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Phytochemical Evaluation of Some Medicinal Plants of Pyuthan District of Nepal

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Abstract

Different parts of eleven medicinal plants used in the traditional medicine in Puthan district were collected. Three different extracts, methanol, 50% aqueous methanol and 70% aqueous acetone extractswere prepared for each plant material. The methanol extractswere screened for the presence of different classes of phytochemicals. Total phenolic and flavonoid content, as well as DPPH free radical scavenging activity, were evaluated. All the investigated extracts contain a high amount of phenolics and flavonoids.The highest amount of phenolics and flavonoids were detected in the methanol extract of bark of *Bauhinia variegate* (355.35+3.69 mg GAE/g and 209.23 ±1.25 mg QE/g extract). Among the eight tested extracts, the highest radical scavenging activity was shown by methanol extract of bark of *B. variegate* (IC^{50} 3.68 µg/ml). The extract having the highest phenolic and flavonoid content showed the lowest IC^{50} demonstrating the positive correlation between radical scavenging activity and total phenolic and flavonoid content.

Keywords

antioxidant, medicinal plant, phytochemical screening, total flavonoid, total phenolic

Introduction

Since ancient times, people have been using plants as a significant source of food and medicine. The health benefits of plants are mainly due to the presence of a large number of bioactive phytochemicals. Current research has confirmed that plants rich in antioxidants play an essential role in preventing cardiovascular diseases, cancers, and neurodegenerative diseases (Dillard & German 2000). Therefore, plant-derived antioxidants are receiving particular attention as they enhance the body's immune system to recognize and destroy cancer cells as well as inhibit the development of angiogenesis necessary for tumor growth. They appear to have both preventative and therapeutic potential in combating cancer (Gutteridge & Halliwell 2010, Turkoglu *et al.,* 2007). Since ancient times, the use of plants as food and remedies is partially attributed to the biological efficacy of secondary metabolites. Among them, phenolic compounds constitute the most abundant class of antioxidants (Serrano *et al.,* 2007, Perez-Jimenez *et al.,* 2008) anti-inflammatory and antibacterial (Harborne & Williams 1992). Numerous epidemiological studies confirmed the significant relationship between the high dietary intake of polyphenols and the reduction of cardiovascular and carcinogenic risk (Ghimire et al., 2011, Kampa *et al.,* 2007). Recently, phenolics have been considered powerful antioxidants in vitro and proved to be more potent antioxidants than Vitamin C, vitamin E, and carotenoids (Rice-Evans et al., 1995, Rice-Evans *et al.,* 1996).

In recent years,people are complementing their treatment with natural supplements to avoid side effects from synthetic materials (Dursum *et al.,* 2004, Nakarni 1976). The formulation of preventive herbal medicines and healthy nutrition requires information about polyphenols content in plant food. Due to the chemical diversity of

polyphenol compounds and the complexity of composition in plant samples, it is costly and inefficient to separate each polyphenol antioxidant and study it individually (Dai & Mumper 2010). Moreover, an integrated total phenolic, flavonoid, and antioxidant power of a complex sample are often more meaningful to evaluate the health benefits because of the cooperative action of antioxidants. Hence, the main aim of the present study is to determine the total phenols, flavonoid content, and antioxidant activity of different plant extracts. The plants selected for this study are commonly used either as food or as traditional medicines in various forms. A literature review revealed that few reports were available on the phenolic, flavonoid content, and antioxidant activity of the plants included in this investigation. These plants could be the potential source of natural antioxidants, which can be used as a nutritional supplement in managing a chronic disease.

