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Original Article

Antioxidant Potential, Urease and Acetylcholine Esterase Inhibitory Activity and Phytochemical Analysis of Selected Medicinal Plants from the Republic of Korea

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Abstract

Background and objectives: Plants are a rich source of bio-functional phytochemicals. The present study was designed to investigate the methanol extracts of selected plants for their phytochemicals, antioxidant activity, urease and acetylcholine esterase (AChE) inhibitory potential.

Methods: Crude methanol extracts of selected ethnopharmacological plants were prepared by a simple maceration procedure. Antioxidant assays, total phenolic and total flavonoid content were determined using colorimetric methods. The urease and AChE inhibitory potential of the extract was investigated using spectroscopy techniques.

Results: Most of the extracts tested positive for alkaloids, saponin, glycosides and terpenoids. The total phenolic and flavonoid content in the extracts ranged from 62.7 ± 6.07 – 172.25 ± 11.8 µg gallic acid equivalent (GAE) and 9.7–60.1 µg quercetin equivalent (QE) per gram dry weight (DW). The maximum GAE and QE content was found in *Coruns officinalis* and *Prunus armeniaca*, at 164.9 ± 5.6 and 60 ± 0.65 g/mg DW, respectively. All medicinal plants showed significant antioxidant activity. *M. kobus* exhibited significant antiradical (DPPH) potential (IC_{50} = 30.77 µg/ml). *F. koreana* showed the maximum total antioxidant capacity when expressed as ascorbic acid equivalent (AAE) (119.1 µg AAE/mg DW). The extracts were evaluated for their inhibitory potential against urease and AChE enzymes. Among all plants, *G. biloba* and *P. mume* exhibited the maximum urease and AChE inhibitory activity with IC_{50} of 45.25 and 16.58 µg/mL, respectively.

Conclusion: The present study showed that methanol extracts of plants can be considered as potential sources of pharmacological importance in terms of phyto-constituents for the treatment of oxidative stress associated ailments, ulcer and Alzheimer's disease.

Keywords: Medicinal plants; Antioxidants; Acetylcholine esterase; Enzyme inhibitors; Urease.

Abbreviations: AChE, acetylcholine esterase; GAE, gallic acid equivalent; QE, quercetin equivalent; DW, dry weight; AAE, ascorbic acid equivalent.

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Introduction

The use of traditional and complementary medicine has increased significantly over the past few years in both developing and devel-

oped countries. Plant-derived drugs remain an important resource to combat serious diseases. Traditional remedies have a long-standing history in many areas of the Republic of Korea and continue to provide useful and applicable tools for treating ailments.[1] Various countries have developed uniquely focused and named medicine systems, such as traditional Chinese medicine in China, traditional Korean medicine in Korea, and oriental medicine in Japan.[2] In Korea, about 69% of the Korean population has been treated with traditional Korean medicine.[3] By contract, in China, traditional Chinese medicine accounts for around 40% of all health care delivered, which is used to treat approximately 200 million patients annually.[4] According to the WHO, about 80% of the world depends on traditional therapies for their primary health care. Cultural acceptability, few side effects, economic affordability, and a high therapeutic index make plants a potential source for complementary therapies.[5]

Natural products obtained from plants may give a new source of treatment for various diseases. Keeping in view the importance of medicinal plants, various research groups are engaged in medicinal plant research.[6,7] The antioxidant activity of plant extracts has become very important in this regard [8,9] due to the prominence of free radicals, or reactive oxygen species, that are responsible for various diseases such as stroke, heart diseases, cancer and arteriosclerosis, as well as for the aging process through the damage to proteins, nucleic acids and lipids.[10]

Despite having an internal antioxidant system in the human body to cope with excessive free radicals, it is recommended to complement this system with exogenous antioxidants.[11] Synthetic antioxidants possess adverse effects such as toxicity and are being increasingly replaced with natural antioxidants.[12] A large number of secondary metabolites derived from plants, such as phenolic compounds and flavonoids, *etc.*, exhibit strong antioxidant potential and free radical scavenging properties. The antioxidant potential of plant extracts is often attributed to the phenolic content, such as that of phenolic acids, flavonoids and phenolic diterpenes.[13]

Despite an exponential increase in the use of herbal products, the current study aimed to investigate the plants of Korea that are often used in herbal medicine. In the course of our investigations, we found that several plants of Korean ethnomedicine possess interesting biological activities that may be of interest across the world.[14,15]

The aim of this work was to continue these investigations and to determine the phytochemical constituents, antioxidant activity (DPPH scavenging assay, and total antioxidant assay), and inhibitory potential of therapeutically important enzymes (*i.e.* acetylcholine esterase and urease) of selected Korean medicinal plants. The active ingredients with these plants may serve as candidates for the management of various ailments like ulcer, Alzheimer's disease and oxidative stress-associated complications.

