

Phytochemical Analysis and Antioxidant Property of Selected Medicinal Plants Native to Cambodia



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Abstract

Cambodian medicinal plants have been used to treat different diseases such as cardiovascular diseases, inflammation, cancers, diabetes and AIDS. This study aimed at conducting the Phytochemical analysis and *in vitro* antioxidant activities of whole plant of *Bryophyllumpinnatum* (Lam.) Kurz, barks of *Dillenia ovata* Wall. ex Hook. f. & Thomson, rhizomes of *Drynariafortunei* (Kunze ex Mett.) J. Sm. and barks of *Lophopetalumwallichii* Kurz. native to Cambodia. All the plants were extracted with ethanol by maceration extraction method. The Phytochemical analysis for alkaloids, phenolic compounds, tannins, flavonoids, coumarins, steroids, terpenoids, cardiac glycosides, essential oils, saponins and resins by using the standard methods. The *in vitro* antioxidant property was evaluated by assessing the DPPH[•] radical scavenging ability. The preliminary Phytochemical evaluation of these species exhibited that the ethanolic extracts of *B. pinnatum* (whole plant), *D. ovata* (barks), *D. fortunei* (rhizomes) and *L. wallichii* (barks) contain alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, cardiac glycosides, saponins and resins. Moreover, the ethanolic extracts of *B. pinnatum* whole plant, *D. ovata* barks, and *D. fortunei* rhizomes possess coumarins and steroids, and of *L. wallichii* barks have essential oils. The *in vitro* antioxidant activity of the species *B. pinnatum* whole plant, *D. ovata* barks, *D. fortunei* rhizomes and *L. wallichii* barks have prominent antioxidant activities. This study suggests the potential source of natural antioxidant in *B. pinnatum*, *D. ovata*, *D. fortunei* and *L. wallichii* native to Cambodia. Further research is highly recommended on the isolation of the antioxidant compounds from these species.

Keywords: *B. pinnatum*; *D. ovate*; *D. fortune*; *L. wallichii*; Phytochemical analysis; *In vitro* antioxidant activity

Introduction

Reactive Oxygen Species (ROS) or free radicals are unstable intermediates formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron, via cleavage of a radical to give another radical and, also via redox reactions [1]. The ROS are categorized into oxygen-centered radicals and oxygen-centered non-radicals. The oxygen-centered radicals include superoxide anion ($\bullet\text{O}_2^-$), hydroxyl radical ($\bullet\text{OH}$), alkoxy radical ($\text{RO}\bullet$) and peroxy radical ($\text{ROO}\bullet$), and nitrogen species. The oxygen-centered non-radicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$), hypochlorous acid (HClO) and ozone (O_3) [2]. Free radicals are also generated either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants,

and industrial chemicals causing oxidative stress which is able to adversely alter lipids, protein and DNA triggering various human complications such as cancers, atherosclerosis, neurodegenerative diseases, diabetes, age-related eye disease and Parkinson's disease [3].

The free radicals can be scavenged by using the antioxidant system including non-enzymatic constituents and a series of antioxidant enzymes. The non-enzymatic constituents include glutathione, selenium, vitamin C and E. The antioxidant enzymes embrace glutathione peroxidase, catalase and superoxide dismutase which are the predominant antioxidant enzymes playing a key role in minimizing the oxidative stress [4]. Several studies reported the free radical scavenging activities of medicinal plants

considered as the natural antioxidant, which is utilized in several medical applications as their assurance of effectiveness and safety [5]. The medicinal plants in form of roots, barks, leaves, seeds and fruits have been used to manage various illnesses since the ancient Cambodian era [6]. Medicinal plants are composed of some bioactive compounds influencing the physiological functions of the human body and these active phyto constituents include alkaloids, terpenes and phenolic compounds [7], biosynthesized by the pathways of acetyl coenzyme A, shikimic acid, mevalonic acid and 1-deoxyulose 5-phosphate in the plant [8]. The Phytochemical is being broadly examined for its ability to provide health benefits, and several reports proved their curative effects on cardiovascular diseases, inflammation, cancers, diabetes and AIDS [9], the underlying mechanism of which is due to their bioactivities as substrates for biochemical reactions, cofactors of enzymatic reactions, and inhibitors of enzymatic reactions [10].