Materials and Methods

Plant materials

Plant materials were collected from Torbang of Pyuthan district in March 2014 based on

Table1. Name of plants, local names, parts used and traditional uses

Name of plants and family	Local name	Parts	Traditional use
1. *Bauhinia variegate*Linnaeus(Fabaceae)	Koiralo	Bark	Tonic, appetizer, astringent, anthelmintic, antidote, anti-dysentery, leucoderma, anal troubles, cough, asthma
2. *Centella Asiatica* Linnaeus Urban (Apiaceae)	Godtapre	Whole plant	Anxiety, gastric, ulcer, blood pressure
3. *Euphorbia royleana Boissier*(Euphorbiaceae)	Siyuri	Stem	Stomach disorder, gastric troubles, leaf paste in cuts and wounds and juice to treat fever
4. *Ficus sarmentosa* Buchanan-Hamilton ex Smith(Moraceae)	Gedulo	Bark	Leaves fodder, fruits edible
5. *Fragaria nubicola*Lindley ex Lacaita(Rosacea)	Bhui-ainselu	Whole plant	Juice of the plant is used in the treatment of profuse menstruation.
6. *Hibiscus rosasinensis*Linnaeus (Malvaceae)	Ghantiful	Flower	Antifertility and juice of flowers are used to treat gonorrhea; powdered roots are used to treat menorrhagia and petals infusion to treat fevers
7. *Melia azedarach*Linnaeus **(**Meliaceae)	Bakaino	Bark, leaf	Leprosy, inflammation, cardiac disorders, fever, thirst, nausea, vomiting, indigestion, aphrodisiac, expectorant, anthelmintic and anti-rheumatic
8.*Terminalia chebula* Retzius (Combretaceae)	Harro	Bark	The fruit is a mild laxative, stomachic, tonic, antispasmodic, the paste is used as an anti-inflammatory, analgesic and wound healing purposes, good appetizer, digestive aid, and liver stimulant
9.*Terminalia bellirica* (Gaertner) Roxburgh (Combretaceae)	Barro	Bark	The bark is used as cardiotonic, with diuretic properties against bronchitis, anemia, sore throat, and eyes inflammation of eyes
10.*Trichilia connaroides*(Wight and Arnott.) Bentvelzen(Meliaceae)	Ankha-Tarawa	Bark	Cholera, arthritis, pharyngitis, tonsillitis
11.*Woodfordia fruticose* (Linnaeus) Kurz (Lythraceae)	Dhairo	Leaf, flower	Boils, diarrhea, dysentery, fever, cough, menstrual disorders, urinary disorders, wounds, swellings, cuts skin diseases rheumatism, leucorrhoea, asthma, liver disorder, and inflammatory conditions

their ethnomedicinal value. The plants were authenticated by comparing with the herbarium specimens deposited at the Central Department of Botany Tribhuvan University, Kathmandu.The voucher specimens were deposited at Research Center for Applied Science and Technology, RECAST. The different parts of the plants like bark, stem, flowers, leaves, twigs were separated, shade dried and ground to a fine powder. The name of plants, local names, parts used, and their traditional usesare givenin Table 1.

Preparation of extracts

Each 20 g of the dried and powdered plant material was extracted with 200 ml methanol in a Soxhlet extractor for 6 hours. The plant residue after extraction with methanol was dried and subjected to extraction with 200 ml of 50% aqueous methanol under reflux for 2 hours. The solvent was evaporated in a rota vapour under reduced pressure to get the respective crude extracts. Similarly, each 20 g of dried and powdered sample was percolated with 200 ml of 70% acetone for 24 hours at room temperature and subjected to ultrasonication for another 30 minutes. The process was repeated for three times. The extract was filtered, and the solvent was evaporated in a rota vapor to get 70% acetone extract.

Phytochemical screening of methanol extracts

The methanol extracts were screened for the presence of different classes of phytochemical applying the standard procedure (Culi 1982).

Estimation of total phenolic content in different extracts

The entire phenolic content in plant extracts was determined by Folin-Ciocalteu colorimetric method based on the oxidation-reduction reactionas described before (Giri & Rajbhandari 2019). The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram dry extract (mg/g).

