traditional utilization, the samples were prepared by water decoction, which was done according to a previous study,[22] or by maceration with ethanol, as described below. Each mixed formulation was macerated for 7 days in 70% ethanol at a ratio of 1:10 (w/v) at 30°C and shaken vigorously at 100 rpm. The resulting solution was filtered using Whatman number one filter paper, and the filtrate was concentrated using a rotary evaporator at 55°C. These dried and concentrated polyherbal extracts were stored at –20°C and dissolved in ethanol to obtain a concentration of 25 mg/mL, unless otherwise stated, before use. The extraction yield of each formulation was calculated against the initial dry weight of the powdered formulation.

Antioxidant-related active constituents

Total phenolic content (TPC)

The Folin-Ciocalteu (FC) method was applied, with limited modifications, to quantify the total phenolic content (TPC) of the polyherbal extracts.[21] In brief, 120 µL (2.5 mg/mL) of each extract was added with 1000 µL of a 10-fold serial dilution of the FC reagent for 5 min Sodium carbonate solution (Ajax Finechem, New Zealand) (1000 µL; 20 % w/v) was then added to this mixture. The solution was thoroughly mixed and was kept in the dark at 25°C for 1.5 hours. The absorbance was measured at 725 nm (Sunrise™ Microplate reader, Tecan Group Ltd., Switzerland). The TPC is given as the milligrams of gallic acid (Sigma-Aldrich Chemie, Germany) per gram of extracts.

Total flavonoid content (TFC)

The aluminum chloride colorimetric procedure was applied to quantify the total flavonoid content (TFC) of each extract, as illustrated by Fattahi and his team,[23] with slight modification. An aliquot of 50 µL of each extract (2.5 mg/mL) was mixed with 300 µL of 5% (w/v) sodium nitrite (Ajax Finechem, New Zealand) and 300 µL of 10% (w/v) aluminum trichloride (Ajax Finechem, New Zealand), followed by the addition of 4 mL of distilled water. The mixture was incubated at 25°C for 6 min. Then, the sodium hydroxide solution (1 M; 2 mL) was added to halt the reaction. The resultant volume was increased to 10 mL by adding distilled water, and the absorbance at 510 nm was read after 10 min. The TFC was calculated from the line graph by using the standard catechin solution, with the result given in mg of catechin equivalent per gram of extract.

Qualitative analysis of antioxidant-related constituents

The effective extract was subjected to an Agilent 1290 Infinity ultra-high-performance liquid chromatography system equipped with a diode array detector (DAD) and electrospray ionization (ESI) mass spectrometry according to a previously published method.[22] The software used for processing acquired data was Agilent Mass Hunter Workstation software (Version B.04.00), Agilent MSC software (Version B.07.00), and the online METLIN database using an accuracy error threshold of ≤5 ppm, as previously mentioned.[24]

In vitro antioxidant capacities

Metal-chelating activity (MCA)

Tel-Çayan and his colleagues[25] detailed the techniques used to measure the chelation ability of ferrous ions by the herbal extracts. Aliquots (250 µL) of the serially diluted extract (0.03–62.50 mg/mL) were mixed with 25 µL iron (II) chloride (2 mM) and 80 µL distilled water. The reaction was started up by adding 50 µL of ferrozine (5 mM). The mixture was incubated at 25°C for 10 min, and the absorbance was measured at 562 nm. Ethylenediaminetetraacetic acid (EDTA) was used as a standard metal chelator. The chelating ability percentage was given by the following:

$$MCA\ (\%) = [(OD_{control} – OD_{sample})/OD_{control}] \times 100$$

This activity was expressed in terms of the 50% inhibition concentration of the ferrous ion ferrozine complex (IC_{50}; mg/mL) for both the standard EDTA and the extracts.

DPPH and ABTS radical scavenging assays

The free radical scavenging activity via a mixed-mode mechanism of the extracts was evaluated according to previous reports on DPPH and ABTS radicals.[21] The free radical scavenging capabilities for both the positive control Trolox and the extracts were presented as the 50% inhibitory concentration of the radicals (IC_{50}; mg/mL). For the DPPH assay, 2-fold dilutions of each sample were made (2500–1.22 µg/mL), and 20 µL of each dilution concentration was added to a 96-well plate containing 180 µL of DPPH solution (80 µM). The plate was shaken for 5 min and kept at 25°C in the dark for 30 min. The decolorization of the DPPH solution was determined by measuring the absorbance at 492 nm.

Similarly, for the ABTS assay, an aliquot of freshly prepared $ABTS^{+}$ solution that was diluted to obtain an absorbance of 0.70±0.05 at 734 nm (200 µL) was mixed with 20 µL of the serially diluted extracts (two-fold: 2500–1.22 µg/mL;) in a 96-well microplate. The plate was immediately incubated at 25°C for 6 min, and the absorbance was recorded at 734 nm.

Ferric-reducing antioxidant power (FRAP) assay

The ability of the extracts to reduce the ferric ion present in the ferric-tripyridyl triazine (TPTZ) complex to ferrous-TPTZ has been described.[21] The 2-fold serially diluted extract (150 µL) was mixed with 1.35 mL of freshly made FRAP reagent. Then, the absorbance of the reaction was measured at 596 nm after incubating at 37°C for 2 hours. The absorbance values were plotted against the ferrous concentrations in the ethanol solution. The ability of the extract to reduce the ferric ion is given as mM of ferrous per mg of extract (µM Fe_2SO_4/mg extract), using a working solution of Fe_2SO_4 as a standard curve.

Superoxide anion radical scavenging assay

The superoxide ion is generated via a combined system of riboflavin, methionine, and illumination, which is evaluated through the formation of purple-colored formazan (NBT^{2+}).[21] A reaction mixture (400 µL) that contained riboflavin (30 µg/mL), methionine (30 µg/mL), and EDTA (20 µg/mL) was mixed with 100 µL of NBT (400 µg/mL), followed by the addition of either a newly prepared 2-fold serial dilution extract or the positive control, catechin, in 0.05 M phosphate buffer at pH 7.4. The light induction was done using fluorescent lamps (20 W) at 25°C for 25 min, and the amount of produced formazan was recorded at 560 nm. The ability of the extracts to scavenge the superoxide ions is presented as the concentration of extract that could inhibit 50% of the superoxide radical (IC_{50}; mg/mL).

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Peroxyl radical scavenging assay

This analysis was carried out with slight modification, as described by Çelik and his colleagues,[26] using a black-walled, clear-bottom, 96-well microplate with Trolox as a positive control. Various concentrations (100–0.2 µg/mL; 25 µL) of the selected extract and the control were mixed with fluorescein solution (0.4 nM; 150 µL) and incubated for 30 min at 37°C. After that, 25 µL of 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) (153 mM) was added, and the degeneration of the fluorescein was instantly read at 485 nm at an interval of 5 min for 1 h 30 min at 37°C. The antioxidant abilities of the extracts are given in µM of Trolox equivalent per µg of the extract (µM of TE/µg of E).

Preliminary testing for cytotoxicity effects

The active extracts were selected based on their antioxidant potential and were tested for their *in vitro* cytotoxic effects on the Vero cell line and the human Caucasian colon adenocarcinoma (Caco-2) ATCC HTB-37 cell line using the green fluorescent protein (GFP)-based assay and the resazurin microplate assay, respectively.[21] The tests were conducted by the National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, Thailand. Ellipticine was included as a positive control.

Effects on lifespan and oxidative biomarkers in Caenorhabditis elegans

Culture conditions and oxidative stress induction for Caenorhabditis elegans

The wild-type strain N2 of *C. elegans* from the UKM Molecular Biology Institute (UMBI) was kept and cultured according to previously described procedures.[18] The nematodes were routinely cultured on a solid nematode growth medium (NGM), which contains heat-killed *Escherichia coli* OP50 as a food source at 20°C. The addition of 5-fluoro-29-deoxyuridine (FUdR; Sigma-Aldrich, St. Louis, MO, USA) (40 µM) to NGM was done to prevent progeny production. All assays were performed in three replicates with age-synchronized animals (L4 stage; at least n=50 per experiment). To induce oxidative stress in *C. elegans*, the nematodes were pre-treated with 0.6 mM hydrogen peroxide (H_2O_2) for 2 h, which resulted in a less than 10% mortality rate compared to the control.[27]

Lifespan study of C. elegans

For the lifespan assays, either age-synchronized nematodes or H_2O_2-pretreated nematodes were transferred to NGM plates containing FUdR, which was supplemented with various concentrations of the tested extract or vehicle control (n=50 nematodes per plate) and incubated at 21°C. The fraction of nematodes that was alive was monitored daily by gently probing with a platinum needle. In contrast, dead nematodes characterized by a straight body and no response upon examination were removed from the plates. The numbers of live and dead nematodes were recorded for calculating their mean lifespans.[27] In addition, to avoid the effect of the tested extract on the food intake of the nematodes, their pharyngeal pumping rate was examined in parallel with the lifespan assay.