However, no much scientific authentication has been made for most of medicinal plants native to Cambodia. To address this lacuna, the present investigation was carried out for the Phytochemical analysis and *in vitro* antioxidant activities of whole plant of *Bryophyllumpinnatum* (Lam.) Kurz (Crassulaceae) (local name: KabelLapoahs), barks of *Dillenia ovata* Wall. ex Hook. f. & Thomson (Dilleniaceae) (local name: Phlu Thom), rhizomes of *Drynariafortunei* (Kunze ex Mett.) J. Sm. (Polypodiaceae) (local name: Baabrak), and barks of *Lophopetalumwallichii* Kurz (Celastraceae) (local name: *Puen Ta Lei*) [11].

Material and Methods

Collection of Plants

The whole plant of *B. pinnatum*, the barks of *D. ovata*, the rhizomes of *Dfortunei* and the barks of *L.wallichii* in the dried form were collected from the local drugstore selling medicinal plants in Phnom Penh, Cambodia, in August 2017. The plants were authenticated with the voucher specimens UPFPT-110071 (*B. pinnatum*), UPFPT-110057 (*D. ovata*), UPFPT-110069 (*D. fortunei*) and UPFPT-110066 (*L. wallichii*) of University of Puthisastra (UP)-Herbarium. The parts of the plant samples were deposited in the UP-Herm barium and the Pharmacognosy Laboratory, Department of Pharmacy, Faculty of Health Sciences, University of Puthisastra, Cambodia, aiming at conducting further investigation.

Preparation of Plant Extracts

The maceration method was applied to the extraction of the plants. Each plant part was powdered and kept in contact with ethanol in the concentration of 157g/3500mL (*B. pinnatum* whole plant), 420g/6100 mL (*D. ovata* barks), 192g/3800mL (*Dfortunei* rhizomes), and 161g/5600mL (*L.wallichii* barks). The supernatants were collected by filtration after 24 hours, and the solvent was evaporated to make the crude extracts. Each crude extract was subjected to the lyophilisation. The residues obtained were stored in airtight bottles in a refrigerator for the phytochemical evaluation

and the *in vitro* antioxidant activity. The plant extraction was carried out at the Laboratory of Biological Pharmacology, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, KhonKaen University, Thailand.

Phytochemical Analysis

The ethanolic extracts of *B. pinnatum* whole plant, *D. ovata* barks, *Dfortunei* rhizomes and *L.wallichii* barks underwent the Phytochemical screening in order to detect the presence or the absence of alkaloids, phenolic compounds, tannins, flavonoids, coumarins, steroids, terpenoids, cardiac glycosides, essential oils, saponins and resins by using the standard methods [12]. This Phytochemical test was evaluated in the Pharmacognosy Laboratory, University of Puthisastra.

Evaluation of Alkaloids (Dragendorff's, Mayer's and Wagner's Tests): The crude extracts were dissolved with 1M-HCl (100mg/10 mL) and subjected to the filtration. The filtrate was loaded equally into four test tubes. One control test tube was added with no reagent, and the rest of test tubes were treated with Dragendorff's, Mayer's or Wagner's reagents. The orange red (Dragendorff), creamy white (Mayer) or reddish brown (Wagner) precipitates demonstrated the presence of Alkaloids [13].

Evaluation of Phenolic Compounds (Ferric Chloride Test): the crude extracts were added with ethanol (100mg/10mL), and the solution was filtrated. Two-milliliter filtrate was pipetted into the test tube, following with the addition of distilled water 5mL. Four drops of 5%-FeCl₃ were dripped into the filtrate. The formation of dark green precipitate showed the presence of Phenolic Compounds [14].

Evaluation of Tannins (Ferric Chloride Test): The ethanol was added to the crude extract (100mg/10mL), and the mixture was filtrated. The two-milliliter filtrate was transferred into the test tube and added with few drops of 0.1%-FeCl₃. The tested group was compared with the control group, which was not added with the reagent. The brownish green coloration interpreted the presence of Tannins [15].