Estimation of total flavonoid content in different extracts

The entire flavonoid content was determined by aluminum chloride colorimetric assay, as described before (Giri & Rajbhandari 2019). The complete flavonoid content of the extracts was expressed as mg quercetin equivalents (QE) per gram of dry extract (mg/g).

Determination of antioxidant activity

Antioxidant activity of the selected extracts was determined by using DPPH free radical as described before (Giri & Rajbhandari 2019). The radical scavenging activity was expressed as the radical scavenging percentage using the equation (1) where; A_S = absorbance of the sample solution, A_b = absorbance of blank and A_C = absorbance of control.

$$\% \, scavenging = \left[\frac{(As - A_b)}{Ac} \right] x100$$

The IC_{50} value is the concentration of the sample required to scavenge 50% of DPPH free radicals. It was calculated from the plotted graph of radical scavenging activity against the concentration of extracts (Giri & Rajbhandari 2019).

Results and Discussion

Extractive values of different extracts

Different extraction approaches were applied, and three different types of extracts were prepared for each sample, such as methanol (Soxhlet), 50% aqueous methanol (reflux), and 70% acetone (ultrasonicated) extracts. This approach of using different polarity solvents has helped achieve the extraction of active substances with different chemical structures. Flavonoid glycosides and more polar aglycons are extracted with methanol or methanol-water mixtures. The high molecular weight tannins are better extracted with aqueous acetone (Kallithraka 1995). Again, conventional long extraction time and high temperature increase the chance of oxidation of phenolics, so ultrasound-assisted extraction technique was applied. The mechanism involves the propagation of the acoustic waves in the kHz range that produce physical, chemical, and mechanical effects disrupting the biological membrane to facilitate the release of extractable compounds (Laborde *et al.,* 1998).

The different extraction approaches provided different extracts in various amounts. The name of plants collected parts and the amount of extract obtained under different extraction conditions are summarized in Table 2.

Table 2. Amount of different extracts obtained from 20 g of dried plant materials

Name of Plant and collected parts	Methanol extract		50% methanol extract		70% acetone extract	
	gram	%	gram	%	gram	%
B. variegate, B	5.58	27.9	4.13	20.65	5.10	25.50
C. assiatica, WP	1.78	8.90	1.41	7.05	1.83	9.15
E. royleana, S	2.31	11.55	1.97	9.85	1.80	9.00
F. sarmentosa, B	0.52	2.60	0.30	1.50	0.41	2.05
F. nubicola, WP	1.67	8.35	1.32	6.60	1.35	6.75
H. rosasinensis, F	3.42	17.10	3.51	17.55	2.57	12.85
*M. azedarach,*B	3.79	18.95	1.52	7.60	1.38	6.90
*M. azedarach,*L	1.82	9.10	1.67	8.35	1.62	8.10
T. bellirica, B	2.42	12.10	2.11	10.55	2.40	12.00
*T. chebula,*B	5.10	25.50	4.37	21.85	3.23	16.15
T. connaroides, B	6.91	34.55	4.54	22.70	4.14	20.70
W. fructicosa, F	2.92	14.60	2.24	11.20	1.99	9.95
W. fructicosa, L	2.23	11.15	2.09	10.45	2.01	10.05

B: bark, **F**: flower,**L**: leaf, **S**: stem, **WP**: whole plant

Phytochemical screening

Most of the phytochemicals present in plant materials are soluble in hot methanol. To get the general view of the phytochemicals present in different plant samples, the methanol extract was subjected to phytochemical screening. The results of the phytochemical screening of methanol extract indicated that alkaloids were present only in few extracts such as *C. Asiatica*, *M. azedarach,* and *T. connaroides.* Flavonoids were present in almost all extracts except in *E. royleana* and *F. sarmentosa.* Terpenoids were absent only in *F. sarmentosa,* while glycosides were absent in *F. sarmentosa, M. azedarach,* and *T. bellirica.* Quinones was absent only in the leaf of *M. azedarach* while reducing sugars were absent in *F. nubicola,* leaf of *M. azedarach***,** *T. connaroides***,** and a leaf of *W. fructicosa.*Polyphenols were absent only in *F. sarmentosa, H. rosa sinensis* and bark of *M. azedarach* while saponins were present only in eight plant extracts, as shown in Table 3. The results of phytochemical screening indicated that the plants selected for this investigation could potentially be sources of different classes of bioactive phytochemicals.