Material and methods

Chemicals and reagents

Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, methanol, acetylcholine esterase, hydrochloric acid, and urease were purchased from Sigma (Chemicals Co. St. Louis, USA). Sodium hydroxide, sodium carbonate, sulfuric acid and neostigmine methylsulfate were purchased from Merck. All the chemicals were of analytical grade.

Collection and extraction of plants

All 20 medicinal plants investigated in this study were purchased from the "Korean Collection of Herbal Extracts", a biotech company in Korea. The extract of the plants was prepared by placing 5 g of each plant material in 50 ml of methanol for 3 days at room temperature with occasional shaking. This process was repeated three times and was followed by the filtration of extracts and concentrated under a vacuum using a rotary evaporator (Eyela Rotary Vacuum Evaporator NN Series and Eyela Digital Waterbath SB-651, Tokyo Rikikai Co. Ltd.) at 35 °C. The filtrates obtained were stored at 4 °C for future use.

Phytochemical analysis

Qualitative phytochemical screening

Preliminary phytochemical screening for the presence of alkaloids, saponin, glycosides, terpenoids was performed according to previously reported procedures.[16]

Alkaloid content

Qualitative analysis of the alkaloid content of the sample extract was performed by adding 250 mg of a sample in 4 ml of 1% HCl. The mixture was then warmed and filtered. Six drops of Mayor's reagents/Dragendorff reagent was added to 1 ml of filtrate. When an orange-/creamish- precipitate was observed, the presence of alkaloids was concluded.

Saponin content

Saponin content in the extract samples was checked using a Frothing test. A half gram of each sample was boiled in 5 ml of distilled water and subsequently cooled and vigorously shaken to produce stable persistent froth.

Cardiac glycoside content

A Keller-Kiliani test was performed to identify cardiac glycosides in each sample. An experimental mixture was prepared by mixing 0.5 g extract, 2 ml glacial acetic acid and a few drops of 1% $FeCl_3$. The solution was then mixed with 1 ml of concentrated H_2SO_4, which resulted in the appearance of green-blue color.

Terpenoid content

The presence of terpenoids was determined through the Liebermann-Burchard reaction. Initially, 1 g of extract sample was dissolved in 4 ml of chloroform. Then, the mixture was filtered and an equal amount of filtrate was mixed with acetic acid, followed the addition of about two drops of sulphuric acid. Terpenoid presence was confirmed through the formation of blue-green ring.

Quantitative phytochemical screening

Determination of phenolic compounds

The total phenolic content of all extracts was determined spectrophotometrically using the Folin-Ciocalteu reagent as previously

described, but with a slight modification.[17] Briefly, 25 µl of the extract (1 mg/ml) was mixed with 115 µl of Folin-Ciocalteu reagent (1:10 v/v distilled water). Subsequently, 120 µl of sodium carbonate (6%) was added to the reaction mixture and the resultant mixtures were incubated for 30 min at room temperature. Methanol was used instead of the sample for blanks and the absorbance of the reaction mixtures was recorded at 725 nm using a microplate reader (OptiMax, Tunable Micro plate Reader). Standard gallic acid solution was prepared by dissolving the mixture in methanol (1 mg/mL). Further, this mixture was diluted to various concentrations, including 25, 50, 100 and 200 µg/mL. The results were obtained from the calibration curve of the standard solution ($y = 0.014x + 0.1093$, $R^2 = 0.9849$). Total phenolic content was determined as mg of gallic acid equivalent (GAE) per gram.

Determination of flavonoid compounds

The flavonoid content of the test samples was determined according to commonly applied spectrophotometric methods and based on the formation of aluminium-flavonoid complexes.[8] Briefly, the reaction mixtures were prepared by combining 10% aluminum chloride, 1 M potassium acetate and distilled water. Sample solutions were then incubated at room temperature for 30 min. Absorbance was recorded using a microplate reader (OptiMax, Tunable Micro plate Reader) at 415 nm. Different concentrations of the Quercetin, including 25, 50, 75 and 100 µg/mL, were prepared from the stock solution of 1 mg/mL for the calibration curve ($y = 0.0276x - 0.066$, $R^2 = 0.9876$). The results were derived from the calibration curve and quercetin equivalents (QEs) were used for the expression of flavonoid content in the methanolic extracts.

