Trop J Nat Prod Res, June 2022; 6(6):868-871 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical Screening and Antioxidant Activity of Longevity Remedy from National Thai Traditional Medicine Scripture (Formulary Special Edition)

Ampa Konsue[1] and Ladachart Taepongsorat[2]*

[1]*Thai Traditional Medicinal Research Unit, Division of Applied Thai Traditional Medicine, Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand*
[2]*Biomedical Unit, Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand*

ARTICLE INFO

Article history:
Received 21 September 2021
Revised 20 May 2022
Accepted 17 June 2022
Published online 02 July 2022

ABSTRACT

A longevity remedy from Thai traditional medicine (formulary special edition) has been linked to body health nourishment, appetizing, muscle pain relief, relaxation, normal excretion, body health improvement, and, most importantly, long-life aging. This longevity remedy has been described to possess anti-aging properties, however, there is no scientific justification to support this. The present study was therefore aimed at conducting a phytochemical screening and evaluating the antioxidant activities of the longevity remedy from Thai traditional medicine (formulary special edition). The wood of *Streblus asper*, *Diospyros rhodocal*yx, and *Albizia proc*era, as well as *Tinospora cordifolia v*ine, *Piper nigrum* fruits, and *Cyperus rotundus* were formulated into longevity remedy. Aqueous, hydro-ethanol, and ethanol extracts were prepared from the longevity remedy. In a phytochemical screening, total phenolic content (TPC) and total flavonoid content (TFC) were measured. The antioxidant activities were examined by 2,2-diphenyl-1-picrylhydrazy (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^{+}$) radical scavenging, and ferric reducing antioxidant power (FRAP) assays. The results revealed that the 50% ethanol extract contained high amounts of TPC (3.617±0.763 mgGE/gExt) and TFC (5.132±0.082 mgQE/gExt). Furthermore, DPPH (IC_{50} = 0.0323±0.0008 mg/mL), ABTS (IC_{50} = 0.0159±0.0004 mg/mL), and FRAP (10.013±0.810 mgTE/gExt) activities were scavenged by this extract according to the techniques used. The findings of this study confirmed the composition of this remedy with TPC and TFC in which it plays a protective role in antioxidant activities.

Keywords: Antioxidant, Flavonoids, Longevity, Phenolic compounds, Thai traditional medicine.

Introduction

The world's population is rapidly aging, posing a threat to economic growth and society by reducing labor force and productivity, decreasing aging life quality, and increasing government spending on aging welfare.[1] Longevity and healthy aging research have the potential to extend human lives and reduce the burden of degenerative diseases, with significant social and economic implications.[2] Aging is a complicated and irreversible degeneration that affects not only body tissues but also body organ physiological processes such as immunological responses, memory loss, and so on. Several studies have found a link between aging and a variety of chronic diseases, such as hypertension, diabetes, atherosclerosis, Alzheimer's disease, Parkinson's disease, and multiple malignancies.[3] Throughout the twentieth century, infectious and communicable diseases indicated that mortality was increasingly concentrated in the elderly, and non-communicable diseases became the leading causes of death.[4] Aging is marked by the buildup of molecular and cellular damage in cells and tissues, resulting in structural and functional abnormalities.[5] Reduced mortality from both communicable and non-communicable diseases has accelerated the global aging population. Longevity aging is associated with a shorter life expectancy due to an increased burden of non-communicable diseases. Aging, health span, and life span are tightly related concepts. While aging is a natural process, stress, poor nutrition, and pollution have all been linked to the increased internal generation of free radicals.[6]

The antioxidant system in plants is amazingly efficient at reducing the effects of oxidative stress.[7] Plant secondary metabolites, such as polyphenols, are a wide and diverse category of chemicals that are abundant in the majority of plants. They protect against reactive oxygen and nitrogen species, ultraviolet radiation, diseases, parasites, and plant predators.[8] Secondary metabolites originating from plants have played a pivotal role in the health care of many ancient and modern societies. Polyphenols, flavonoids, alkaloids, terpenoids, steroids, and other plant-derived natural compounds have a wide range of pharmacological effects, including antioxidation. Antioxidant compounds have the potential to mitigate the effects of free radicals, making them a valuable health-protective component.[9] *Streblus asper* wood, *Diospyros rhodocal*yx wood, *Albizia proc*era wood, *Tinospora cordifolia v*ine, *Piper nigrum* fruits, and *Cyperus rotundus* make up a lifespan treatment from Thai traditional medicine (formulary special edition). Tubers have traditionally been utilized for health promotion purposes, including nourishment, appetizing, muscle pain reduction, relaxation, normal excretion, health improvement, and, most notably, long-life aging. Although this longevity remedy was indicated to be anti-aging, there is no scientific evidence to support this.