Total phenolic and flavonoid content in different extracts

The total phenolic content in plant extracts was determined by using the Folin-Ciocalteu colorimetric method. It is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/ phosphotungstic acid complexes to form blue colored complexes $(PMoW_{11}O_{40})^{-4}$ that is determined spectrophotometrically at 760 nm. The total phenolic content in different extracts was calculated from the calibration curve using the regression equation ($Y=0.014x$, $R^2= 0.9894$) followed by the formula $C=cV/m$ and expressed as mg gallic acid equivalents (GAE) per g of extract in dry weight (mg/g).Total flavonoid content in plant extracts was determined by aluminum chloride in alkaline conditions. Total Flavonoids content of the extracts was calculated from the regression equation of the calibration curve ($y=0.004x$; $R^2=0.995$) and expressed as mg quercetin equivalent (QE) per gram extract (mg/g).

The results indicated that the total phenolic and total flavonoid content depend on the plant materials as well as the extraction method

Table 3. Phytochemical screening of methanol extracts

Name of plants	Alkaloid	Flavonoid	Terpenoid	Glycoside	Quinone	Reducing sugar	Polyphenol	Saponin
B. variegata **B**	-	+	+	+	+	+	+	+
C. assiatica **WP**	+	+	+	+	+	+	+	-
E. royleana **S**	-	-	+	+	+	+	+	+
F. sarmentosa **B**	-	-	+	-	+	+	-	-
F. nubicola **WP**	-	+	+	+	+	-	+	+
H. rosasinensis **F**	-	+	+	+	+	+	-	+
*M. azedarach.***B**	+	+	+	-	+	+	-	-
M. azedarach **L**	+	+	+	+	-	-	+	-
T. bellirica **B**	-	+	+	-	+	+	+	+
T. chebula **B**	-	+	+	+	+	+	+	+
T. connaroides ***B***	+	+	+	+	+	-	+	+
W. fructicosa **F**	-	+	+	+	+	+	+	-
W. fructicosa **L**	-	+	+	+	+	-	+	+

B: bark, F: flower, L: leaf, S: stem, WP: whole plant, (+) positive, (-) negative

applied. Total phenolic content is relatively high in methanol and 70% acetone extracts than in 50% methanol extracts. Total flavonoid contents in methanol extracts were found to be relatively high when compared with that of 50% aqueous methanol and 70% acetone extracts.The methanol extract of the bark of *B. variegate* showed the highest amount of phenolics (355.35±3.69 mg GAE/g extract) and flavonoids (209.23±1.26 mg QE/g extract followed by the leaves of *W. Fructicosa* (313.57±4.22 mg GAE/g extract) while the lowest amount was found in the barkof *F. sarmentosa* (63.92±3.96 mg GAE/g extract). Total flavonoid contents in methanol extracts were found to be relatively high when compared with that of 50% aqueous methanol and 70% acetone

Table 4. Total phenolic and flavonoid content in methanol extracts and their ratio