Antioxidant activity potential

DPPH free radical scavenging assay

The free radical scavenging capacity of the samples was measured by the DPPH method, as described previously but with slight modifications.[18] Briefly, stock solutions of the test samples (10 mg/ml) were prepared and DPPH (150µM) was dissolved in methanol. Test samples were allowed to react with stable free radical DPPH in the dark for 30 min at room temperature. Ascorbic acid was used as a positive control. Methanol containing DPPH was used as a blank. After incubation, the optical density of the test samples was recorded at 517 nm using a microplate reader ($OPTI_{Max}$, Tunable Micro plate Reader; wavelength range 340–850 nm; for 96-well plates). The percent radical scavenging activity (% RSA) of the samples was determined in comparison with a methanol-treated control using the following formula, where A.E is the optical density of the extract and A.B the optical density of the blank.

$$\%RSA = \left(1 - \frac{A.E}{A.B}\right) * 100$$

Total antioxidant capacity assay

The total antioxidant capacity of the extracts was evaluated using the phosphomolybdenum method[18] and expressed as an equivalent of ascorbic acid (AAE). Briefly, 100 µl of the test sample was initially mixed with 400 µl of reagent solution [ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (0.6 M)]. A reaction mixture was then incubated at 95 °C for 90 minutes. Subsequently, the mixture was cooled and the absorbance was measured at 695nm using a microplate reader ($OPTI_{Max}$, Tunable Microplate Reader; wavelength range 340–850 nm; for 96-well plates).

Enzyme inhibitory activities

Acetylcholine esterase inhibition assay

The acetylcholine esterase inhibition activity of all extracts was carried out according to a standard procedure.[19] Briefly, the assay solution consisted of 180 µL of 50 mM tris-HCl buffer (pH 8.0) (0.1 M sodium chloride and 0.02 M magnesium chloride) containing 20 µL of an enzyme (AChE, acetylcholine hydrolase, EC 3.1.1.7, acetylcholine esterase from human erythrocytes) solution (0.03 U/mL). The increasing concentration of extract samples (10 µL) was added to the assay solution and pre-incubated for 30 minutes at 4 °C. In this reaction mixture, 20 µL of 5,5′-dithio-bis(2-nitrobenzoic acid) (0.3 mM) and acetylthiocholine iodide (1.8 mM) were added and incubated at 37 °C for 10 minutes. The absorbance of the assay mixture was then recorded at 412 nm. For blanks, all components and conditions were kept the same except for the use of acetylcholine esterase. The assay measurements were carried out using a micro plate reader (OptiMax, Tunable). The reaction rates were compared and the percent inhibition due to the presence of test inhibitors was calculated. Neostigmine methylsulfate was used as a reference inhibitor. The experiment was repeated three times for each concentration.

Urease inhibition assay

The urease inhibitory activity was determined by measuring the amount of ammonia produced by the indophenols method, as described by Phull *et al.*[20] The assay mixture, containing 20 µL of enzyme (5 U/mL), 40 µL of buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M $LiCl_2$, pH 8.2), and 20 µL of test samples were incubated for 30 minutes at 37 °C in 96-well plates. Subsequently, 50 µL each of phenol reagent (1% w/v phenol, and 0.005% w/v sodium nitroprusside) and alkali reagent (0.5% w/v NaOH, and 0.1% active chloride NaOCl) was added to each well. Assay mixtures were left for 20 minutes at room temperature. The absorbance at 625 nm was then measured using a microplate reader ($OPTI_{Max}$, Tunable Micro plate Reader; wavelength range 340–850 nm; for 96-well plates). Thiourea was used as the standard inhibitor of urease. The experiment was performed three times and the percentage inhibition was calculated using the following formula, where Ab of sample and Ab of control denote the absorbance in the presence and absence of sample, respectively.

$$\%\ inhibition\ of\ urease\ activity = \frac{Ab\ of\ control - Ab\ of\ extract\ sample}{Ab\ of\ control} * 100$$

Statistical analysis

The data are expressed as means ± standard error of three individual experiments (n = 3). The GraphPad Prism version 5.0 for windows (GraphPad software, San Diego, CA, USA) was used for

Table 1. Medicinal plants species investigated in this study for various biological activities

S. No	Plant name	Family	Plant material used
1	*Acer palmatum thumb*	Sapindaceae	Bark
2	*Aesculus turbinate*	Sapindaceae	Bark
3	*Forsythia koreana*	Oleaceae	Bark
4	*Ginkgo biloba*	Ginkgoaceae	Bark
5	*Elaeagnus multiflora*	Elaeagnaceae	Bark
6	*Magnolia kobus*	Magnoliaceae	Bark
7	*Lagerstroemia indica*	Lythraceae	Bark
8	*Abeliophyllum distichum nakai*	Oleaceae	Bark
9	*Paulownia coreana*	Paulowniaceae	Bark
10	*Rhododendron schlippenbachii maxim*	Ericaceae	Bark
11	*Coruns officinalis*	Cornaceae	Bark
12	*Parthenocissus tricuspidata*	Vitaceae	Bark
13	*Zelkov aserrata makino*	Ulmaceae	Bark
14	*Ligustrum obtusifolium*	Oleaceae	Bark
15	*Alnus hirsute*	Betulaceae	Bark
16	*Paeonia suffruticosa*	Paeoniaceae	Bark
17	*Prunus armeniaca*	Rosacee	Bark
18	*Prunus serrulata*	Rosaceae	Bark
19	*Prunus mume*	Rosaceae	Bark
20	*Cercis chinensis*	Fabaceae	Bark

Notes: The bark material of the plants was obtained from the "Korean Collection of Herbal Extracts", a biotech company in Korea.

the calculation of IC_{50}.