Determination of oxidative stress markers and antioxidant enzymes

The nematodes were cultured and treated, as mentioned above, and used for the determination of the levels of oxidative stress markers (lipofuscin and protein carbonyl levels) and the activities of antioxidant enzymes (catalases [CAT] and superoxide dismutase [SOD]).

The level of lipofuscin was quantified as an indicator of oxidative stress in the nematodes from each group, which were harvested from the plates and mounted on a 2% agarose pad containing 0.1% sodium azide. The nematodes were then observed with an Olympus BX53 digital fluorescence microscope (Olympus, Japan) coupled with the Olympus cellSens Standard software package for measuring the fluorescence intensity by a filter set to 03 (BP; λ_{exc}=450–490 nm; λ_{em} >510 nm) with 1.8 s exposure times.[27]

The treated *C. elegans* (1×10^6 cells/mL) were lysed using 200 µL of radioimmunoprecipitation assay (RIPA) lysis buffer containing a protease inhibitor cocktail (cOmplete™, EDTA-free Protease Inhibitor Cocktail, Sigma, USA). The protein concentration of the *C. elegans* cell lysate supernatant was determined using the Bradford assay. As an indicator of protein oxidation in the supernatant, the protein carbonyl level was also evaluated as previously described using Cayman's protein carbonyl colorimetric assay kit (Cayman Chemical, USA). The protein carbonyl content was measured at an optical density of 360 nm.

After the treatment, the nematodes in each group were separately harvested and diluted to 1×10^6 cells/mL and then homogenized at 12,500 *g* for 2 min at 4°C. The resultant solution was centrifuged at 14,000 rpm for 10 min at 4°C. The clear supernatants were carefully collected. The SOD activity was immediately measured by recording the inhibitory capability of the supernatant in the photochemical reduction of NBT *via* the riboflavin/methionine system. The activity of CAT in the supernatant was monitored by detecting the decomposition of hydrogen peroxide (30 mM) at 240 nm.[28]

Statistical analysis

The results of the antioxidant assays performed in triplicates are given as the mean ± the standard deviation (SD). The data analyses were statistically carried out with the Statistical Package for the Social Sciences software (SPSS 17) for Windows. Pearson's correlation test determined the relationship between different assays, and *p* values lower than 0.05 were determined to be significant. The results were analyzed by the Student's t-test for correspondence within two means. One-way ANOVA with Tukey's HSD as post hoc tests were implored to analyze the variation between samples. A variance was considered statistically significant if the *p* values were lower than 0.05.

Table 1: Ingredients and proportions of Thai traditional polyherbal formulation used as rejuvenators

Remedies No	Herbal components (Family)	Parts used	Materia medica voucher
THP-R001	*Allium sativum* L. (Amaryllidaceae)	Bulb	MTM08-04
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Morinda citrifolia L. (Rubiaceae)	Fruit	MTM08-65
	Piper nigrum L. (Piperaceae)	Fruit	MTM08-78
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R002	*Boesenbergia rotunda* (L.) Mansf. (Zingiberaceae)	Rhizomes	MTM08-18
	Curcuma longa L. (Zingiberaceae)	Rhizomes	MTM08-30
	Curcuma zedoaria (Christm.) Roscoe (Zingiberaceae)	Rhizomes	MTM08-31
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33

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	Maclura cochinchinensis (Lour.) Corner (Moraceae)	Wood	MTM08-56
	Piper nigrum L. (Piperaceae)	Fruit	MTM08-78
	Zingiber montanum (J. Koenig) Link ex A. Dietr. Zingiberaceae)	Rhizomes	MTM08-97
THP-R003	*Amomum testaceum* Ridl. (Zingiberaceae)	Fruit	MTM08-09
	Angelica dahurica (Hoffm.) Benth. & Hook. f. ex Franch & Sav. (Apiaceae)	Root	MTM08-11
	Ardisia polycephala Wall. ex A. DC. (Primulaceae)	Fruit	MTM08-13
	Aristolochia sp. (Aristolochiaceae)	Root	MTM08-14
	Atractylodes lancea (Thunb.) DC. (Asteraceae)	Rhizomes	MTM08-16
	Clausena excavata Burm. f. (Rutaceae)	Root	MTM08-27
	Cuminum cyminum L. (Apiaceae)	Fruit	MTM08-29
	Curcuma zedoaria (Christm.) Roscoe (Zingiberaceae)	Rhizomes	MTM08-31
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Leonurus sibiricus L. (Lamiaceae)	Whole plant	MTM08-52
	Lepidium sativum L. (Brassicaceae)	Seed	MTM08-53
	Atractylodes lancea (Thunb.) DC. (Asteraceae)	Rhizomes	MTM08-16
	Micromelum falcatum Lour. (Rutaceae)	Wood	MTM08-62
	Myristica fragrans Houtt. (Myristicaceae)	Aril, Seed	MTM08-66
	Nigella sativa L. (Ranunculaceae)	Seed	MTM08-68
	Petroselinum crispum (Mill.) Nyman ex A.W. Hill (Apiaceae)	Fruit	MTM08-71
	Pimpinella anisum L. (Apiaceae)	Fruit	MTM08-74
	Piper cubeba L. f. (Piperaceae)	Fruit	MTM08-76
	Pistacia integerrima Stew. ex Brandis (Pistaciaceae)	Grail	MTM08-81
	Syzygium aromaticum (L.) Merr. & L. M. Perry (Myrtaceae)	Flower	MTM08-89
	Terminalia chebula Retz. (Combretaceae)	Gail	MTM08-92
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95
	Zanthoxylum rhetsa (Roxb.) DC. (Rutaceae)	Fruit	MTM08-96
THP-R004	*Aegle marmelos* (L.) Corrêa ex Roxb. (Rutaceae)	Fruit	MTM08-01
	Amomum testaceum Ridl (Zingiberaceae)	Fruit	MTM08-09
	Angelica sinensis (Oliv.) Diels (Apiaceae)	Root	MTM08-12
	Carthamus tinctorius L. (Asteraceae)	Flower	MTM08-23
	Cinnamomum bejolghota (Buch.-Ham.) Sweet (Lauraceae)	Stem bark	MTM08-24
	Clausena excavata Burm. f. (Rutaceae)	Root	MTM08-27
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Ferula assa-foetida L. (Apiaceae)	Oleo-gum resin	MTM08-43
	Glycyrrhiza glabra L. (Fabaceae)	Root	MTM08-46
	Myristica fragrans Houtt. (Myristicaceae)	Aril, Fruit	MTM08-66
	Piper nigrum L. (Piperaceae)	Fruit	MTM08-76
	Syzygium aromaticum (L.) Merr. & L. M. Perry (Myrtaceae)	Flower	MTM08-89
THP-R005	*Aegle marmelos* (L.) Corrêa ex Roxb. (Rutaceae)	Fruit	MTM08-01
	Anethum graveolens L. (Apiaceae)	Fruit	MTM08-10

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	Angelica dahurica (Hoffm.) Benth. & Hook. f. ex Franch & Sav (Apiaceae)	Root	MTM08-11
	Angelica sinensis (Oliv.) Diels (Apiaceae)	Root	MTM08-12
	Artemisia annua L.(Asteraceae)	Leaf	MTM08-15
	Atractylodes lancea (Thunb.) DC. (Asteraceae)	Rhizomes	MTM08-16
	Cananga odorata (Lam.) Hook. f. & Thomson (Annonaceae)	Flower	MTM08-22
	Cinnamomum bejolghota (Buch.-Ham.) Sweet (Lauraceae)	Bark	MTM08-24
	Cinnamomum parthenoxylon (Jack) Meisn. (Lauraceae)	Wood	MTM08-25
	Cuminum cyminum L. (Apiaceae)	Fruit	MTM08-29
	Curcuma zedoaria (Christm.) Roscoe (Zingiberaceae)	Rhizomes	MTM08-31
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Dracaena cochinchinensis (Lour.) S. C. Chen (Asparagaceae)	Wood	MTM08-38
	Excoecaria agallocha L. (Euphorbiaceae)	Wood	MTM08-42
	Foeniculum vulgare Mill. (Apiaceae)	Fruit	MTM08-45
THP-R005 (Continued)	*Jasminum sambac* (L.) Aiton (Oleaceae)	Flower	MTM08-49
	Kaempferia galanga L. (Zingiberaceae)	Rhizomes	MTM08-50
	Lepidium sativum L. (Brassicaceae)	Seed	MTM08-53
	Ligusticum chuanxiong Hort (Apiaceae)	Rhizomes	MTM08-55
	Magnolia champaca (L.) Baill. Ex Pierre var. *champaca* (Magnoliaceae)	Flower	MTM08-58
	Mammea siamensis (Miq.) T. Anderson (Calophyllaceae)	Flower	MTM08-59
	Mesua ferrea L. (Calophyllaceae)	Flower	MTM08-61
	Mimusops elengi L. (Sapotaceae)	Flower, Wood	MTM08-63
	Nelumbo nucifera Gaertn. (Nelumbonaceae)	Flowers	MTM08-67
	Nigella sativa L. (Ranunculaceae)	Seed	MTM08-68
	Piper interruptum Opiz (Piperaceae)	Stem	MTM08-77
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Piper sarmentosum Roxb. (Piperaceae)	Root	MTM08-80
	Plumbago indica L. (Plumbaginaceae)	Root	MTM08-82
	Santalum album L. (Santalaceae)	Woo	MTM08-86
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R006	*Alstonia scholaris* (L.) R. Br. (Apocynaceae)	Bark	MTM08-07
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Diospyros rhodocalyx Kurz (Ebenaceae)	Bark	MTM08-37
	Prismatomeris tetrandra (Roxb.) K. Schum. (Rubiaceae)	Whole plant	MTM08-83
	Senna alata (L.) Roxb. (Fabaceae)	Leaf	MTM08-87
	Streblus asper Lour. (Moraceae)	Seed	MTM08-88
THP-R007	*Carthamus tinctorius* L. (Asteraceae)	Flower	MTM08-23
	Morinda citrifolia L. (Rubiaceae)	Leaf	MTM08-65