Name of plants and collected parts	**TP content in methanol extracts (mg GAE/g extract)**	**TF content in methanol extracts (mg QE/g extract)**	**The ratio of TF/TP**
B. variegate, B	355.35 ± 3.69	209.23 ± 1.26	0.588
C. Asiatica, WP	100.71 ± 2.37	41.15 ± 0.22	0.408
E. royleana, S	138.21 ± 2.74	11.08 ± 0.21	0.080
F. sarmentosa, B	63.92 ± 3.96	5.64 ± 0.51	0.088
F. nubicola, WP	166.02 ± 2.11	11.72 ± 0.37	0.070
H. rosasinensis, F	175.00 ± 4.49	14.16 ± 0.20	0.080
M. azedarach, B	177.50 ± 4.08	54.09 ± 1.04	0.304
M. azedarach, L	107.35 ± 2.19	13.01 ± 0.33	0.121
T. bellirica, B	250.60 ± 4.21	48.59 ± 0.84	0.193
T. chebula, B	296.42 ± 3.11	137.43 ± 1.25	0.463
T. connaroides, B	282.50 ± 4.98	129.10 ±1.05	0.456
W. fructicosa, F	270.72 ± 0.11	13.28 ± 0.11	0.048
W. fructicosa, L	313.57 ± 4.22	62.30 ± 0.85	0.198

B: bark, F: flower, L: leaf, S: stem, WP: whole plant

extracts. The highest amount of total flavonoid was found in the methanol extract of the bark of *B. variegate* (209.23±1.26 mg QE/g extract). Methanol extracts of the bark of *T. chebula* (137.43±1.26 mg QE/g extract) and *T. connaroid* (129.10±1.05 mg QE/g extract) contain a high amount of flavonoids. The lowest amount was found in the bark of *F. sarmentosa* (5.64 ±0.51 mg QE/g extract). The ratio of total flavonoid to total phenolic content was found to be different in different extracts. The greatest ratio was observed in the case of *B. variegate* (0.558), and the lowest ratio was observed in the case of flowers of *W. fructicosa* (0.048). This indicated that 55.8% of the total phenolic in *B. variegate* and 4.8% of total phenolic in *W. fructicosa* are flavonoids. The total phenolic and flavonoid contentin methanol extracts of different plant materials and their ratio are given in Table 4.

In the case of 50% aqueous methanol extracts, the highest amount of phenolic compounds was detected in the flowers of *W. fructicosa* (289.39 ± 3.52 mg GAE/g extract) and the lowest amount in the flower of *H. rosa-sinensis* (17.71 ± 4.11 mg GAE/g extract). In comparison to the phenolic content, the flavonoid content was found to be relatively low. The ratio of total flavonoid to total phenolic content was found to be different in different extracts. The greatest ratio was observed in the case of *C. Asiatica* (0.393), and the lowest ratio was observed in *T. connaroides* (0.081), which indicated that 39.3% of total phenol*s in C. Asiatica* are flavonoids. Only 8.1% of the total phenolic in *T. connaroides*are flavonoids. The results are shown in Table 5.

In the series of 70% acetone extracts, the bark of *B. variegate* (315.71 ± 4.74 mg GAE/g extract) has the highest phenolic content while the lowest amount was found in *E. royleana* (68.28 ± 4.16 mg GAE/g extract). The highest flavonoid was found in the bark of *B. variegate* (206.41 ± 2.22 mg QE/g extract), and the lowest amount of flavonoid was found in the stem of *E. royleana* (3.65 ± 0.38 mg QE/g extract). The ratio of total flavonoid to total phenolic content was found to be different.The greatest ratio was observed in the case of*C. Asiatica* (0.939), and the lowest ratio was observed in the case of *E. royleana* (0.053) which indicated 93.9% of total phenolics are flavonoids in the case of *C. Asiatica*, and only 5.3% of total phenolics are flavonoids in the case of *E. royleana.* The results are shown in Table 6.