Therefore, the aim of this study was to conduct a phytochemical screening and evaluate the antioxidant activities of longevity remedy from Thai traditional medicine (formulary special edition).

*Corresponding author. E mail: ladachart@msu.ac.th
Tel: +66 43 722 393

Citation: Konsue A and Taepongsorat L. Phytochemical Screening and Antioxidant Activity of Longevity Remedy from National Thai Traditional Medicine Scripture (Formulary Special Edition). Trop J Nat Prod Res. 2022; 6(6):868-871. doi.org/10.26538/tjnpr/v6i6.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Source of plant materials

The plants, which include *Streblus asper* wood (code: MSU.MED-SA0001/LT), *Diospyros rhodoca*lyx wood (code: MSU.MED-DR0001/LT), *Albizia proc*era wood (code: MSU.MED-AP0001/LT) , *Tinospora cordifolia v*ine (code: MSU.MED-TC0001/LT), *Piper nigrum* fruits (code: MSU.MED-PN0001/LT), and *Cyperus rotundus* (code: MSU.MED-CR0001/LT) rhizomes used in the formulation of the remedy, were collected from Maha Sarakham province in northeastern Thailand between October and December 2020. The specimens were identified and deposited at the Faculty of Medicine, Mahasarakham University, Thailand. The plant materials were cleaned and dried in a hot air oven at 60°C for three days, and then blended into fine powder.

Preparation of plant extracts

The aqueous extract of the remedy was prepared by boiling 100 g of the sample twice in 1000 mL of distilled water for 10 minutes each time. The hydro-ethanolic and ethanolic extracts were macerated for 7 days in 50 and 95% ethanol, respectively (100 g per 400 mL). The residual powder was removed by using filter paper (Whatman, Germany). The filtrate was evaporated using a rotary evaporator (Heidolph Laborota 4000, Germany) and freeze-dried to obtain a dark brown extract. The extracts were stored in the refrigerator at -20°C until they were needed.[10]

Determination of total phenolic content

Total phenolic content was determined according to a modified procedure. The sample (100 µL) was oxidized with 500 µL of 0.2 N Folin-Ciocalteu's reagent and neutralized by adding 400 µL of 7.5% Na_2CO_3. After mixing and incubating at room temperature for 30 minutes, the absorbance was measured at 765 nm. The results were expressed as gallic acid equivalents (mgGE/gExt).[10]

Estimation of total flavonoid content

Flavonoid content was estimated using the aluminum chloride colorimetric method. The plant extract was mixed with 100 µL of 5% aluminum chloride (w/v), 400 µL of 2.5% Na_2NO_3. After 5 min, 500 µL of 5% $AlCl_3$ was added. The mixture was allowed to stand at room temperature for 10 min. Two milliliters were added to the solution. The absorbance was measured at 415 nm. The TFC was calculated from a standard quercetin equivalent (mgQE/gExt).[10]

Evaluation of antioxidant activities

DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacities of wheat extracts were estimated by the reduction of the reaction color between DPPH solution and sample extracts as previously described by the prior method. DPPH was dissolved in ethanol to a concentration of 0.039 mg/mL. The plant extract at various concentrations was diluted with distilled water to get a sample solution. One hundred microliters of the sample solution was combined with a 900 µL DPPH (0.1 mM) working solution. After a 30-minute reaction, the mixture was kept in the dark at room temperature. The absorbance of the solution was then measured at 515 nm. Trolox® and ascorbic acid were used as standard chemicals. Blanks were run in each assay. DPPH radical ability was expressed as inhibitory concentration (IC_{50}; mg/mL) and the inhibition percentage was calculated using the following formula:

% DPPH radical scavenging activity = $[(A_0-A_1)/ A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.[11]