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	Ocimum tenuiflorum L. (Lamiaceae)	Whole plant	MTM08-69
	Pandanus amaryllifolius Roxb. (Pandanaceae)	Leaf	MTM08-70
	Piper sarmentosum Roxb. (Piperaceae)	Root	MTM08-80
THP-R008	*Aegle marmelos* (L.) Corrêa ex Roxb. (Rutaceae)	Fruit	MTM08-01
	Anethum graveolens L. (Apiaceae)	Fruit	MTM08-10
	Angelica dahurica (Hoffm.) Benth. & Hook. f. ex Franch & Sav (Apiaceae)	Root	MTM08-11
	Angelica sinensis (Oliv.) Diels (Apiaceae)	Root	MTM08-12
	Artemisia annua L. (Asteraceae)	Leaf	MTM08-15
	Atractylodes lancea (Thunb.) DC. (Asteraceae)	Rhizomes	MTM08-16
	Cinnamomum verum J. Presl (Lauraceae)	Bark	MTM08-26
	Cuminum cyminum L. (Apiaceae)	Fruit	MTM08-29
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Foeniculum vulgare Mill. (Apiaceae)	Fruit	MTM08-45
	Lepidium sativum L. (Brassicaceae)	Seed	MTM08-53
	Ligusticum chuanxiong Hort (Apiaceae)	Rhizomes	MTM08-55
	Momordica charantia L. (Cucurbitaceae)	Whole plant	MTM08-64
	Nigella sativa L. (Ranunculaceae)	Seed	MTM08-68
	Phyllanthus urinaria L. (Phyllanthaceae)	Whole plant	MTM08-73
	Piper interruptum Opiz (Piperaceae)	Stem	MTM08-77
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Piper sarmentosum Roxb. (Piperaceae)	Root	MTM08-80
	Plumbago indica L. (Plumbaginaceae)	Root	MTM08-82
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R009	*Azadirachta indica* A. Juss. (Meliaceae)	Leaf	MTM08-17
	Caesalpinia bonduc (L.) Roxb. (Fabaceae)	Leaf	MTM08-20
	Piper nigrum L. (Piperaceae)	Fruit	MTM08-78
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Piper sarmentosum Roxb. (Piperaceae)	Fruit	MTM08-80
	Plumbago indica L. (Plumbaginaceae)	Root	MTM08-82
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R010	*Aegle marmelos* (L.) Corrêa ex Roxb. (Rutaceae)	Fruit	MTM08-01
	Coriandrum sativum L (Apiaceae)	Fruit	MTM08-28
	Curcuma zedoaria (Christm.) Roscoe (Zingiberaceae)	Rhizomes	MTM08-31
	Cyperus involucratus Rottb. (Cyperaceae)	Rhizomes	MTM08-32
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Gymnopetalum chinense (Lour.) Merr. (Cucurbitaceae)	Fruit	MTM08-48
	Mesua ferrea L. (Calophyllaceae)	Flower	MTM08-61
	Mimusops elengi L. (Sapotaceae)	Flower	MTM08-63
	Nelumbo nucifera Gaertn. (Nelumbonaceae)	Pollen	MTM08-67
	Piper interruptum Opiz (Piperaceae)	Stem	MTM08-77
	Piper nigrum L. (Piperaceae)	Fruit	MTM08-78
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Piper sarmentosum Roxb. (Piperaceae)	Root	MTM08-80

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THP-R010 (Continued)	*Plumbago indica* L. (Plumbaginaceae)	Rhizomes	MTM08-82
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R011	*Albizia myriophylla* Benth. (Fabaceae)	Stem	MTM08-02
	Albizia procera (Roxb.) Benth. (Fabaceae)	Bark	MTM08-03
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Diospyros rhodocalyx Kurz (Ebenaceae)	Bark	MTM08-37
	Elephantopus scaber L. (Asteraceae)	Whole plant	MTM08-40
	Glycyrrhiza glabra L. (Fabaceae)	Root	MTM08-46
	Kaempferia parviflora Wall. ex Baker (Zingiberaceae)	Rhizomes	MTM08-51
	Pueraria candollei Wall. ex Benth. var. *mirifica*(Airy Shaw & Suvat.) Niyomdham (Fabaceae)	Tuber	MTM08-84
	Streblus asper Lour. (Moraceae)	Seed	MTM08-88
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95
THP-R012	*Alternanthera bettzickiana* (Regel) G. Nicholson (Amaranthaceae)	Whole plant	MTM08-08
	Caesalpinia sappan L. (Fabaceae)	Wood	MTM08-21
	Maclura cochinchinensis (Lour.) Corner (Moraceae)	Wood	MTM08-56
THP-R013	*Aegle marmelos* (L.) Corrêa ex Roxb. (Rutaceae)	Fruit	MTM08-01
	Boesenbergia rotunda (L.) Mansf. (Zingiberaceae)	Rhizomes	MTM08-18
	Caesalpinia sappan L. (Fabaceae)	Wood	MTM08-21
	Carthamus tinctorius L. (Asteraceae)	Flower	MTM08-23
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Morinda citrifolia L. (Rubiaceae)	Fruit	MTM08-65
	Piper interruptum Opiz (Piperaceae)	Stem	MTM08-77
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Piper sarmentosum Roxb. (Piperaceae)	Root	MTM08-80
	Plumbago indica L. (Plumbaginaceae)	Root	MTM08-82
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R014	*Derris scandens* (Roxb.) Benth (Fabaceae)	Stem	MTM08-34
	Derris trifoliata Lour. (Fabaceae)	Wood	MTM08-35
	Lepionurus sylvestris Blume (Opiliaceae)	Wood, Root	MTM08-34
	Salacia chinensis L. (Celastraceae)	Wood	MTM08-85
THP-R015	*Aegle marmelos* (L.) Corrêa ex Roxb. (Rutaceae)	Fruit	MTM08-01
	Cyperus involucratus Rottb (Cyperaceae)	Rhizomes	MTM08-32
	Phyllanthus emblica L. (Phyllanthaceae)	Fruit	MTM08-72
	Piper interruptum Opiz (Piperaceae)	Stem	MTM08-77
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Piper sarmentosum Roxb. (Piperaceae)	Root	MTM08-80
	Plumbago indica L. (Plumbaginaceae)	Root	MTM08-82
	Terminalia bellirica (Gaertn.) Roxb. (Combretaceae)	Fruit	MTM08-91
	Terminalia chebula Retz. (Combretaceae)	Fruit	MTM08-92
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95

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	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R016	*Allium sativum* L. (Amaryllidaceae)	Blub	MTM08-04
	Alpinia galanga (L.) Willd. (Zingiberaceae)	Rhizomes	MTM08-05
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Maerua siamensis (Kurz) Pax (Capparaceae)	Root	MTM08-57
	Phyllanthus emblica L. (Phyllanthaceae)	Fruit	MTM08-72
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Terminalia arjuna (Roxb. Ex DC.) Wight & Arn(Combretaceae)	Fruit	MTM08-90
	Terminalia bellerica (Gaertn.) Roxb. (Combretaceae)	Fruit	MTM08-91
	Terminalia citrina (Gaertn.) Roxb. ex Fleming (Combretaceae)	Fruit	MTM08-93
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizomes	MTM08-98
THP-R017	*Albizia procera* (Roxb.) Benth. (Fabaceae)	Wood	MTM08-03
	Borassus flabellifer L. (Arecaceae)	Root	MTM08-19
	Cyperus rotundus L. (Cyperaceae)	Rhizomes	MTM08-33
	Diospyros rhodocalyx Kurz (Ebenaceae)	Wood	MTM08-36
	Mansonia gagei J. R. Drumm. ex Prain (Malvaceae)	Wood	MTM08-60
	Myristica fragrans Houtt. (Myristicaceae)	Seed	MTM08-66
	Piper interruptum Opiz (Piperaceae)	Stem	MTM08-77
	Piper betle L. (Piperaceae)	Leaf	MTM08-75
	Piper nigrum L. (Piperaceae)	Seed, Stem	MTM08-78
	Piper retrofractum Vahl (Piperaceae)	Fruit	MTM08-79
	Piper sarmentosum Roxb. (Piperaceae)	Root, Leaf	MTM08-80
	Plumbago indica L. (Plumbaginaceae)	Root	MTM08-82
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	stem	MTM08-95
	Zingiber officinale Roscoe (Zingiberaceae)	Rhizome	MTM08-98
THP-R018	*Albizia myriophylla* Benth. (Fabaceae)	Root	MTM08-02
	Alstonia macrophylla Wall. ex G. Don (Apocynaceae)	Wood	MTM08-06
	Eurycoma longifolia Jack (Simaroubaceae)	Wood/Root	MTM08-41
	Prismatomeris tetrandra (Roxb.) K. Schum. (Rubiaceae)	Whole plant	MTM08-83
	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95
THP-R019	*Diospyros toposia* Ham. var. toposioides (King & Gamble) Phengklai (Ebenaceae)	Bark	MTM08-37
	Dryopteris syrmatica O. kze (polypodiaceae)	Rhizomes	MTM08-39
	Ficus foveolata Wall. (Moraceae)	Stem	MTM08-44
	Goniothalamus macrophyllus (Blume) Hook. f. & Thomson (Annonaceae)	Root, Wood	MTM08-47
THP-R020	*Eurycoma longifolia* Jack (Simaroubaceae)	Root, Wood	MTM08-41
	Tinospora baenzigeri Forman (Menispermaceae)	Stem	MTM08-94