It was reported that *B. variegate* is rich in polyphenols (Shahana *et al.,* 2017). The methanol extract of *B. variegate* bark collected from Syangja has been investigated for total phenols, flavonoid content, and antioxidant activity. In

Table 5. Total phenolic and flavonoid content in 50% methanol extracts and their ratio

Name of plants and collected parts	**TP content in 50% methanol extracts (mg GAE/g extract)**	**TF content in 50% methanol extracts (mg QE/g extract)**	**Ratio of TF/TP**
B. variegate, B	148.35 ± 1.61	41.75 ± 0.84	0.281
C. Asiatica, WP	97.42 ± 2.08	38.31 ± 0.84	0.393
E. royleana, S	41.84 ± 5.63	4.61 ± 0.51	0.110
F. sarmentosa, B	37.35 ± 5.47	6.21 ± 0.11	0.166
F. nubicola, WP	50.38 ± 1.23	10.63 ± 0.42	0.211
H. rosasinensis, F	17.71 ± 4.11	6.21 ± 0.11	0.350
M. azedarach, B	38.33 ± 3.89	10.51 ± 0.44	0.274
M. azedarach, L	58.10 ± 5.83	7.81 ± 2.01	0.134
T. bellirica, B	88.60 ± 2.02	7.62 ± 0.38	0.086
T. chebula, B	138.47 ± 5.01	51.53 ± 0.44	0.372
T. connaroides, B	96.90 ± 2.48	7.94 ± 0.44	0.081
W. fructicosa, F	289.39 ± 3.52	28.33 ± 0.25	0.097
W. fructicosa, L	94.96 ± 5.44	31.41 ± 0.84	0.330

B: bark, F: flower, L: leaf, S: stem, WP: whole plant

Table 6. Total phenolic and flavonoid content in 70% acetone extracts and their ratio

Name of plants and collected parts	TP content in 70% acetone extracts (mg GAE/g extract)	TF content in 70% acetone extracts (mg QE/g extract)	Ratio of TF/TP
B. variegate, B	315.71 ± 4.73	206.41 ± 2.22	0.653
C. Asiatica, WP	141.10 ± 5.60	132.56 ± 1.05	0.939
E. royleana, S	68.28 ± 4.16	3.65 ± 0.37	0.053
F. sarmentosa, B	70.53 ± 1.70	6.21 ± 0.55	0.088
F. nubicola, WP	166.21 ± 2.19	125.25 ± 1.32	0.753
H. rosasinensis, F	136.09 ± 5.24	8.52 ± 0.33	0.062
M. azedarach, B	129.91 ± 4.37	21.150 ± 1.11	0.162
M. azedarach, L	266.28 ± 3.85	19.23 ±1.28	0.072
T. bellirica, B	163.21 ± 2.11	74.35 ± 2.56	0.455
T. chebula, B	259.99 ± 2.85	89.10 ± 2.12	0.342
T. connaroides, B	249.63 ± 2.34	151.53 ± 2.34	0.607
W. fructicosa, F	237.85 ± 3.71	72.30 ± 0.89	0.303
W. fructicosa, L	266.35 ± 3.78	52.43 ± 1.69	0.196

comparison to our results, the reported values of phenolics (156.30±0.30 mgGAE/g extract) and flavonoids (16.04±1.04 mg QE/g extract) were relatively low (Sharma *et al.,* 2015). In our investigation, *W. Fructicosa* showed the presence of a high amount of phenolics. The flowers contain 24.1%, leaves contain 12-20%, and bark contains 20-27% of tannins (Rastogi & Mehrotra 1999). Total phenol content in methanol extract of flower (470±20 µg/ml) and leaves (613±7.63 µg/ml) of *W. fruticosa* collected from Maharastra has been reported (Chaturvedi *et al.,* 2012). Our investigation revealed that bark of *T. chebula* and *T. bellirica* were found to contain a relatively high amount of phenolics. However, only fruits of both plants have been investigated for total phenol and antioxidant activity (Genwali *et al.,* 2013), and reports on the bark are not available. *T. connaroid* was reported to contain norterpenoids (Wang *et al.,* 2013), and reports related to phenolic and flavonoid estimation are not available. The aqueous and ethanolic extracts of *C. Asiatica* have been investigated for total phenolic, flavonoid, and antioxidant activity (Pittella *et al.,* 2009; Polash *et al*., 2017). It was reported that only 4.58±0.44 mg/g polyphenols were found in 37% aqueous ethanol extract of *C. Asiatica* (Gunathilak *et al.,* 2019) collected from Sri Lanka which was very low than our findings (100.71 ±2.37 mg GAE/g). The total phenolic/flavonoid in methanol extracts of *E. royleana* (56.97±0.27/43.98±0.79 µg/g) collected from Pakisthan was reported (Ashraf *et al.,* 2015), which were different from our findings (138.21 ± 2.74/11.08 ± 0.21). From *F. sarmentosa,* some flavonoids have been isolated (Wang *et al.,* 2010); however, phenolic and flavonoid content is not reported. The phenol content of the methanolic, aqueous and ethyl acetate extract of *F. nubicola* collected from Kashmir was found to be 843.68 ±13.28 mg/g, 596.6 ± 8.54 mg/g and 411.1 ± 9.61 mg/g respectively (Anees *et al.,* 2018). The reported phenolic content in methanol extract was found to be higher than our findings (166.02 ± 2.11). *Hibiscus rosa-sinensis* collected from Pakisthan has also been investigated for total phenol, flavonoid, and antioxidant activity (Khan *et al*., 2014). In *M. azedarach,* only the ethanol extract of leaves has been evaluated for their total phenolic contents and antioxidant activity (Ahmed *et al.,* 2012); however, a report on the bark is not available.