$ABTS^+$ radical scavenging assay

In the $ABTS^+$ assay, the plant extract is allowed to react with $ABTS^+$, a model stable free radical derived from 2,2-azinobis (3-ethylvenzothiazolin-6-sulphonic acid). The $ABTS^+$ (900 µL) was added to the extract (100 µL) and thoroughly mixed. The mixture was kept at room temperature for 6 minutes, and absorbance was immediately measured at 734 nm. Trolox® and ascorbic acid solution in 80% ethanol were prepared and assayed under the same conditions. ABTS scavenging ability was expressed as IC_{50} (mg/mL) and the inhibition percentage was calculated using the following formula:

% ABTS radical scavenging activity = $[(A_0-A_1)/ A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.[11]

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of the medicinal plant was estimated spectrophotometrically following the method described by Puangpornpitag, Tankitijano, Sumalee, and Konsue.[12] This reaction was monitored by measuring the change in absorbance at 593 nm. The ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°C. Using a 1-5 mL variable micropipette, a freshly prepared working FRAP reagent was pipetted into 5 µL of the correctly diluted plant sample and carefully mixed. When the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to its ferrous (Fe^{2+}) form, a strong blue color complex was formed, and the absorbance at 593 nm was measured against a reagent blank (3.995 mL FRAP reagent, 5 µL pure water) after 30 minutes at 37°C. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. The concentrations of $FeSO_4$ were in turn plotted against the concentrations of the standard antioxidant Trolox®. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and were expressed as mg of Trolox equivalent per gram of the sample.

Statistical analysis

All data were expressed as mean ± standard deviation of mean (SD) from five separate experiments (n = 5). Statistical analysis was carried out using the Statistical Package for Social Sciences (version 23). Mean separation was determined with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered to be significant.

Results and Discussion

The 50% ethanolic extract from the longevity remedy extract contains much more TPC and TFC than the aqueous and 95% ethanolic extracts (Table 1). Some literature report that one of the plant compositions in this recipe, *A. procera* contains highly phenolic compounds that contribute to the determination of total phenolic contents. The ethyl acetate fraction had the highest antioxidant activity and total phenolic content. This extract may be utilized to prevent a variety of diseases associated with free radicals.[13] Antidiabetic, hypolipidemic, hepatoprotective, and antibacterial effects have been reported for *C. rotundus*, a plant component. It contains secondary metabolites such as flavonoids, tannins, glycosides, monoterpenes, sesquiterpenes, sitosterol, alkaloids, saponins, terpenoids, essential oils, starch, carbohydrates, protein, and isolated amino acids. This indicates the increased interest in the medicinal potential and chemical ingredients of longevity drugs.[14]

Many secondary metabolites were found in the remedy, including flavonoids (visnagin, khellin, ammiol, isorhamnetin, and tricin) as well as phenolic chemicals.[15] The total phenolic content of the rhizome of the plant was determined using water and 50% ethanol extracts, as well as the activity of free radical scavenging using DPPH and FRAP assays.

Table 1: The total phenolic content (TPC) and total flavonoid content (TFC) of various extracts from the longevity drug

Sample	TPC (mgGE/gExt)	TFC (mgQE/gExt)
Aqueous extract	2.159 ± 0.205^b	1.356 ± 0.025^a
50% ethanolic extract	3.617 ± 0.763^c	5.132 ± 0.082^b
95% ethanolic extract	1.392 ± 0.241^a	1.700 ± 0.098^a

The TPC was measured with gallic acid equivalent (mgGE/gExt). TFC was measured with quercetin equivalent (mgQE/gExt). Different letters in each column indicate significant difference at $p < 0.5$.

Table 2: The antioxidant activities of various extracts from the longevity remedy.