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	Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae)	Stem	MTM08-95

Results and Discussion

Preliminary antioxidant activity results

Using an ethanol maceration technique that mimics a standard method for the preparation of herbal medicine, the extraction yields were found to be in the range of 3.25–14.60%, as given in Table 2. Among the tested extracts, the ethanol extract of THP-R016 yielded the highest phenolic contents (651.2±2.1 mg gallic acid per g extract [GAE mg/g]), which might be responsible for the potent antioxidant. It appears that the extracts of THP-R012 and THP-R019 exhibited moderately high phenolic contents of 629.6±8.2 and 534.7±12.1 GAE mg/g, respectively. Additionally, the TFC, which represents the major group of plant-derived phenolic compounds, was examined. The results are presented in Table 2 as the mass in milligrams of catechin standard per mass of extract in grams (CAE mg/g). The extract of THP-R012 possessed the highest value (382.9±6.2 CAE mg/g), followed by THP-R019 and THP-R017, with TFC values of 300.6±2.2 and 268.0±0.9 CAE mg/g, respectively.

In parallel with the examinations of antioxidant-related compounds, the extracts were subjected to the evaluation of their properties as either chain-breaking antioxidants or chelator antioxidants. Within all the formulations shown in Table 3, THP-R009 exhibited the highest chelating power with an IC_{50} of 0.33±0.01 mg/mL, followed by THP-R007, THP-R016, and THP-R015, which produced IC_{50} values of 0.48±0.01, 0.56±0.01, and 0.64±0.01 mg/mL, respectively. However, their metal-chelating abilities were found to be approximately 16- to 32-times lower than that of the standard chelator, EDTA. It should be noted that THP-R016 and THP-R012 strongly act as chain-breaking antioxidants, which was determined by the FRAP assay and scavenging assays against DPPH and ABTS radicals (Table 2). THP-R016 had the highest FRAP (191.44±4.39 µmol Fe_2SO_4/mg extract) and the ability to scavenge the DPPH radical (IC_{50}=0.13±0.03 mg/mL), accompanied by the extracts of THP-R012, THP-R014, THP-R015, THP-R017, and THP-R019. It is worth noting that the ABTS scavenging capacity of THP-R012 (IC_{50}=0.12 mg/mL), which is higher than that of THP-R016 (IC_{50}=0.20 mg/mL) and THP-R019 (IC_{50}=0.29 mg/mL), was higher than that of the standard Trolox (IC_{50}=0.35 mg/mL). Except for the chelating ability, the formulations with high phenolic and flavonoid contents had high chain-breaking antioxidant capacities, as shown in Table 4.

The current investigation confirmed that among the 20 tonifying polyherbal mixtures used by folk healers in Phatthalung and Songkhla, THP-R016 (or Phy-Blica-O) possessed remarkable antioxidant capacities as a primary antioxidant that neutralizes the reactive species by transferring an electron or one hydrogen atom. According to the literature,[5,22]

Table 2: Extraction yield, total phenolic, and total flavonoid contents of ethanol extract of 20 combined polyherbal formulations.

Remedies	% yield (w/w)	Total phenolic content mg gallic acid/g extract	Total flavonoid content mg catechin/g extract
THP-R001	5.11	137.2±2.9	114.5±2.8
THP-R002	11.11	382.0±8.3	232.9±3.6
THP-R003	6.62	284.2±5.4	119.2±1.3
THP-R004	9.98	270.4±7.3	163.9±0.9
THP-R005	7.69	165.2±2.8	71.7±1.8
THP-R006	5.11	97.0±0.4	92.9±0.4
THP-R007	5.48	85.4±0.7	42.3±0.9
THP-R008	6.16	132.3±1.6	65.6±0.1
THP-R009	7.77	118.6±3.2	119.3±1.0
THP-R010	5.85	145.8±4.1	79.1±0.2
THP-R011	3.87	147.3±3.8	55.2±1.2
THP-R012	3.95	629.6±8.2	382.9±6.7
THP-R013	6.29	208.5±7.1	47.3±0.6
THP-R014	6.46	355.9±6.8	153.8±0.4
THP-R015	12.59	347.1±5.6	100.8±3.2
THP-R016	14.60	651.2±2.1	79.4±0.4
THP-R017	8.88	344.5±11.7	268.0±0.9
THP-R018	3.25	114.2±3.1	38.6±0.6
THP-R019	5.61	534.7±12.1	300.6±2.2
THP-R020	5.10	123.1±3.2	49.8±3.8

Phy-Blica-O is made from equal parts of 11 medicinal plants: *Allium sativum*, *Alpinia galanga*, *Cyperus rotundus*, and *Maerua siamensis*, *Piper retrofractum*, *Phyllanthus emblica*, *Terminalia arjuna*, *Terminalia bellirica*, *Terminalia citrina*, *Tinospora crispa*, and *Zingiber officinale*. All its herbal components, except for *Maerua siamensis*, have been found to act as free radical scavengers,[29–36]

thereby playing the main role in the reactive oxygen species scavenging activities of Phy-Blica-O. Among these, the administration of extracts obtained from *Allium sativum*, *Zingiber officinale*, *Piper retrofractum*, *Terminalia citrina*, *Terminalia bellirica*, *Phyllanthus emblica*, *Tinospora crispa*, or *Terminalia arjuna* has been found to attenuate the antioxidant status in animal models.[31,34,35,37–42] Clinical studies have also revealed that *Phyllanthus emblica* or *Terminalia arjuna* consumption significantly changes the oxidative stress biomarkers in patients.[43,44] Although Phy-Blica-O is made by combining equal amounts of the medicinal plant, it is likely that *Phyllanthus emblica* and *Terminalia arjuna* were its active ingredients, according to the evidence mentioned above

Scavenging of reactive oxygen radicals, cytotoxic effects, and proposed active ingredients of the effective formulations

In addition to their antioxidant capability, as mentioned above, the toxicity as well as the free radical scavenging ability toward peroxyl and superoxide radicals, which represent abundant ROS radicals, were evaluated to determine the most promising extracts. All formulation extracts had a very low cytotoxic effect on normal cells (Vero cells), with an IC_{50} value of >50 µg/mL. Based on the American National Cancer Institute (NCI), these extracts were inactive against Caco-2 cells; their IC_{50} values were found to be higher than 50 µg/mL in this preliminary assay (Table 5). Among the eight selected polyherbal ethanol extracts, THP-R012, THP-R019, and THP-R016 scavenged superoxide anions, with an IC_{50} value ranging from 40 to 80 µg/mL, in a dose-dependent manner, as presented in Figure 1.

According to its highest phenolic content, remarkable scavenging ability, and good metal chelating properties, the ethanol extract of THP-R016 (or Phy-Blica-O) was tested for its scavenging ability against peroxyl radicals. The Phy-Blica-O ethanol extract possessed peroxyl radical scavenging capacity in a concentration-dependent manner comparable to that of Trolox. It produced an ORAC value of 37.87±1.73 µM Trolox/g extract (Figure 2). To mimic its traditional administrative method, as previously published, [21] Phy-Blica-O water extract was subjected to the qualitative analysis of antioxidant-related compounds by HPLC-DAD-ESI-MS in negative mode. The results revealed that there are 21 known compounds found with the database with a match score of at least 90. Among these compounds, 7 antioxidant-related compounds, which are 1-*O*-galloylglycerol (RT=0.83), methyl-gallate (RT=2.05), ellagic acid (RT=10.75), shoyuflavone B (RT=10.85), acetylcaranine (RT=15.10), sericoside (RT=15.66), and (±)-naringenin (RT=17.89), were detected in this water extract.