The quality and quantity secondary metabolites depend on several factors such as genetic diversity within the species, climatic, ecological and geographical conditions of the collection sites, developmental status, physiological conditions, harvesting time as well as analytical methods and samples preparations (Tatsis *et al*., 2007; Wojdylo *et al*., 2017). In comparison to the literature data, our investigation revealed that our

Table 7. IC_{50} values in the DPPH assay, total phenolic, and full flavonoid content

Plants	Extracts	IC_{50} µg/ml	TPC (mg GAE/g)	TFC (mg QE/g)
B. variegate B	Methanol	3.68	355.35 ± 3.69	209.23 ± 1.25
M. azedarach B	Methanol	55.68	177.50 ± 4.08	54.09 ± 1.03
F. sarmentosa B	Methanol	129.59	63.92 ± 3.96	5.64 ± 0.51
B. variegate B	70%Acetone	7.66	315.71 ± 4.73	206.41 ± 2.21
C. assiatica WP	70%Acetone	28.67	141.10 ± 55.60	132.56 ± 1.05
F. sarmentosa B	70%Acetone	267.38	70.53 ± 1.70	6.21 ± 0.55
W.fructicosa F	50% Methanol	20.32	289.39 ± 3.52	28.33 ± 0.25
H. rosasinensis F	50% Methanol	261.84	17.71 ± 4.11	5.21 ± 0.11

samples contained high amounts of phenolics and flavonoids. So the plants from Puthan could be a good source of bioactive phytochemicals.

Antioxidant activity of different extracts

DPPH assay was carried out for methanol, 50% methanol, and 70% acetone extracts of selected plants. The IC_{50} values of various extracts are given in Table 7, which revealed that the extracts with the highest phenolic and flavonoid content showed the most significant radical scavenging activity. Among the nine extracts selected for the DPPH assay, the highest radical scavenging activity was shown by methanol extract of *B. variegata* (IC_{50} 3.68 µg/ml) and the lowest was demonstrated by 70% acetone extract of bark of *F. sarmentosa* (IC_{50} 267.38µg/ml). It can also be stated that the scavenging effects of extracts are not limited to phenolic compounds. However, it may be due to the presence of other antioxidants in the extracts such as volatile oils and carotenoids or vitamins.

Conclusions

The results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants. The plants selected for this investigation are the rich sources of phenolic and flavonoid compounds. They were easily extractable with methanol and 70% acetone rather than 50% methanol. Some of these plants could show potent antioxidant activity, which provides prophylaxis against various diseases like heart diseases, stroke, arteriosclerosis, and cancers. The bioassay-guided fractionation to characterize and isolate the antioxidant constituents is needed.

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