Evaluation of Flavonoids (Ammonium Test): The crude extracts were dissolved in chloroform 2 mL and taken into the test tube. One milliliter of 1%-NH₄ was added into it. The mixture was shaken vigorously. The yellow color observed in the ammonia layer demonstrated the presence of Flavonoids [16].

Evaluation of Coumarins (NaOH Test): the crude extracts with the addition of ethanol in the concentration of 100mg/10mL were filtrated. The two-milliliter of filtrate was loaded into the test tube and added with 3mL of 10%-NaOH. The yellow coloration represented the presence of Coumarins [17].

Evaluation of Steroids (Liebermann-Burchard Test): The crude extract 100 mg was dissolved in the chloroform 2 mL

and filtered into the test tube. The mixture was added with 1 mL of glacial acetic acid, followed by carefully the addition of 1ml of H_2SO_4 along the side of the test tube. The greenish color indicated the presence of Steroids [18].

Evaluation of Terpenoids (Salkowski's Test): The crude extract of 100 mg was dissolved in the chloroform 5mL and filtered into the test tube. The mixture was added carefully with 3mL of H_2SO_4 along the side of the test tube. The reddish brown color at the interface of the two phases characterized the presence of Terpenoids [19].

Evaluation of Cardiac Glycosides (Keller-Kiliani's Test): The glacial acetic acid 2 mL was mixed with 2 drops of 2%- $FeCl_3$. The crude extract 100 mg was dissolved in this solution in the test tube. The mixture was added with 1 mL of H_2SO_4 along the side of the test tube. The brown ring at the interface indicated the presence of Cardenolides, and the violet-green ring below the brown ring in the acetic acid layer represented Glycoside. These together characterized Cardiac Glycosides [19,20].

Evaluation of Essential Oils (NaOH-HCl Test): In the test tube, the filtrate 2 mL of the extract was added with 100 μ l of 1M-NaOH, followed by the addition of 3 drops of 1M-HCl. The mixture was shaken. The white precipitate demonstrated the presence of Essential Oils [21].

Evaluation of Saponins (Froth Test): the distilled water 15mL were added to 100mg of the crude extract and filtered into the test tube. The mixture was shaken for 10min until the formation of stable persistent froth. Formation of stables five-minute persistent froth indicated the presence of Saponins [22].

Evaluation of Resins (Turbidity Test): Ten milliliter of distilled water were added to 200 mg of the crude extract and

filtered into the test tube, and the mixture was observed. The occurrence of turbidity showed the presence of Resins [21].

DPPH Radical Scavenging Assay: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method is a rapid and sensitive procedure to observe the antioxidant activity of plant extracts. The stock solutions of the extracts were prepared in ethanol to achieve the concentration of 1mg/ml. The dilutions were made to obtain the concentrations of 100, 250, 500, 750 and 1000 μ g/mL (*B. pinnatum* whole plant); 2, 4, 6, 8 and 10 μ g/mL (*D. ovata* barks); 150, 200, 250, 300 and 350 μ g/mL (*D. fortunei* rhizomes); and 20, 30, 40, 50 and 60 μ g/mL (*L. wallichii* barks). The diluted solutions (100 μ L each) were mixed with 100 μ L of ethanolic solution of DPPH (200 μ M). The mixture was left for 30min in the darkness at room temperature, and the absorbance was recorded at 550nm. The experiment was replicated in three independent assays. The solution without samples and with DPPH and ethanol was used as negative control, and the Trolox (30 μ M) was used as a positive control. The inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{\text{negative control}} - A_{\text{test}}) / (A_{\text{negative control}} - A_{\text{control}})] \times 100$$

Where $A_{\text{negative control}}$ is the absorbance of the negative control (DPPH + EtOH) and A_{test} is the absorbance of sample extracts. A_{control} is the absorbance of the control (Et OH alone). All tests were run in triplicates (n=3), and average values were calculated [23]. The assay was carried out in the Laboratory of Biological Pharmacology, KhonKaen University. The IC_{50} parameter was used for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50% [23].