Phenolics and flavonoids, the major subgroup, are the most abundant secondary metabolites associated with the antioxidant properties of medicinal plants.[45] The remarkable free radical scavenging activities and chelating ability observed from both ethanol and water extracts of Phy-Blica-O are possibly due to their high contents of phenolics and flavonoids. The scavenging properties of phenolics mediated by phenolic hydroxyl groups can either neutralize free radicals through hydrogen- or electron-donation or delocalize an unpaired electron by the extended conjugated aromatic system.[46] In addition to the phenolics, it is well-described that at least three structural features of flavonoids are able to transfer a single electron or a donate hydrogen to stabilize free radicals, including an *o*-dihydroxy structure in the B ring, hydroxyl groups on the A and C rings, and a double bond between C2 and C3 combined with the oxo-C4 on the C ring.[45] It should be noted that several phenolics and flavonoids found in Phy-Blica-O, among these, ellagic acid and naringenin, were found to be potent biological makers responsible for its antioxidant activity due to their remarkable capacity to scavenge detrimental ROS, as reported in prior studies.[47,48] Research done on naringenin has revealed that this flavonoid has not only ROS scavenging properties, but it also possesses an inhibitory effect toward prooxidant enzymes, thereby exhibiting protective effects against oxidative stress disorders, as reviewed by Zaidun and colleagues.[47] A well-known plant-derived polyphenol detected in Phy-Blica-O, ellagic acid, were remarkably ameliorated age-related diseases including Alzheimer's and Parkinson's diseases, which possibly mediated by its neuroprotective effects.[49,50] Even though both naringenin and ellagic acid contents need to be quantified, the finding of these compounds in Phy-Blica-O partially supports its traditional claimed use as a nourishing formula.

Effects of Phy-Blica-O on lifespan, oxidative stress markers, and antioxidant enzymes of wild-type Caenorhabditis elegans under normal culture and H_2O_2 induced oxidative stress conditions

As given in Table 6, the nematodes have a mean lifespan of 17.38±2.49 days under untreated conditions. There was a significant increase in the mean lifespan of *C. elegans* upon treatment of Phy-Blica-O water extract, which was found to be 22.48±0.19, 21.56±0.45, and 21.53±0.14 days after treatment with 0.039, 0.156, and 0.625 mg/mL of the extract, respectively. As previously reported, the lifespan enhancement of the nematodes is associated with a calorie restriction; therefore, we investigated the effect of Phy-Blica-O on the rate of pharyngeal pumping, which represents the amount of food intake of *C. elegans*. There are no statistically significant differences in the pharyngeal pumping rates of *C. elegans* between groups, which were 172.7±1.9 pumps/min in the untreated group and 171.7±0.9, 169.3±1.2, and 170.0±1.7 pumps/min in groups supplemented with Phy-Blica-O water extract at concentrations of 0.039, 0.156, and 0.625 mg/mL, respectively. There were no significant changes in the pumping rate of the nematodes after two days of incubation with all Phy-Blica-O concentrations, which were 177.0±2.1 (0.039 mg/mL), 172.3±4.1 (0.156 mg/mL), and 178.3±2.3 (0.625 mg/mL) pumps/mins, as compared to the untreated control (176.7±2.3 pumps/mins). Exposure to an oxidative stress inducer (H_2O_2) significantly reduced the mean lifespan of the nematodes to 14.69±0.99 days. In contrast, all tested concentrations of Phy-Blica-O have failed to extend the lifespan of *C. elegans* under this oxidative stress condition

As it was clearly demonstrated that Phy-Blica-O possessed an *in vitro* antioxidant capacity and increased the *C. elegans* lifespan, we postulated that the oxidative status of the nematodes possibly interfered with the treatment of this herbal mixture. As shown in Figure 3, treating nematodes with Phy-Blica-O at 0.039 mg/mL did not cause significant changes in the levels of oxidative stress markers (lipofuscin and protein carbonyl content) and antioxidant enzymes (CAT and SOD). Pre-treating the nematodes with H_2O_2 significantly increased the levels of lipofuscin and the protein carbonyl content, whereas treating these nematodes with the extract tended to reduce the levels of the oxidative stress markers and increase the level of SOD.

A well-recognized nematode model, *C. elegans* has been chosen to explore the antiaging effect of the herbal formulation because of its small body size, short lifespan, completely sequenced genome in which 60–80% of its genes are associated with humans, and at least 83% of its proteome have human homologous.[51] Several studies have demonstrated that their lifespan extension abilities and protective effects against age-related diseases of tonifying polyherbal mixtures are partially related to their antioxidant effects.[14–17] This tonifying agent uses as a whole-body tonic [21] and significantly extends the lifespan of *C. elegans* under the normal growth condition. The extract slightly reduced the accumulation of lipofuscin and enhanced the level of SOD production of *C. elegans*. Similarly, an increase in the mean lifespan of *C. elegans* was observed when treated with ellagic acid [52], *Allium sativum* [53], and *Zingiber officinale*[54]. Even though naringenin failed to extend the lifespan of *C. elegans* [55], it was highlighted as a promising hepatoprotective agent that could decrease oxidative biomarkers, such as levels of lipid peroxidation and protein carbonylation, and increase the status of antioxidant defenses in other animal models.[56–58] Furthermore, ellagic acid actively exhibited protective effects partly through the enhancement of antioxidant enzyme activities, such as the activities of glutathione peroxidase, glutathione-S-transferase, CAT, and SOD,[59] which is in agreement with the results obtained in this present work.

However, only slightly increased lifespans of the nematodes were observed after treatment with Phy-Blica-O under H_2O_2-induced oxidative stress conditions. This is possibly due to the lack of repair mechanisms of the decoction on oxidative stress-damaged biomolecules caused by H_2O_2. In prior studies,[60,61] two herbal mixtures, which have ingredients similar to Phy-Blica-O, were found to enhance the mean survival of *C. elegans*. The water extract of a polyherbal formula named Jatu-Phala-Tiga, prescribed as a tonifying medicine for the liver and body fluids and made from *Phyllanthus*

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emblica, *Terminalia arjuna*, *Terminalia chebula*, and *Terminalia bellirica*, could enhance the lifespan of the nematodes under normal growth conditions. Still, it did not extend their lifespan under H_2O_2-induced oxidative stress.[60] Conversely, the Ayurvedic formula consisting of *Berberis aristata*, *Cyperus rotundus*, *Cedrus deodara*, *Phyllanthus emblica*, *Terminalia chebula*, and *Terminalia bellirica* enhanced the survival rate of *C. elegans* in the oxidative stress condition induced by juglone.[61] Based on the acquired results, it has been demonstrated for the first time that the traditional tonifying formula, Phy-Blica-O, significantly enhances the lifespan of *C. elegans*, possibly through its potent antioxidant capacities and high phenolic and flavonoid contents, thereby modulating the oxidative stress status and promoting antioxidant enzymes
.

Table 3: DPPH and ABTS radical scavenging capacities, ferric reducing antioxidant power, and metal chelating activity of ethanol extracts prepared from 20 combined polyherbal formulations.

Remedies	**Metal chelating activity (IC_{50}**±SD) (mg/mL)**	**Ferric reducing mg gallic acid/g extract antioxidant power**	**Free radical scavenging capacity mg catechin/g extract (IC_{50}*±SD) (mg/mL)**	
		(Fe_2SO_4 equivalent) *	DPPH	ABTS
THP-R001	2.90±0.06	41.18±0.880	NA	1.54±0.02
THP-R002	1.49±0.04	53.93±0.63	0.62±0.01	0.41±0.00
THP-R003	0.95±0.01	134.15±2.70	0.73±0.02	0.46±0.01
THP-R004	1.80±0.07	148.33±0.65	1.83±0.39	0.63±0.01
THP-R005	1.90±0.03	60.20±1.10	0.83±0.39	0.91±0.02
THP-R006	0.68±0.06	47.06±1.07	NA	0.63±0.01
THP-R007	0.48±0.01	19.01±0.98	NA	2.49±0.01
THP-R008	0.70±0.01	39.80±0.28	1.64±0.06	0.90±0.02
THP-R009	0.33±0.01	53.43±2.86	2.21±0.13	1.61±0.02
THP-R010	1.70±0.01	48.68±0.88	NA	1.46±0.00
THP-R011	1.05±0.0	37.41±0.46	2.35±0.10	0.87±0.03
THP-R012	2.31±0.02	189.27±2.44	0.18±0.01	0.12±0.00
THP-R013	NA	75.21±2.18	1.93±0.23	0.85±0.01
THP-R014	0.73±0.00	97.50±1.12	0.45±0.03	0.38±0.00
THP-R015	0.64±0.01	166.95±1.51	0.18±0.01	0.43±0.02
THP-R016	0.56±0.01	191.44±4.39	0.13±0.03	0.20±0.00
THP-R017	2.72±0.02	174.13±2.10	0.55±0.00	0.57±0.00
THP-R018	1.39±0.02	30.83±0.50	NA	1.99±0.05
THP-R019	1.05±0.05	168.22±2.55	0.26±0.01	0.29±0.00
THP-R020	1.51±0.07	41.47±0.24	NA	1.72±0.02
Trolox	NA	NA	0.09±0.01	0.35±0.01
EDTA	0.02±0.00	NA	NA	NA

NA = Not applicable
* Fe_2SO_4 equivalent expressed as µM Fe_2SO_4 equivalents per mg of extract

Table 4: The correlation analysis of total antioxidant capacities and main active ingredients of ethanol extracts prepared from 20 polyherbal medicines used as rejuvenators.