Results

Twenty selected medicinal plants from the Republic of Korea were obtained from the "Korean Collection of Herbal Extracts" and were screened for phytochemical, antioxidant and therapeutically important enzyme inhibition potential. The selected Korean medicinal plants are summarized in Table 1.

Qualitative and quantitative phytochemical screening

All the medicinal plants showed positive results for the presence of alkaloids, while most of the plants were positive for saponin, glycosides and terpenoids. The presence of phytochemicals in each extract is depicted in Table 2.

The total phenolic content (TPC) of the medicinal plants are presented in Table 2. Varied content was observed in all plant extracts and ranged from 62.7 ± 6.07 to172.25 ± 11.8 µg GAE/g dry weight (DW). The highest TPC was recorded for *Coruns officinalis* (164.9 ± 5.6) and *Abeliophyllum distichum nakai* (158 ± 7.53), followed by *Ginkgo biloba* (157.7 ± 2.82), *Forsythia koreana* (145.7 ± 3) and *Paulownia coreana* (142.16 ± 7.53). By contrast, *P. tricuspidata*has exhibited the lowest phenolic content (62.7 ± 6.07 µg GAE/g DW) among all plant extracts.

The total flavonoid content (TFC) ranged from 9.7–60.1 µg QE/g DW, as shown in Table 2. The highest TFC was recorded for *Prunus armeniaca* (60 ± 0.65) and *Ligustrum obtusifolium* (56 ± 3.3), followed by *Alnus hirsute* (51.8 ± 2.40 and *Acer palmatum thumb* (51.6 ± 4.04). On the other hand, *Rhododendron schlippenbachii maxim* (9.7 ± 0.56) exhibited the lowest TFC among all medicinal plants.

Antioxidant potential

The DPPH free radical scavenging activity of the medicinal plants was measured at 1,000 µg/ml, the results of which are illustrated in Figure 1. All plants showed antioxidant activity ranging from 11.2 to 92.3%. Among all plant extracts, only six plants were found to have <70% scavenging potential. Those plants that showed more than 85% scavenging potential were further diluted, and the scavenging activity was examined in order to calculate the IC_{50} values. Thereafter, the extract of *M. kobus, P. tricuspidata* revealed IC_{50} values of 30.77 and 32.94 µg/ml respectively. The other two extracts of *F. koreana* and *Z. serratamakino* exhibited IC_{50} values of 100 µg/ml and 95µg/ml, respectively. Furthermore, varied contents of the AAE were observed, ranging from 11.4–119.1 mg AAE/g DW extract. The total alkaloid content (TAC) of the medicinal plants is depicted in Figure 2. The highest TAC was observed for *F. koreana* (119.1 mg AAE/g DW). The TAC of the crude methanolic extracts was measured spectrophotometrically using the phosphomolybdenum method. The present study demonstrated that most plants