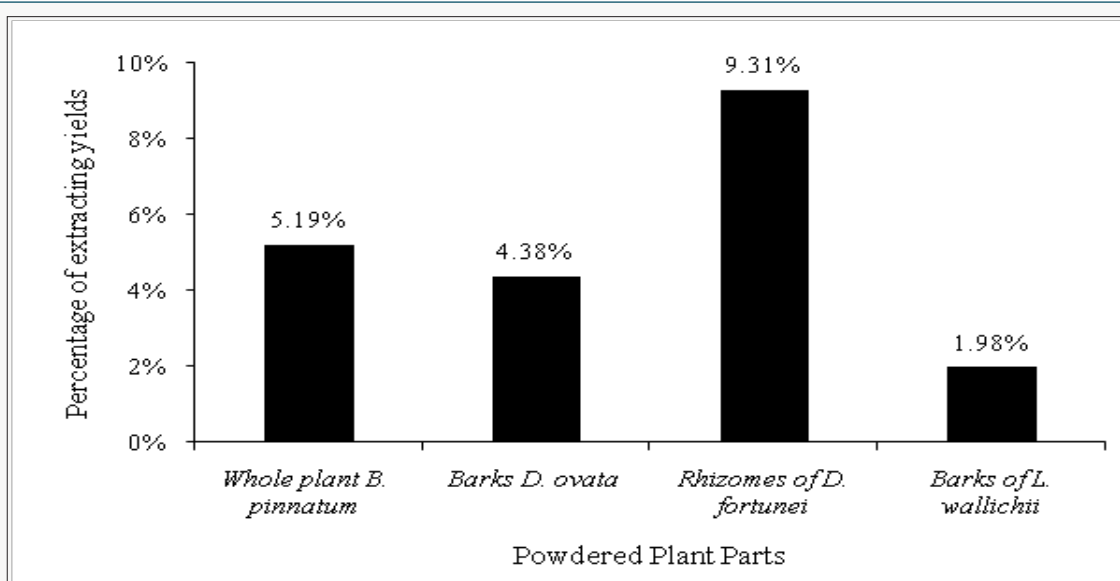


Figure 1: Ethanolic extracting yields (%) of four plant parts.

Results and Discussion

Extracting Yields (%) of Powdered Plants

The present study revealed that the ethanolic extracting yield of *D. fortunei* rhizomes (9.31%) showed the highest value, following by the yields of *B. pinnatum* whole plant (5.19%), *D. ovata* barks (4.38%) and *L. wallichii* barks (1.98%) successively (Figure 1). The maceration method is considered as one of the general techniques of medicinal plant extraction, and ethanol is widely used as a solvent to extract the plant-derived chemicals [24]. Several reports showed the different extracting values of different Cambodian plant extracts [25,26], which is in consistence with our study indicating different percentages of extracting yield. The technique of maceration extraction is commonly used at the drug manufacturing enterprise line [27].

Preliminary Phytochemical Evaluation

The current study indicated that the ethanolic extracts of *B. pinnatum* whole plant, *D. ovata* barks, *D. fortunei* rhizomes contained alkaloids, phenolic compounds, tannins, flavonoids,

Table 1: Phytochemical screening of the ethanolic extracts of *B. pinnatum* whole plant, *D. ovata* barks, *D. fortunei* rhizomes and *L. wallichii* barks.

Phytochemicals	Types of Tests	Whole plant of <i>B. pinnatum</i>	Barks of <i>D. ovata</i>	Rhizomes of <i>D. fortunei</i>	Barks of <i>L. wallichii</i>
Alkaloids	Dragendorff	+ve	+ve	+ve	+ve
	Mayer	+ve	+ve	+ve	+ve
	Wagner	+ve	+ve	+ve	+ve
Phenolic compounds	Ferric chloride	+ve	+ve	+ve	+ve
Tannins	Ferric chloride	+ve	+ve	+ve	+ve
Flavonoids	Ammonium	+ve	+ve	+ve	+ve
Coumarins	NaOH	+ve	+ve	+ve	-ve
Steroids	Liebermann-Burchard	+ve	+ve	+ve	-ve
Terpenoids	Salkowski	+ve	+ve	+ve	+ve
Cardiac glycosides	Keller-Kiliani	+ve	+ve	+ve	+ve
Essential oils	NaOH-HCl	-ve	-ve	-ve	+ve
Saponins	Froth	+ve	+ve	+ve	+ve
Resins	Turbidity	+ve	+ve	+ve	+ve