Person's Correlation (P value)					
Water	ABTS	FRAP	MCA	TPC	TFC
DPPH	0.927 (0.000)	0.875 (0.000)	0.384 (0.095)	0.923 (0.000)	0.650 (0.002)
ABTS		0.813 (0.000)	0.309 (0.185)	0.945 (0.000)	0.713 (0.000)
FRAP			0.445 (0.049)	0.853 (0.000)	0.631 (0.003)
MCA				0.340 (0.143)	0.155 (0.513)
TPC					0.704 (0.001)

* Antioxidant capacities of the extracts were expressed in terms of their inhibitory activity against $ABTS^{\bullet+}$ and DPPH radicals (% inhibition at 0.6 mg/mL), ferric reducing antioxidant power (FRAP; µM Fe2SO4/mg extract), and ferrous ions metal chelating activity (MCA; % chelating activity at 3.5 mg/mL). ** Total phenolic content (TPC) and total flavonoid content (TFC) expressed as mg of gallic acid equivalent per g extract and mg catechin equivalent per g extract, respectively.

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Table 5: Cytotoxicity effects of selected ethanol extracts of Thai polyherbal remedies on African green monkey kidney (Vero) cells and human caucasian colon adenocarcinoma (Caco-2) ATCC HTB-37 cell line

Remedies	Cytotoxicity (IC_{50}; µg/mL)	
	Vero cells	Caco-2 cells
THP-ROO2	>50	>50
THP-ROO3	>50	>50
THP-RO12	>50	>50
THP-RO14	>50	>50
THP-RO15	>50	>50
THP-RO16	>50	>50
THP-RO17	43.2	>50
THP-RO19	>50	>50
Ellipticine	0.7	21.4

Table 6: Effects of Phy-Blica-O (THP-R016) on mean lifespans of *Caenorhabditis elegans* N2 wild-type under normal growth conditions and after 0.6 mM hydrogen peroxide (H_2O_2)-mediated oxidative stress.

Concentrations (mg/mL)	Mean lifespan (Days±SD)	
	Normal growth condition	H_2O_2-induced oxidative stress
Untreated control	17.38±2.49[b]	14.69±0.99[ns]
0.039	22.48±0.19[a]	15.68±0.090[ns]
0.156	21.56±0.45[a]	15.20±1.09[ns]
0.625	21.53±0.14[a]	15.28±0.90[ns]
1.25	16.53±0.13[b]	-
2.5	13.62±0.47[b]	-
5	9.22±0.25[c]	-

[a-c;] Values in the same column with different superscripts are significantly different (p<0.05).

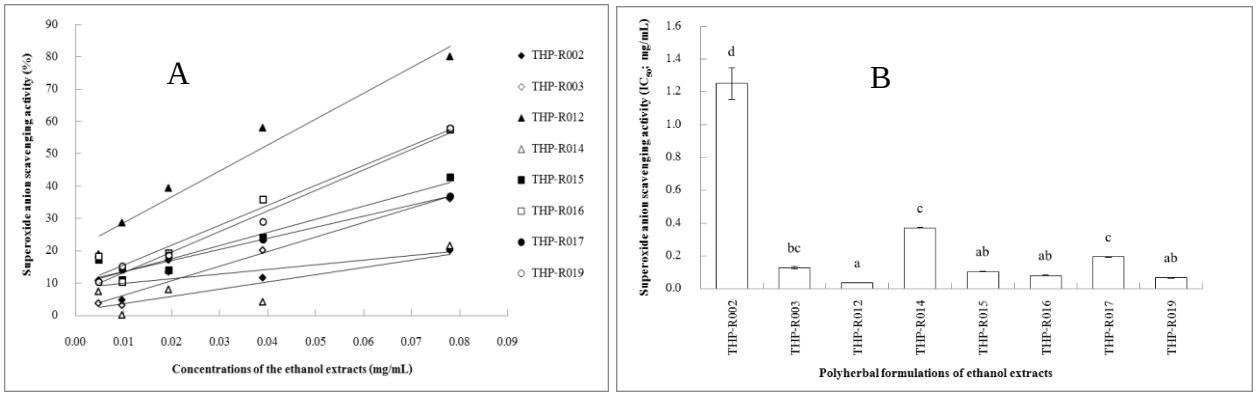

Figure 1: Superoxide anion scavenging activity of selected ethanol extracts in a dose-dependent manner (A) and IC_{50} values (mg/mL) are expressed as mean±SD (B). Different letters indicate statistically significant differences at p<0.01.

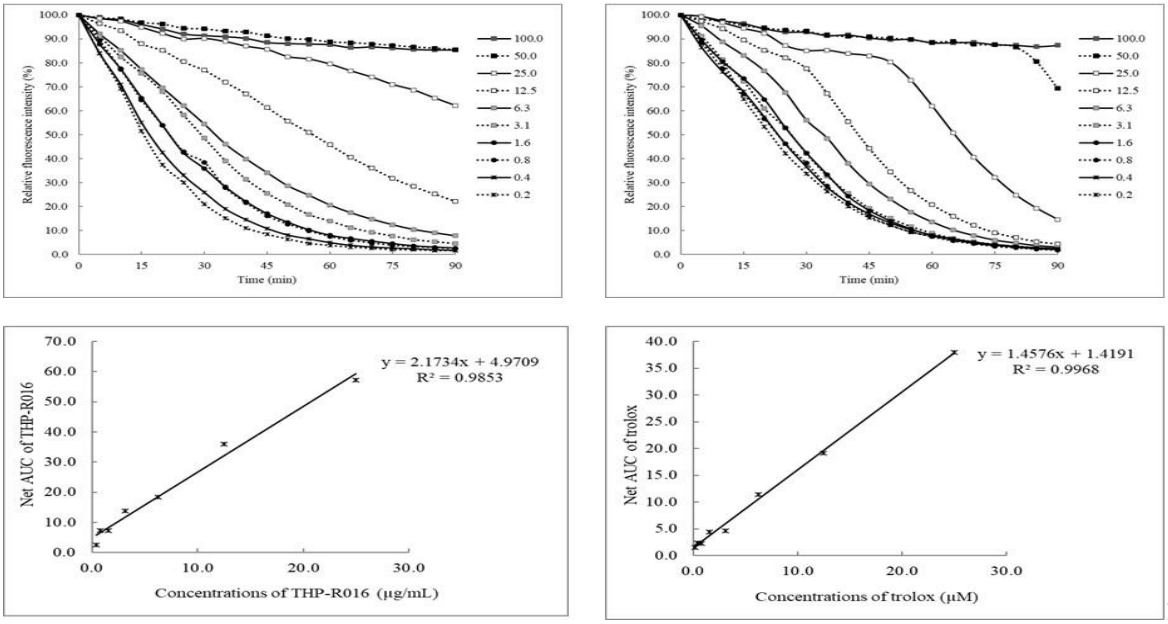

Figure 2: Anti-peroxyl radical activity, fluorescence decay curves of fluorescein in the presence of various concentrations of Phy-Blica-O (THP-R016) ethanol extract (left panel), and the reference antioxidant Trolox (right panel).

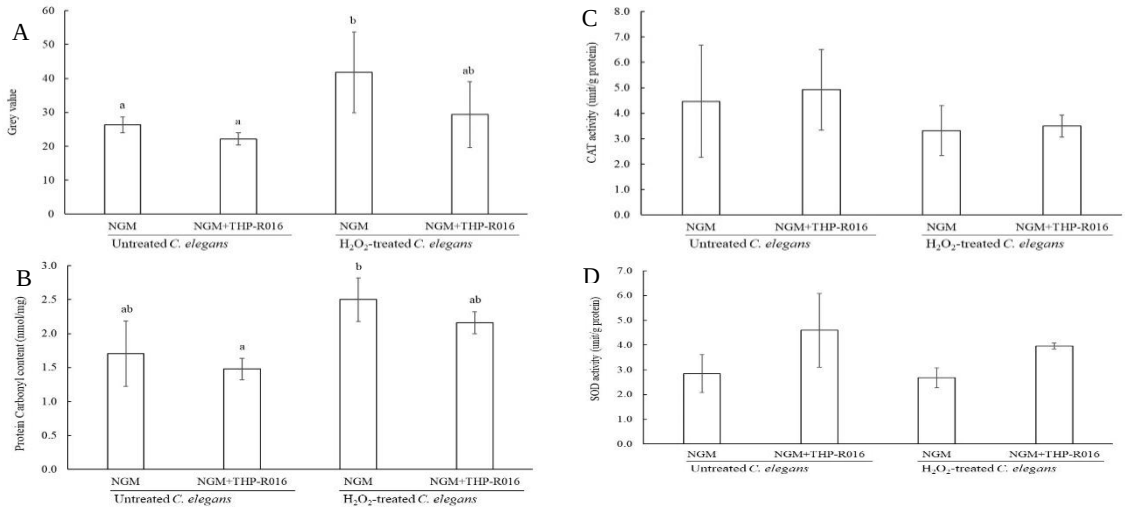

Figure 3: Levels of oxidative stress markers which are the accumulation of lipofuscin granule (A) and protein carbonyl content (B) and antioxidant enzymes, catalase (CAT; C) and superoxide dismutase (SOD; D) of *Caenorhabditis elegans* growth on Nematode Growth Medium (NGM) plates with or without the supplementation of Phy-Blica-O (THP-R016) at a concentration of 39 µg/mL. The nematodes under normal growth and oxidative stress-induced condition by pre-exposed with 0.6 mM hydrogen peroxide (H_2O_2) for 2 hr. were used.