Table 2. Qualitative and quantitative phytochemical analysis of medicinal plants

S. No	Plant name	Quantitative analysis (mg/g)		Qualitative analysis			
		TPC	TFC	Alk.	Sap.	Gly.	Terp.
1	*Acer palmatum thumb*	114.9 ± 5.72	36.5 ± 2.10	+++	++	++	+++
2	*Aesculus turbinate*	98.80 ± 10.28	36.5 ± 2.10	+	++	+++	+
3	*Forsythia koreana*	145.7 ± 3.40	32.8 ± 5.90	+++	+	–	++
4	*Ginkgo biloba*	157.7 ± 2.82	33.4 ± 2.61	++	–	++	++
5	*Elaeagnus multiflora*	114.3 ± 5.92	40.2 ± 1.72	+	–	+	+
6	*Magnolia kobus*	124.16 ± 4.9	20.8 ± 2.29	+++	+	++	–
7	*Lagerstroemia indica*	105.73 ± 4.5	13.9 ± 2.55	++	+	–	+++
8	*Abeliophyllum distichum nakai*	158 ± 7.53	36 ± 1.530	++	++	+	++
9	*Paulownia coreana*	142.16 ± 7.53	40.5 ± 1.74	+++	–	++	+
10	*Rhododendron schlippenbachii maxim*	111.3 ± 4.03	9.7 ± 0.56	++	+	+	++
11	*Coruns officinalis*	164.9 ± 5.6	29.2 ± 1.55	++	++	++	–
12	*Parthenocissus tricuspidata*	62.7 ± 6.07	32.6 ± 2.97	++	+	+++	++
13	*Zelkov aserrata makino*	86.8 ± 4.9	30.2 ± 2.31	+++	++	++	+
14	*Ligustrum obtusifolium*	77.06 ± 6.5	56 ± 3.350	+	+++	++	+++
15	*Alnus hirsute*	140 ± 5.67	51.8 ± 2.4	+++	++	+	+++
16	*Paeonia suffruticosa*	126.3 ± 3.81	40.7 ± 2.05	+++	++	–	++
17	*Prunus armeniaca*	172.25 ± 11.8	60 ± 0.65	++	+	–	++
18	*Prunus serrulata*	139.9 ± 3.89	40.9 ± 2.4	+	–	++	–
19	*Prunus mume*	83.8 ± 5.85	19 ± 2.80	+	++	++	+++
20	*Cercis chinensis*	94.4 ± 3.53	20.9 ± 1.58	++	+	–	++

Notes: TPC: Total phenolic content; TFC: Total flavonoid content; Alk.: alkaloids; Sap.: Saponins; Gly.: Glycosides; Terp.: Terpinoids; +++: highly present; ++: moderately present; +: weekly present; –: not detected.

exhibited the highest antioxidant capacity for phosphomolybdate reduction.

Urease and acetylcholine esterase inhibitory activity

All plant extracts were assessed for their acetylcholine esterase inhibitory potential, the results of which are depicted in Figure 3. All the extracts showed a strong inhibitory effect except for *R. schlippenbachii maxim*. The highest percent inhibitory effect was observed for *P. mume* (91 ± 3), *A. distichum nakai* (89 ± 3.5) and *P. coreana* (85 ± 2.1). The IC_{50} was also calculated for thse plants that showing >80% inhibitory effect. *A. distichumnakai*, *P. coreana* and *P. mume* were found to be the most active among all the tested plants and exhibited IC_{50} values of 32.38, 21.65, 32.74 and 16.58 µg/mL, respectively. However, certain medicinal plants exhibited very low inhibitory potential. The lowest inhibitory effect was observed for *P. armeniaca* (3 ± 0.1%), followed by *A. palmatum thumb* (5 ± 0.2%) and *A. turbinate* (7 ± 0.51%).

The results for urease inhibitory potential are depicted in Figure 4. The results demonstrate that *P. suffruticosa*, *P. serrulata*, *P. mume*, *P. tricuspidata* and *Z. serratamakino* were found to be more active compared to the other plants, exhibiting IC_{50} values of less the 100 µg/ml. Furthermore, *G. biloba* (IC_{50} = 45.25 µg/mL) and *M. Kobus* (IC_{50} = 56.49 µg/mL) were found to have the maximum urease inhibition activity among all plants examined.

Discussion

Plants provide a generous source of valuable bioactive and pharmacological substances. Secondary metabolites such as phytochemicals are found in the plant kingdom, and are being used as a nutritional source, but also act as antioxidants and provide protection against various disorders.[21] Crude methanolic extract of 20 medicinal plants were investigated in the current work for the presence of the alkaloids, saponins, glycosides and other important secondary metabolites. Various medicinal agents have been reported from nature to cure and prevent various ailments.[22] Phytochemicals are not only important in pharmaceuticals but also play a significant role in the food industry. As shown in Table 1, almost all medicinal plants examined in this work tested positive in our qualitative analysis of phytochemicals (alkaloids, saponins, glycosides and triterneoids). There was some variation in the metabolites observed in each extract and may be attributed to the habitat and genetic makeup of specific plant species.[23] Plant materials rich in phenolic compounds are increasingly being used in the food industry because they retard the oxidative degradation of lipids and improve the quality and nutritional value of food.[24]

Phenolic compounds are also considered secondary metabolites and are derived from phenylalanine and tyrosine, which occur universally in plants.[25] This finding is in agreement with other studies that have shown that the methanol extract exhibited the highest total phenolic content.[26] Flavonoids are very important constituents of