Note: +ve = Positive (present); -ve = Negative (absent)

DPPH Radical Scavenging Activity

The free radical scavenging activity of the ethanolic extract of different plants was tested by DPPH radical method using Trolox as a positive control. The DPPH assay provides information on the reactivity of the test extracts with a stable free radical giving an absorption band at 550nm [32]. The concentration of *B. pinnatum* whole plant ranged from 100-1000µg/mL, of *D. ovata* barks ranged from 2-10µg/mL, of *D. fortunei* rhizomes ranged from 150-350µg/mL, and of *L. wallichii* barks ranged from 20-60µg/mL. The ethanolic extract of *B. pinnatum* whole plant inhibited the free radical in dose-dependent manner ($R_2 = 0.9068$) with the inhibition percentage 17.72, 39.69, 65.35, 83.31 and 84.57% at the concentration of

coumarins, steroids, terpenoids, cardiac glycosides, saponins and resins, but showed the negative test of essential oils. The ethanolic extract of *L. wallichii* barks demonstrated the positive tests of alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, cardiac glycosides, essential oils, saponins and resins; however, the coumarins and the steroids were not positively tested (Table 1). Phytochemical are secondary metabolites that enable plants to overcome temporary or continuous threats integral to their environment, which is beneficial to the human in term of medical treatment [28]. The plant-derived anticancerous drugs such as etoposide and taxol have been applied for years in the clinical use and play a crucial role in the development of the novel drug entities for human [29]. The medicinal plants contributing towards the ethnomedicine have been broadly screened for their Phytochemicals including alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin and cardiac glycoside [30]. They are associated with protection from and/or treatment of chronic diseases such as heart disease, cancers, diabetes, and hypertension as well as other medical conditions [31].

100, 250, 500, 750 and 1000µg/mL respectively (Figure 2). The ethanolic extract of *D. ovata* barks inhibited the free radical in dose-dependent manner ($R_2=0.9361$) with the inhibition percentage 14.23, 32.77, 59.07, 60.35 and 75.06% at the concentration of 2,4,6,8 and 10µg/mL respectively (Figure 3). The ethanolic extract of *D. fortunei* rhizomes inhibited the free radical in dose-dependent manner ($R_2 = 0.991$) with the inhibition percentage 28.58, 34.6, 44.41, 50.36 and 56.23% at the concentration of 150, 200, 250, 300 and 350µg/mL respectively (Figure 4). The ethanolic extract of *L. wallichii* barks inhibited the free radical in dose-dependent manner ($R_2 = 0.9648$) with the inhibition percentage 27.41, 53.28, 60.21, 75.53 and 87.32% at the concentration of 20, 30, 40, 50 and 60µg/

mL respectively (Figure 5). The IC₅₀ of the ethanolic extracts of *B. pinnatum* whole plant, *D. ovata* barks, *D. fortunei* rhizomes and *L. wallichii* barks accounted for 412.45, 6.23, 300.45, and 32.43 μg/mL respectively (Table 2). Several studies reported the free radical scavenging potential of medicinal plant extracts [33-35]. The

antioxidant plant drugs play an important role in the prevention and treatment of various disorders, caused by oxidative stress, such as cancers, cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative diseases [36].