Conclusion

In summary, *in vitro* studies revealed that Phy-Blica-O remarkably acts as a primary antioxidant that neutralizes reactive species by donating an electron or one hydrogen atom, possibly due to its herbal components, in particular, *Allium sativum*, *Phyllanthus emblica*, and *Terminalia arjuna*, and its chemical constituents, ellagic acid, and naringenin. Phy-Blica-O was found to enhance the longevity of *C. elegans*, to slightly promote the activity of the antioxidant enzyme, SOD, and to subsequently reduce the age-related oxidative stress marker, lipofuscin, in *C. elegans*. These results support the traditional uses of Phy-Blica-O and provide an opportunity for the development of traditional and natural pharmaceutical products for possibly lengthening lifespans and delaying age-related disorders.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Franceschi C, Garagnani P, Morsiani C, Conte M, Santoro A, Grignolio A, Monti D, Capri M, Salvioli S. The continuum of aging and age-related diseases: Common mechanisms but different rates. Front Med (Lausanne). 2018; 5:61.
2. Mariotto AB, Yabroff KR, Shao Y, Feuer EJ, Brown ML. Projections of the cost of cancer care in the United States: 2010-2020. J Natl Cancer Inst. 2011; 103(2):117-128.
3. Alzheimer's Association. Alzheimer's Disease Facts and Figures. Alzheimers Dement. 2020; 16(3): 1-94.
4. Pomatto LCD and Davies KJA. Adaptive homeostasis and the free radical theory of ageing. Free Radic Biol Med. 2018; 124:420-430.
5. Rejeski WJ and Fanning J. Models and theories of health behavior and clinical interventions in aging: a contemporary, integrative approach. Clin Interv Aging. 2019; 14:1007-1019.
6. Omidifar N, Moghadami M, Mousavi SM, Hashemi SA, Gholami A, Shokripour M, Sohrabi Z. Trends in natural nutrients for oxidative stress and cell senescence. Oxid Med Cell Longev. 2021; 2021: 7501424.
7. Rashrash M, Schommer JC, Brown LM. Prevalence and predictors of herbal medicine use among adults in the United States. J Patient Exp. 2017; 4(3):108-113.
8. Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan. 2016; 31(8):984-991.
9. Samtiya M, Aluko RE, Dhewa T, Moreno-Rojas JM. Potential health benefits of plant food-derived bioactive components: an overview. Foods. 2021; 10(4): 839.
10. Maugeri A, Barchitta M, Mazzone MG, Giuliano F, Basile G, Agodi A. Resveratrol modulates SIRT1 and DNMT functions and restores LINE-1 methylation levels in ARPE-19 cells under oxidative stress and inflammation. Int J Mol Sci. 2018; 19(7):2118.
11. Maiti P and Dunbar GL. Use of curcumin, a natural polyphenol for targeting molecular pathways in treating age-related neurodegenerative diseases. Int J Mol Sci. 2018; 19(6):1-42.
12. Anand David AV, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: a bioactive flavonoid, Pharmacogn. Rev. Pharmacogn Rev. 2016; 10(20):84-89.

13. Cascella M, Bimonte S, Muzio MR, Schiavone V, Cuomo A. The efficacy of Epigallocatechin-3-gallate (green tea) in the treatment of Alzheimer's disease: an overview of pre-clinical studies and translational perspectives in clinical practice. Infect Agent Cancer. 2017; 12:36.
14. Meng F, Li J, Rao Y, Wang W, Fu Y. A Chinese herbal formula, Gengnianchun, ameliorates β-amyloid peptide toxicity in a *Caenorhabditis elegans* model of Alzheimer's disease. Evid Based Complement Alternat Med. 2017; 2017: 7480980.
15. Zeng L, Yang Z, Yun T, Fan S, Pei Z, Chen Z, Sun C, Xu F. Antiaging effect of a Jianpi-yangwei formula in *Caenorhabditis elegan*. BMC Complement Altern Med. 2019; 19(1):313.
16. Zeng L, Sun C, Pei Z, Yun T, Fan S, Long S, Wu T, Chen Z, Yang Z, Xu F. Liangyi Gao extends lifespan and exerts an antiaging effect in *Caenorhabditis elegans* by modulating DAF-16/FOXO. Biogerontol. 2019; 20(5):665-676.
17. Chen W, Wang J, Shi J, Yang X, Yang P, Wang N, Yang S, Xie T, Yang H, Zhang M, Wang H, Fei J. Longevity effect of Liuwei Dihuang in both *Caenorhabditis elegans* and aged mice. Aging Dis. 2019; 10(3):578-591.
18. The National Essential Drug List Committee. The National List of Essential Drugs:Last update 19th March 2018 (List of Herbal Medicinal Products). [Online]. 2018 [cited 2021 Nov 29]. Available from: http://ndi.fda.moph.go.th/uploads/main_drug_file/20171021185635.pdf.
19. Maneenoon K, Khuniad C, Teanuan Y, Saedan N, Prom-In S, Rukleng N, Kongpool W, Pinsook P, Wongwiwat W. Ethnomedicinal plants used by traditional healers in Phatthalung Province, Peninsular Thailand. J Ethnobiol Ethnomed. 2015; 11:43.
20. Neamsuvan O, Komonhiran P, Boonming K. Medicinal plants used for hypertension treatment by folk healers in Songkhla province, Thailand. J Ethnopharmacol. 2018; 214: 58-70.
21. Chanthasri W, Puangkeaw N, Kunworarath N, Jaisamut P, Limsuwan S, Maneenoon K, Choochana P, Chusri S. Antioxidant capacities and total phenolic contents of 20 polyherbal remedies used as tonics by folk healers in Phatthalung and Songkhla provinces, Thailand. BMC Complement Altern Med. 2018; 18(1):73.
22. Issuriya A, Puangkeaw N, Choochana P, Jaisamut P, Kunworarath N, Maneenoon K, Limsuwan S, Kaewmanee T, Chusri S. Safety and antioxidant potential of traditional thai poly-herbal tea 'Phy-Blica-D' used as a rejuvenation formula. Pharmacogn Res. 2019; 11(3):295-303.
23. El-Sayed M, Metwally N, Ibrahim I, Abdel-Hady H, Abdel-Wahab BS. Antioxidant activity, total phenolic and flavonoid contents of *Petroselinum crispum* Mill. J Appl Life Sci Int. 2018; 19(2):1-7.
24. Sun D, Yan Q, Xu X, Shen W, Xu C, Tan J, Zhang H, Li L, Cheng H. LC-MS/MS analysis and evaluation of the anti-inflammatory activity of components from BushenHuoxue decoction. Pharm Biol. 2017; 55(1):937-945.
25. Tel-Çayan G, Çayan F, Deveci E, Duru ME. Phenolic profile, antioxidant and cholinesterase inhibitory activities of four Trametes species: *T. bicolor*, *T. pubescens*, *T.* suaveolens, and *T. versicolor*. J Food Meas Charact. 2021; 2021:1-9.
26. Çelik SE, Bekdeşer B, Apak R. A novel colorimetric sensor for measuring hydroperoxide content and peroxyl radical scavenging activity using starch-stabilized gold nanoparticles. Talanta. 2019; 196:32-38.
27. Aan GJ, Zainudin MS, Karim NA, Ngah WZ. Effect of the tocotrienol-rich fraction on the lifespan and oxidative biomarkers in *Caenorhabditis elegans* under oxidative stress. Clinics (Sao Paulo). 2013; 68(5):599-604. .
28. Jubri Z, Rahim NB, Aan GJ. Manuka honey protects middle-aged rats from oxidative damage. Clinics (Sao Paulo). 2013; 68(11):1446-1454.
29. Abdul Qadir M, Shahzadi SK, Bashir A, Munir A, Shahzad S. Evaluation of phenolic compounds and antioxidant and antimicrobial activities of some common herbs. Int J Anal Chem. 2017; 2017:3475738.
30. Kilani-Jaziri S, Neffati A, Limem I, Boubaker J, Skandrani I, Sghair MB, Bouhlel I, Bhouri W, Mariotte AM, Ghedira K, Franca MGD, Chekir-Ghedira L. Relationship correlation of antioxidant and antiproliferative capacity of *Cyperus rotundus* products towards K562 erythroleukemia cells. Chem Biol Interact. 2009; 181(1):85-94.
31. Mahaldar K, Hossain A, Islam F, Islam S, Islam MA, Shahriar M, Rahman MM. Antioxidant and hepatoprotective activity of *Piper retrofractum* against Paracetamol-induced hepatotoxicity in Sprague-Dawley rat. Nat Prod Res. 2020; 34(22): 3219-3225.
32. Naik GH, Priyadarsini KI, Bhagirathi RG, Mishra B, Mishra KP, Banavalikar MM, Mohan H. *In vitro* antioxidant studies and free radical reactions of triphala, an ayurvedic formulation and its constituents. Phytother Res. 2005; 19(7):582-586.
33. Khan A, Nazar H, Sabir SM, Irshad M, Awan SI, Abbas R, Akram M, Khaliq A, Rocha JB, Ahmad SD, Malik F. Antioxidant activity and inhibitory effect of some commonly used medicinal plants against lipid per-oxidation in mice brain. Afr J Tradit Complement Altern Med. 2014; 11(5):83-90.
34. Tanaka M, Kishimoto Y, Sasaki M, Sato A, Kamiya T, Kondo K, Iida K. *Terminalia bellirica* (Gaertn.) Roxb. extract and gallic Acid attenuate LPS-induced inflammation and oxidative stress via MAPK/NF-kappaB and Akt/AMPK/Nrf2 pathways. Oxid Med Cell Longev. 2018; 2018:9364364.
35. Rajkumari J, Dyavaiah M, Sudharshan SJ, Busi S. Evaluation of *in vivo* antioxidant potential of *Syzygium jambos* (L.) Alston and *Terminalia citrina* Roxb. towards oxidative stress response in *Saccharomyces cerevisiae*. J Food Sci Technol. 2018; 55(11):4432-4439.
36. Ahmad W, Jantan I, Bukhari SN. *Tinospora crispa* (L.) Hook. f. & Thomson: a review of its ethnobotanical, phytochemical, and pharmacological aspects. Front Pharmacol. 2016; 7:59.
37. Naji KM, Al-Shaibani ES, Alhadi FA, Al-Soudi SA, D'Souza M R. Hepatoprotective and antioxidant effects of single clove garlic against CCl_4-induced hepatic damage in rabbits. BMC Complement Altern Med. 2017; 17:411.
38. Kadir FA, Othman F, Abdulla MA, Hussan F, Hassandarvish P. Effect of *Tinospora crispa* on thioacetamide-induced liver cirrhosis in rats. Indian J Pharmacol. 2011; 43(1):64-68.
39. Nwozo SO, Osunmadewa DA, Oyinloye BE. Anti-fatty liver effects of oils from *Zingiber officinale* and *Curcuma longa* on ethanol-induced fatty liver in rats. J Integr Med. 2014; 12(1):59-65.
40. Abdel-Hafeez EH, Ahmad AK, Kamal AM, Abdellatif MZ, Abdelgelil NH. *In vivo* antiprotozoan effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) extracts on experimentally infected mice with