Sample	DPPH (IC_{50} = mg/mL)	ABTS (IC_{50} = mg/mL)	FRAP (mg = TE/gExt)
Aqueous extract	0.1195 ± 0.0091^{e}	0.0391 ± 0.0032^{e}	5.093 ± 0.100^{b}
50% ethanolic extract	0.0323 ± 0.0008^{c}	0.0159 ± 0.0004^{c}	10.013 ± 0.810^{c}
95% ethanolic extract	0.0556 ± 0.0015^{d}	0.0213 ± 0.0006^{d}	4.734 ± 0.975^{a}
Ascorbic acid	0.0007 ± 0.0001^{a}	0.0004 ± 0.00001^{a}	-
Trolox®	0.0037 ± 0.0002^{b}	0.0030 ± 0.00002^{b}	-

DPPH: 2,2-diphenyl-1-picrylhydrazy; $ABTS^{+}$: 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate); FRAP: Ferric reducing antioxidant power. The DPPH radical scavenging, $ABTS^{+}$ and FRAP assays were used to measure the antioxidant capacities. Trolox® and ascorbic acid were employed as standard substances. Different letters in each column indicated significant difference at $p < 0.5$.

The rhizome contained a high level of polyphenols, as well as a high FRAP and the same ability to scavenge DPPH free radicals.[16] In addition, *T. crispa*, which contains flavonoids and some secondary metabolites, could be a potential source of drugs that in the future may serve for the production of synthetically improved therapeutic agents.[17] Apigenin is a flavonoid component that was isolated from the least polar fractions of a *T. crispa* ethyl acetate extract using column chromatography followed by recycling preparative HPLC and demonstrated free radical scavenging activity.[18]

On the DPPH, ABTS, and FRAP assays (Table 2), the 50% ethanolic extract had a positive effect on free radical scavenging. The plant in the longevity recipe, *S. asper* is a promising natural source for neuroprotective agents with antibacterial and antioxidant properties, as well as AChE inhibitors, which will aid in the creation of new natural chemicals in anti-aging therapies.[19] In diabetics, *S. asper* extract reduced blood glucose levels and improved DPPH radical scavenging.[20] Studies have found that some plants in the *S. asper* recipe have antiallergic, antifilarial, cardiotonic, antimicrobial, anti-Parkinson, hepato-protective, anti-diabetic, anti-inflammatory, antiviral, and analgesic properties.[21] The essential oil from *C. rotundus* rhizomes demonstrated high antioxidant activity, DNA damage protection, and cytotoxic effects on the human neuroblastoma SH-SY5Y cell, as well as antibacterial activity against a variety of foodborne pathogens. The scavenging activity of essential oil from *C. rotundus* rhizomes on DPPH and ABTS radicals showed concentration-dependent scavenging of the DPPH and ABTS radicals at certain concentrations. This observation indicates that the antioxidant activity of essential oils could be mediated by the direct trapping of free radicals through hydrogen or electron transfers.[22] The plant composition in this remedy, *T. crispa*, was reported to have high antioxidant activity and polyphenolic concentration in some research. This remedy was effective in reducing diabetes complications. The effects of the components in the herbs were discovered to be extremely appropriate to develop further when investigating the composition of substances. This is because the mechanism of action from the components in these herbs can assist in reducing blood sugar levels and diabetes issues.[23]

Conclusion

The findings of this study reveal that the longevity remedy contains TPC and TFC, which play a protective role in antioxidant activities. It suggests that health-promoting substances with antioxidant and anti-aging benefits for human body health could be produced.

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.

Acknowledgements

The study was funded by the Scholarships for Research Promotion and Development of Staff in the Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand.