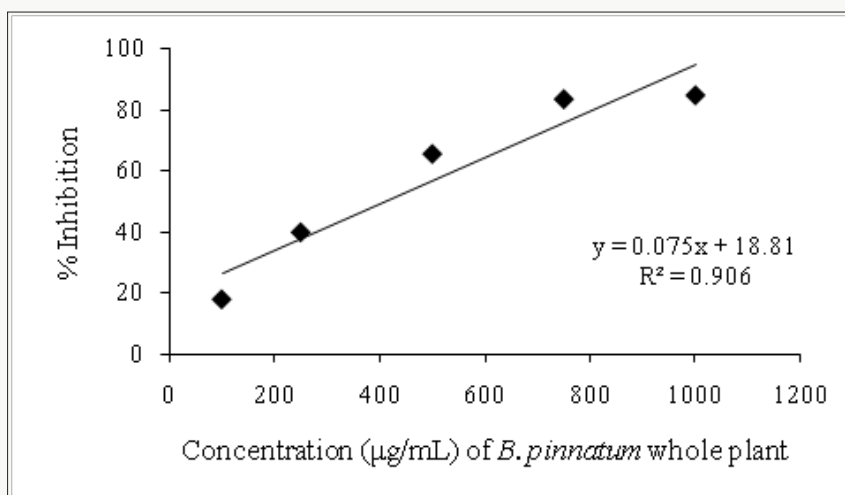


Figure 2: Free radical scavenging activity of ethanolic extract of *B. pinnatum* whole plant.

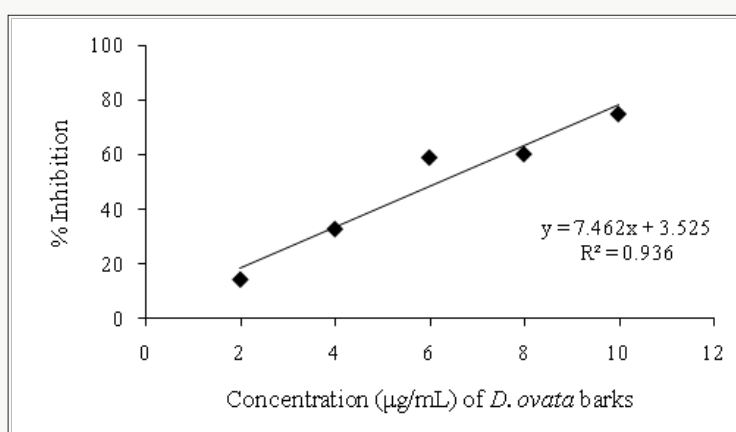


Figure 3: Free radical scavenging activity of ethanolic extract of *D. ovata* barks.

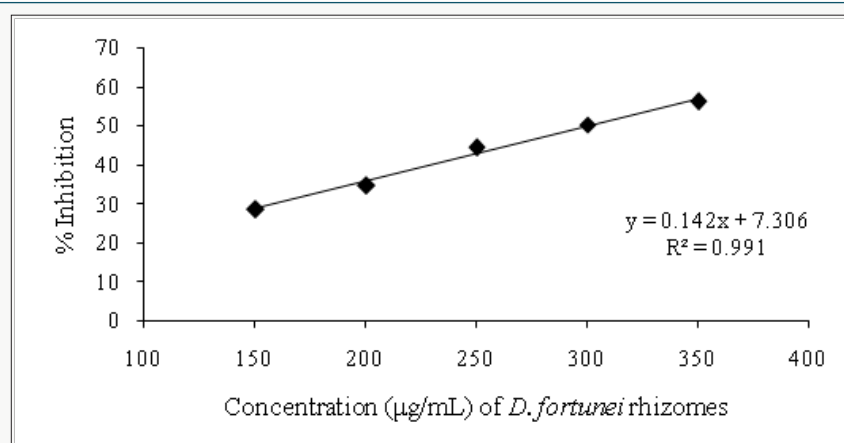


Figure 4: Free radical scavenging activity of ethanolic extract of *D. fortunei* rhizomes