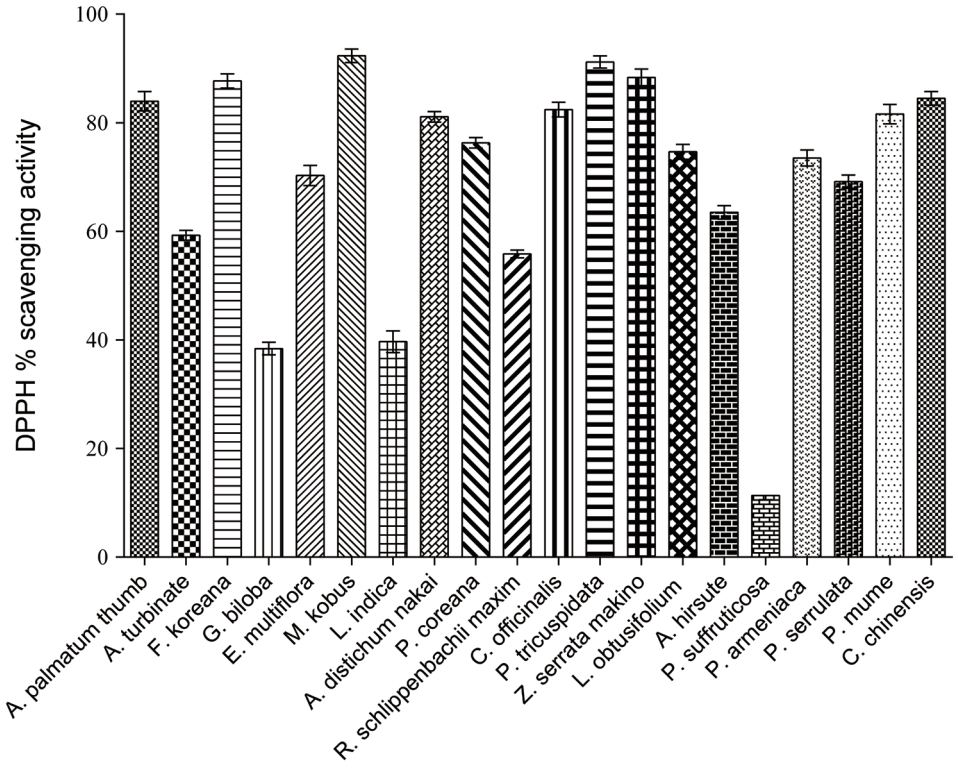

Fig. 1. DPPH scavenging potential of methanol extract of selected plants. Values are presented as mean ± standard error from triplicate investigations.

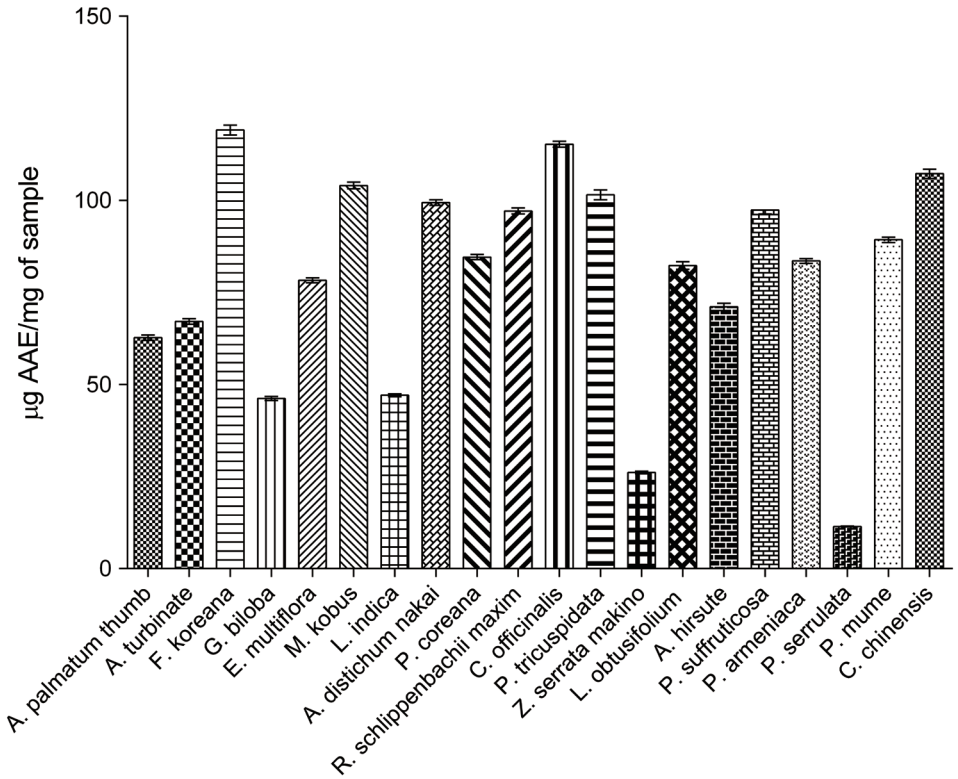

Fig. 2. Total antioxidant capacity (µg AAE/mg sample) assessment in the methanol extract of selected plants. Values are presented as mean ± standard error from triplicate investigations.