References

1. Putson C and Pornsirivatcharasin K. Solutions to the problem of an aging society in Thailand. Hum Soc Sci Res Pro Net. 2018; 1(1):25-35.
2. Taormina G, Ferrante F, Vieni S, Grassi N, Russo A, Mirisola MG. Longevity: lesson from model organisms. Genes. 2019; 10(518):1-20.
3. Kong SZ, Li JC, Li SD, Liao MN, Li CP, Zheng PJ, Guo MHi, Tan WX, Zheng ZH, Hu Z. Anti-aging effect of chitosan oligosaccharide on D-galactose-induced sub-acute aging in mice. Mar Drugs. 2018; 16(181):1-13.
4. Ostan R, Monti D, Gueresi P, Bussolotto M, Franceschi C, Baggio G. Cardiovascular disease (CVD) type 2 diabetes (t2d) cancer neurodegenerative diseases disability stress and spousal bereavement gender, aging and longevity in humans: an update of an intriguing/neglected scenario paving the way to a gender-specific medicine. Clin Sci. 2016; 130(19):1711-1725.
5. Carmona JJ and Michan S. Biology of Healthy Aging and Longevity. Rev Inves Clin. 2016; 68(1):7-16.
6. Kunugi H and Ali AM. Royal jelly and its components promote healthy aging and longevity: from animal models to humans. Int J Mol Sci. 2019; 20(4662):1-26.
7. Puthur JT. Antioxidants and cellular antioxidation mechanism in plants. South Indian J Bio Sci. 2016; 2(1); 14-17.
8. Mojzer EB, Hrnˇciˇc M, Škerget M, Knez Ž, Bren U. Polyphenols: Extraction methods, antioxidative action, bioavailability, and anticarcinogenic effects. Mol. 2016; 21(901):1-38.
9. Patel M, Verma R, Srivastav P. Antioxidant activity of *Eclipta alba* extract. J Med Plant Stud. 2016; 4(5):92-98.
10. Chanhan P, Konsue A, Nammatra R. Effect of an agricultural model of using fertilizer, harvesting time, and extraction method on phytochemical contents and antioxidant activities from mulberry leaves grown in Maha Sarakham Province, Thailand. Pharmacogn J. 2019; 11(3):531-535.
11. Taepongsorat L and Konsue A. Biological screening of tri-jannarose as a recipe from Thai traditional medicine. Phcog Res. 2019; 11(2):110-114.
12. Puangpornpitag D, Tankitijano P, Sumalee A, Konsue A. Phytochemical screening and antioxidant activities of the seedling extracts from Inca Peanut *Plukenetia volubilis*. Pharmacogn J. 2021; 13(1):1-7.
13. khatoon M, Islam E, Islam R, Rahman AA, Alam AHMK, Khondkar P, Rashid M, Parvin S. Estimation of total phenol

and in vitro antioxidant activity of *Albizia procera* leaves. BMC Res Notes. 2013; 6(21):1-7.
14. Kumar M, Rani M, Meher B. Review on pharmacology and phytochemistry of *Cyperus rotundus* L. Curr Res Pharm Sci. 2017; 07(01):11-15.
15. Al-Snafi AE. A review on Cyperus rotundus a potential medicinal plant. IOSR J Pharm. 2016; 6(7):32-48.
16. Safriani N, Erfiza NM, Arpi N. Antioxidant activities of *Cyperus rotundus* L. rhizome and *Areca catechu* L. seed. Int J Adv Sci Eng Inf Technol. 2016; 6(3):285-288.
17. Mahalle D and Gupta A. Comparative qualitative phytochemical analysis of the different parts of *Tinospora crispa*: A contribution to sustainable use of the plant species. J Drug Deliv Ther. 2019; 9(4):795-798.
18. Ismail M and Choudhary MI. Compounds isolated from *Tinospora crispa*. Chem Nat Commun. 2016; 52(6):1151-1153.
19. Prasansuklab A, Theerasri A, Payne M, Ung AT, Tencomnao T. Acid-base fractions separated from *Streblus asper* leaf ethanolic extract exhibited antibacterial, antioxidant, antiacetylcholinesterase, and neuroprotective activities. BMC Compl Altern Med. 2018; 18(223):1-13.
20. Rahman MO, Alqahtani AB, Huda SB, Siddiqui SA, Noman OM, Nasr F, Hassan A, Islam SN. *Streblus asper* attenuates alloxan-induced diabetes in rats and demonstrates antioxidant and cytotoxic effects. Pharm Biol. 2021; 59(1):1058-1064.
21. Neekhra S, Awasthi H, Singh DCP. Potential therapeutic use of *Streblus asper*: a review. Int J Res Dev Pharm Life Sci. 2017; 6(7):2845-2849.
22. Hu QP, Cao XM, Hao DL, Zhang L. Chemical composition, antioxidant, DNA damage protective, cytotoxic and antibacterial activities of *Cyperusrotundus* rhizomes essential oil against foodborne pathogens. Sci Rep. 2017; 7(45231):1-9.
23. Pumthong G, Junlatat J, Thiantongin P, Chaweerak S. Antioxidant activity and total phenolic compounds of herbs and traditional recipes to reduce complications of diabetes. J Health Sci Res Ubon Rachathani Univ. 2019; 8(1):8-16.