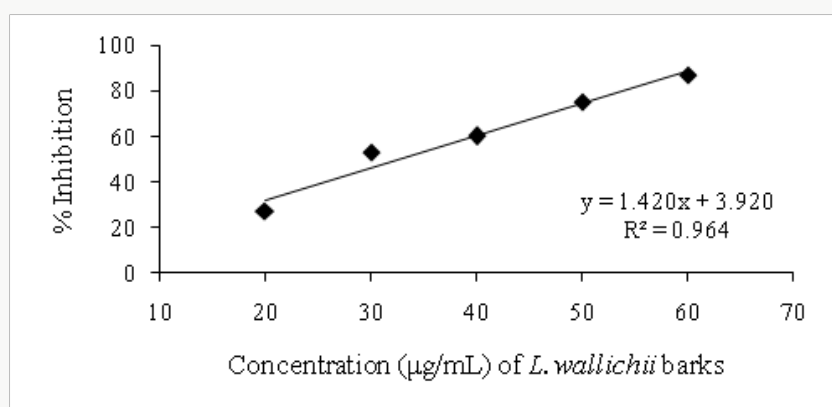


Figure 5: Free radical scavenging activity of ethanolic extract of *L. wallichii* barks.

Table 2: Inhibition Concentration (IC₅₀) of the selected medicinal plants.

Medicinal Plants	Khmer Names	Plant Parts	IC ₅₀ (µg/mL)
<i>B. pinnatum</i>	KabelLapoahs	Whole plant	412.45
<i>D. ovata</i>	Phlu Thom	Barks	6.23
<i>D. fortunei</i>	Baabrak	Rhizomes	300.45
<i>L. wallichii</i>	Puen Ta Lei	Barks	32.43

Conclusion

The preliminary phytochemical evaluation of these species exhibited that the ethanolic extracts of *B. pinnatum* (whole plant), *D. ovata* (barks), *D. fortunei* (rhizomes) and *L. wallichii* (barks) contain alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, cardiac glycosides, saponins and resins. Moreover, the ethanolic extracts of *B. pinnatum* whole plant, *D. ovata* barks, and *D. fortunei* rhizomes possess coumarins and steroids, and of *L. wallichii* barks have essential oils. The *in vitro* antioxidant activity of the species *B. pinnatum* whole plant, *D. ovata* barks, *D. fortunei* rhizomes and *L. wallichii* barks have prominent antioxidant activities. This study suggests the potential source of natural antioxidant in *B. pinnatum*, *D. ovata*, *D. fortunei* and *L. wallichii* native to Cambodia. Further research is highly recommended on the isolation of the antioxidant compounds from these species.

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References

- Pham Huy LA, He H, Pham Huy C (2008) Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science* 4(2): 89-96.
- Sisein EA (2014) Biochemistry of free radicals and antioxidants. *Scholars Academic Journal of Biosciences* 2(2): 110-118.
- Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. *Pharmacognosy Review* 4(8): 118-126.
- El Bahr SM (2013) Biochemistry of free radicals and oxidative stress. *Science International* 1(5): 111-117.
- Al Snafi AE (2016) Medicinal plants with antioxidant and free radical scavenging effects (part 2): plant based review. *IOSR Journal of Pharmacy* 6(7): 62-82.
- Ka S, Ka M, Ka N, Chhuor (2015) Cambodia: medical anthropology. *Anthropology* 3(2): 1-4.
- Kennedy DO, Wightman EL (2011) Herbal extracts and Phytochemical: plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition* 2(1): 32-50.
- Kabera JN, Semana E, Mussa AR, He X (2014) Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *Journal of Pharmacy and Pharmacology* 2(7): 377-392.
- Saxena M, Saxena J, Nema R, Singh D, Gupta A (2013) Photochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry* 1(6): 168-182.
- Dillard CJ, German JB (2000) Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture* 80(12): 1744-1756.
- Kham L (2004) Medicinal plants of Cambodia: habitat, chemical constituents and ethno botanical uses. Bendigo Scientific Press, Golden Square, Australia.