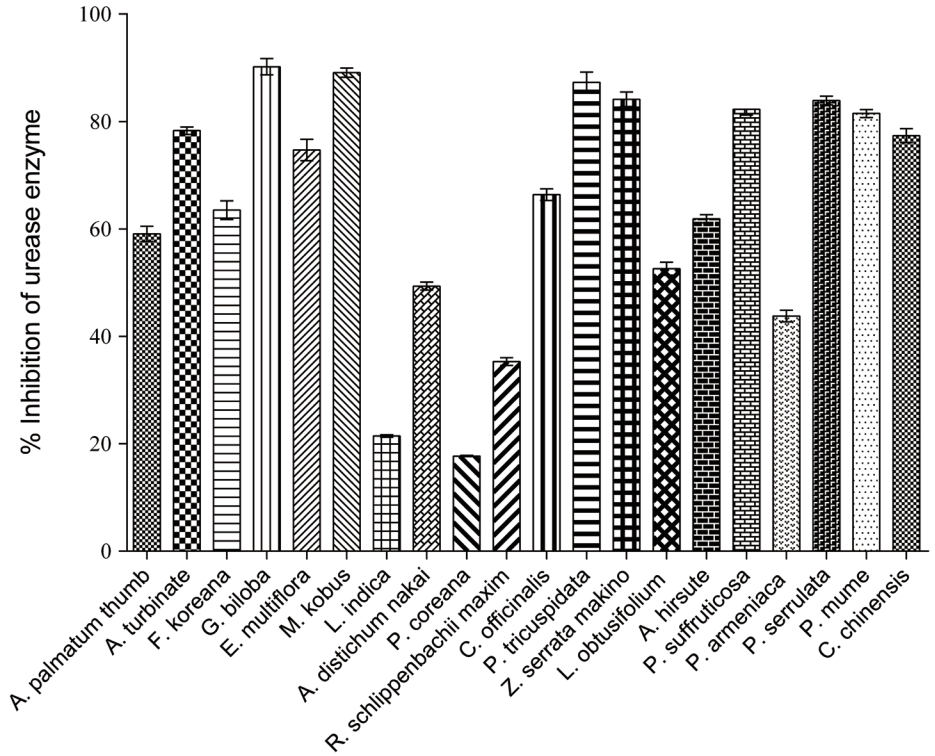

Fig. 3. Urease inhibitory activity of selected plants. Values are presented as mean ± standard error from triplicate investigations.

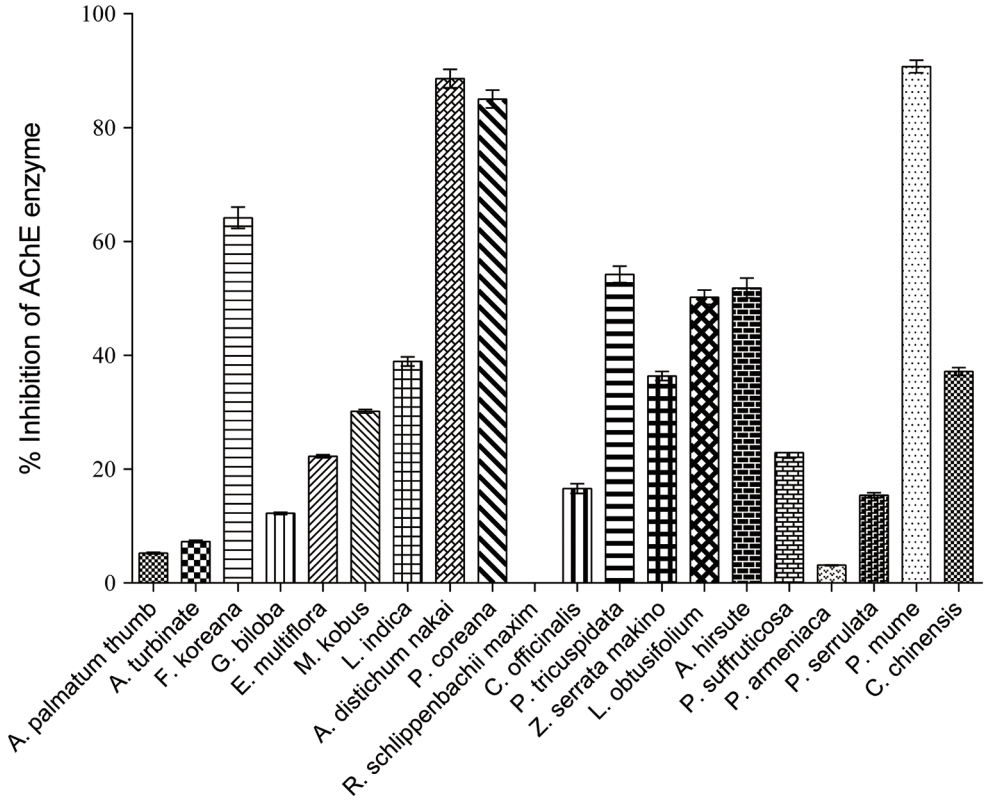

Fig. 4. Acetylcholine esterase (AChE) inhibitory activity of selected plants. Values are presented as mean ± standard error from triplicate investigations.