Blastocystis spp. Parasitol Res. 2015; 114(9):3439-3444.
41. Tasanarong A, Kongkham S, Itharat A. Antioxidant effect of *Phyllanthus emblica* extract prevents contrast-induced acute kidney injury. BMC Complement Altern Med. 2014; 14:138.
42. Gauthaman K, Banerjee SK, Dinda AK, Ghosh CC, Maulik SK. *Terminalia arjuna* (Roxb.) protects rabbit heart against ischemic-reperfusion injury: role of antioxidant enzymes and heat shock protein. J Ethnopharmacol. 2005; 96(3):403-409.
43. Maulik SK, Wilson V, Seth S, Bhargava B, Dua P, Ramakrishnan S, Katiyar CK. Clinical efficacy of water extract of stem bark of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. in patients of chronic heart failure: a double-blind, randomized controlled trial. Phytomedicine. 2016; 23(11):1211-1219.
44. Usharani P, Merugu PL, Nutalapati C. Evaluation of the effects of a standardized aqueous extract of *Phyllanthus emblica* fruits on endothelial dysfunction, oxidative stress, systemic inflammation and lipid profile in subjects with metabolic syndrome: a randomised, double blind, placebo controlled clinical study. BMC Complement Altern Med. 2019; 19(1):97.
45. Salehi B, Azzini E, Zucca P, Maria Varoni E, V. Anil Kumar N, Dini L, Panzarini E, Rajkovic J, Valere Tsouh Fokou P, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, El Beyrouthy M, Setzer W, Polito L, Iriti M, Sureda A, Magdalena Quetglas-Llabrés M, Martorell M, Martins N, Sharifi-Rad M, M. Estevinho L, Sharifi-Rad J. Plant-derived bioactives and oxidative stress-related disorders: a key trend towards healthy aging and longevity promotion. Appl Sci. 2020; 10(3):947.
46. Dai J and Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15(10):7313-7352.
47. Zaidun NH, Thent ZC, Latiff AA. Combating oxidative stress disorders with citrus flavonoid: Naringenin. Life Sci. 2018; 208:111-122.
48. Priyadarsini KI, Khopde SM, Kumar SS, Mohan H. Free radical studies of ellagic acid, a natural phenolic antioxidant. J Agric Food Chem. 2002; 50(7):2200-2206. .
49. Sarkaki A, Farbood Y, Dolatshahi M, Mansouri SM, Khodadadi A. Neuroprotective effects of ellagic acid in a rat model of Parkinson's disease. Acta Med Iran. 2016; 54(8):494-502.
50. Kiasalari Z, Heydarifard R, Khalili M, Afshin-Majd S, Baluchnejadmojarad T, Zahedi E, Sanaierad A, Roghani M. Ellagic acid ameliorates learning and memory deficits in a rat model of Alzheimer's disease: an exploration of underlying mechanisms. Psychopharmacol. 2017; 234(12):1841-1852.
51. Lai CH, Chou CY, Ch'ang LY, Liu CS, Lin W. Identification of novel human genes evolutionarily
62. .
conserved in *Caenorhabditis elegans* by comparative proteomics. Genome Res. 2000; 10(5):703-713.
52. Saul N, Pietsch K, Stürzenbaum SR, Menzel R, Steinberg CE. Diversity of polyphenol action in *Caenorhabditis elegans*: between toxicity and longevity. J Nat Prod. 2011; 74(8):1713-1720.
53. Huang CH, Hsu FY, Wu YH, Zhong L, Tseng MY, Kuo CJ, Hsu AL, Liang SS, Chiou SH. Analysis of lifespan-promoting effect of garlic extract by an integrated metabolo-proteomics approach. J Nutr Biochem. 2015; 26(8):808-817.
54. Lee EB, Kim JH, Kim YJ, Noh YJ, Kim SJ, Hwang IH, Kim DK.Lifespan-extending property of 6-shogaol from *Zingiber officinale* Roscoe in *Caenorhabditis elegans*. Arch Pharm Res. 2018; 41(7):743-752.
55. Grünz G, Haas K, Soukup S, Klingenspor M, Kulling SE, Daniel H, Spanier B. Structural features and bioavailability of four flavonoids and their implications for lifespan-extending and antioxidant actions in *C. elegans*. Mech Ageing Dev. 2012; 133(1):1-10.
56. Hernández-Aquino E, Zarco N, Casas-Grajales S, Ramos-Tovar E, Flores-Beltrán RE, Arauz J, Shibayama M, Favari L, Tsutsumi V, Segovia J, Muriel P. Naringenin prevents experimental liver fibrosis by blocking TGFbeta-Smad3 and JNK-Smad3 pathways. World J Gastroenterol. 2017; 23(24):4354-4368.
57. Hermenean A, Ardelean A, Stan M, Hadaruga N, Mihali CV, Costache M, Dinischiotu A. Antioxidant and hepatoprotective effects of naringenin and its beta-cyclodextrin formulation in mice intoxicated with carbon tetrachloride: a comparative study. J Med Food. 2014; 17(6):670-677.
58. Yen FL, Wu TH, Lin LT, Cham TM, Lin CC. Naringenin-loaded nanoparticles improve the physicochemical properties and the hepatoprotective effects of naringenin in orally-administered rats with CCl(4)-induced acute liver failure. Pharm Res. 2009; 26(4):893-902.
59. Vijaya PV, Kalai SP, Sravani S. Protective effect of ellagic acid against TCDD-induced renal oxidative stress: modulation of CYP1A1 activity and antioxidant defense mechanisms. Mol Biol Rep. 2014; 41(7):4223-4232.
60. Wetchakul P, Goon JA, Adekoya AE, Olatunji OJ, Ruangchuay S, Jaisamut P, Issuriya A, Kunworarath N, Limsuwan S, Chusri S. Traditional tonifying polyherbal infusion, Jatu-Phala-Tiga, exerts antioxidant activities and extends lifespan of *Caenorhabditis elegans*. BMC Compl Altern Med. 2019; 19(1):209.
61. Rathor L, Pant A, Awasthi H, Mani D, Pandey R. An antidiabetic polyherbal phytomedicine confers stress resistance and extends lifespan in *Caenorhabditis elegans*. Biogerontol. 2017; 18(1):131-147