12. Harborne JB (1984) phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd., London.
13. Humayun S, Ibrar M, Barkatullah, Ahmad I (2012) Comparison of three extracts of *Fumeria indica* for the evaluation of cytotoxic and phytotoxic activities. International Journal of Biosciences 2(12): 112-119.
14. Raaman N (2006) Phytochemical techniques. New India Publishing, New Delhi.
15. Karthiashwaran K, Mirunalimni S, Dhamodharan G, Krishnaveni M and arulmozhi V (2010) Phytochemical investigation of methanolic extract of the leaves of *Pergulariadaemia*. Journal of Biological Sciences 10(3): 242-246.
16. Sheel R, Nisha K, Kumar J (2014) Preliminary phytochemical screening of methanolic extract of *Clerodendroniifortunalum*. IOSR Journal of Applied Chemistry 7(1): 10-13.
17. Sawant RS, Godghate AG (2013) Qualitative phytochemical screening of rhizomes of *Curcuma longa* Linn. International Journal of Science. Environment and Technology 2(4): 634-641.
18. Bargah RK (2015) Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringapterygysperma Gaertn.* Journal of Pharmacognosy and Phytochemistry 4(1): 07-09.
19. Ajiboye BO, Ibkun EO, Edobor G, Ojo AO, Onikanni SA (2013) Qualitative and quantitative analysis of phytochemicals in *Senecionbiafrae* leaf. International Journal of Invention and Pharmaceutical Sciences 1(5): 428-432.
20. Jaradat N, Hussien F, Ali AA (2015) Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Dencne. Journal of Materials and Environmental Science 6(6): 1771-1778.
21. Mir SA, Mishra AK, Reshi ZA, Sharma MP (2013) Preliminary phytochemical screening of some pteridophytes from District Shopian (J & K). International Journal of Pharmacy and Pharmaceutical Sciences 5(4): 632-637.
22. Djaafar Z, Ridha OM (2014) Phytochemical study of selected medicinal plant, *Solanumnigrum*, the Algerian Dessert. International Letters of Chemistry, Physics and Astronomy 1: 25-30.
23. Soni A, Sosa S (2013) Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. Journal of Pharmacognosy and Phytochemistry 2(4): 22-29.
24. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011) Phytochemical screening and extraction: a review. International Pharmaceutica Scientia 1(1): 98-106.
25. Oeung S, Nov V, Ung H, Roum K, Yin V, et al. (2017) Phytochemical analysis of different extracts of leaves of *Nicotianatabacum* L. of Cambodia. Asian Journal of Pharmacognosy 1(3): 18-26.
26. Nov V, Oeung S, Ung H, Roum K, Yin V, et al. (2017) Organoleptic and phytochemical evaluation of different extracts of *Vernoniacinerea* L. whole plant of Cambodia. Asian Pharm NET 2017Kuala Lumpur, Malaysia.
27. Azwanida NN (2015) A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants 4:3.
28. Molyneux RJ, Lee ST, Gardner DR, Panter KE, James LF (2007) Phytochemicals: the good, the bad and the ugly? Phytochemistry 68(22-24): 2973-2985.
29. Phillipson JD (2001) Phytochemistry and medicinal plants. Phytochemistry 56(3): 237-243.
30. Edeoga HO, Okwu DE, Mbaebie BO (2005) Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 4(7): 685-688.
31. Craig WJ (1997) Phytochemicals: guardians of our health. Journal of the American Diabetic Association 97(10): 199-204.
32. Rao DB, Rao PK, Sumitra DJ, Rao TR (2011) Phytochemical screening and antioxidant evaluation of some Indian medicinal plants. Journal of Pharmacy Research 4(7): 2082-2084.
33. Senguttuvan J, Paulsamy S, Karthika K (2014) Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochoerisradicata* L. for *in-vitro* antioxidant activities. Asian Pacific Journal of Tropical Biomedicine 4(1): S359-S367.
34. Dluya T, Daniel D, Yusuf U (2017) *In-vitro* antioxidant activity and phytochemical evaluation of five medicinal plants extract. The Pharmaceutical and Chemical Journal 4(5): 73-82.
35. Chandra S, Saklani S, Mishra AP, Agrawal RK (2016) *In-vitro* antioxidant activity and phytochemical screening of Garhwal Himalaya medicinal plants. International Journal of Medical Research & Health Sciences 5(8): 35-43.
36. Zhang Y, Gan R, Li S, Zhou Y, Li A, et al. (2015) Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules 20(12): 21138-21156.



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