plants and influence various biological activities such as antioxidant and enzyme inhibitory activity. A significant variation of TFC was observed across the medicinal plants investigated here. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants.[27–29] Significant variation of flavonoid content was observed among the plants investigated, however, all plants exhibited a good amount of TFC. Antioxidant activities of the extracts were correlated with phenolic and flavonoids, as observed with simple linear regression analysis, with the correlations varying among both assays. A relative correlation was found for the total antioxidant assay, as well as the phenolic and flavonoids content, implicating their involvement in antioxidant activities. A study carried out by Li and others[30] supports our findings regarding the slight correlation between DPPH activity and TFC. The nature of the plant and the mechanism of the assay determines the correlation among bioassays and bioactive compounds.[31] Overall, the weak linear correlation between flavonoids and % scavenging of DPPH free radicals might be due to such a reason. Some plants showed higher antioxidant activities, but also exhibited lower phenolic and flavonoid content. This phenomenon might be due to the involvement of other secondary metabolites in antioxidant assays.[8,31,32]

Acetylcholine esterase and urease inhibitory assay results showed the significant inhibitory potential of plant extracts. Acetylcholine esterase has a major role in cholinergic synapses and at low substrate concentrations, acetylcholine esterase accelerates the hydrolysis of acetylcholine.[33] In neurodegenerative disorders like Alzheimer's disease, the role of the cholinergic system is very influential. These disorders cause memory deterioration and the decline of other cognitive functions.[34] Recently, acetylcholinesterase inhibitors such as galantaminehydrobromide, rivastigmine tartrate and donepezil hydrochloride drugs have been approved for Alzheimer's disease treatment.[35]

Urease is an enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate. Significant increases in pH lead to negative effects of urease activity in humans which often arise from gastrointestinal and urinary tract infections.[36] In the case of *Helicobacter pylori,* ureases are possibly involved in stomach cancer and peptic ulcers.[37] These enzymes play a crucial role during the development of hepatic encephalopathy, urolithiasis, urinary catheter encrustation and pyelonephritis.[38]

Medicinal plants are some of the important natural sources of useful active constituents, and various medicinal plants can be used in drugs used to treat specific diseases. Being of natural origin, these substances can be used to isolate bioactive constituents and to design compounds that have lower side effects.

Future directions

Plants have a long history of use in the treatment of various ailments, and have been reported to be biologically active and contain a variety of bioactive constituents. In the present study, as a preliminary approach, twenty plant extracts were investigated for their phytochemical content, and then antioxidant, and urease and AChE enzyme inhibitory potential. It is important to mention that the chemical and quantitative analysis carried out was applied to general groups such as total phenolics. Furthermore, the activity of extracts generally depends on the collective chemical function of the various chemical constituents. Therefore, it is necessary for researchers to probe further into the chemical constituents of these extracts. Such a process will involve both a qualitative approach using sophisticated techniques like GC-MS to identify active compounds, and bio-guided quantitative approaches to isolate active constituents, and characterize and explore the mechanism of action.

Conclusion

The current study investigates the medicinal importance of selected plants and the effective use of the extracts. Methanolic extract from various plant species was explored for antioxidant, phytochemical and enzyme inhibition capacities. Significant antioxidant potential may be attributed to the phenolic and flavonoid constituents of these extracts. Specifically, that of *M. kobus*, *P. tricuspidata*, *F. koreana* showed promising antioxidant activity. Among all samples investigated, *G. biloba* and *P. mume* exhibited remarkable enzyme inhibitory potential when assessed against urease and AChE enzymes, respectively. This result indicates significant promise for pharmacological applications. The current findings regarding antioxidants and the inhibition of therapeutically important enzymes are of great interest in the pharmaceutical industry. Herein, such results necessitate further pharmacological characterization and activity-guided isolation of active phyto-constituents.

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Data sharing statement

No additional data are available.

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Conflict of interest

The authors declare that they have no any conflict of interests.

Author contributions

Study design, performance, analysis and interpretation of data, and write the manuscript (ARP, MR, AA), critically revised the manuscript (AM, TT), designing, execution of the study (HJP, SYS). All authors have made a significant contribution to this study and have approved the final manuscript.

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