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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT CAPACITY OF SOME SELECTED MEDICINAL PLANTS OF BANGLADESH

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Abstract

The leaves of twelve medicinal plants, namely *Andrographis paniculata, Blumea lacera, Caesalpinia bonduc, Codariocalyx motorius, Hyptis suaveolens, Vitex trifolia, Centella asiatica, Cynodon dactylon, Portulaca oleracea, Hyptis capitata, Acmella paniculata* and *Lippia alba* were selected to investigate the presence and status of different phytochemicals along with their antioxidant activity. All of these medicinal plants contained high amounts of different phytochemicals. The phenolic content ranged from 118.98 to 288.62 mg GAE/100g FW, flavonoids from 95.19 to 198.51 mg QE/100g FW, FRAP values from 0.4523 to 3.4390 µmol Fe2+/g FW, IC50 values for scavenging DPPH radicals from 3.376 to 38.56 mg/ml, vitamin C from 10.418 to 41.763 mg/100g FW, total chlorophyll from 0.7818 to 4.2185 mg/100g FW, and carotenoids from 0.4614 to 1.8557 µg/g FW. Among these, *H. suaveolens* and *H. capitata* showed the best performances regarding most of the parameters. Based on the results of the present investigation, it may be concluded that these medicinal plants possess excellent amounts of different phytochemicals and are a good source of antioxidants, indicating their potential in ethnomedicinal studies and therapeutic uses.

Introduction

Bangladesh is a sub-tropical country and is quite rich in naturally available medicinal plants. Approximately, 1500 plant species are expected as medicinal, and already 747 plants are listed (Uddin and Lee 2020). People depend on medicinal plants for their primary aid due to the easy availability of medicinal plants. It is very important to find out these medicinal plants and their phytochemical constituents which have been used by these people for many years (Kumar *et al.* 2021).

Medicinal plants have been used in traditional medicine systems across the world for their healing properties. The compounds found in these plants can have diverse biological activities, such as anti-inflammatory, antioxidant, antimicrobial, and anticancer effects (Islam *et al.* 2018). Studying the phytochemical composition of medicinal plants helps in understanding their potential health benefits. The necessity of phytochemical investigation of medicinal plants is a time-demanding activity to ensure the proper and justified use of these medicinal plants. Twelve traditionally important medicinal plants from different families were selected to investigate the targeted phytochemicals present in the plants as well as to find out the antioxidant capacity and finally to screen out the most suitable medicinal plants with higher phenolics, flavonoids, and antioxidant activity.

Materials and Methods

Twelve widely used medicinal plants, including Kalomegh (*Andrographis paniculata*), Shial mutra (*Blumea lacera*), Nata (*Caesalpinia bonduc*), Turi chandal (*Codariocalyx motorius*), Tokma (*Hyptis suaveolens*), Sagor nishinda (*Vitex trifolia*), Thankuni (*Centella asiatica*), Durba grass (*Cynodon dactylon*), Nunia (*Portulaca oleracea*), Holkhusha (*Hyptis capitata*), Surjokonna

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(*Acmella paniculata*) and Motmotia (*Lippia alba*) were selected for this experiment. The leaves of these plants were used to investigate the phytochemicals and antioxidant activities. The materials were collected from the Bangladesh Agricultural University Botanical Garden, located at Latitude 24° 43' 27.8" N and Longitude 90° 26' 30.6" E.

Chlorophyll determination was performed according to Litchenthaler (1987) with slight modification. Total phenolics content (TPC) was determined on a fresh weight basis according to Adebooye *et al.* (2008). Total flavonoid content was estimated following the aluminum chloride colorimetric method according to the method explained in Aryal *et al.* (2019). The quercetin standard curve was prepared ($y = 0.001x + 0.020$, $R^2 = 0.999$) with different concentrations of quercetin (0-400 ppm) along with the other reagents.

The antioxidant activity of the plant sample was determined through the reducing powers of the plant extract using the Ferric Reducing Antioxidant Power (FRAP) assay that expresses the reduction of Fe^{3+} to Fe^{2+} according to Benzie and Strain (1999). Finally, the FRAP value of the sample was expressed as µmol Fe (II)/g FW of leaves. IC_{50} is the amount of plant extracts required to scavenge 50% of DPPH free radicals and was calculated by plotting the % inhibition against the sample concentration (Chithiraikumar *et al.* 2017). Vitamin-C contents of the fresh leaves were measured by UV spectrophotometric method according to Desai and Desai 2019.

The experimental data were analyzed statistically using Minitab software (version 20.1.2). DMRT was also performed to find out whether the treatment means were statistically similar or different.

Results and Discussion

The results of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content in selected medicinal plants are tabulated in Table 1. *Codariocalyx motorius* exhibited the highest chlorophyll a content (1.890 mg/100gFW), followed by V*itex trifolia* (1.79 mg/100gFW), and *Hyptis suaveolens* (1.750 mg/100gFW). The lowest chlorophyll a content was found in *Portulaca oleracea* (0.594 mg/100gFW). Chlorophyll b content was highest in *Lippia alba* (2.551 mg/100gFW) followed by *Cynodon dactylon* (2.368 mg/100gFW), indicating a higher proportion of this pigment relative to others. *Acmella paniculata* and *Codariocalyx motorius* also showed significant chlorophyll b levels (2.286 mg/100gFW and 1.985mg/100gFW, respectively), while *Caesalpinia bonduc* had the lowest (0.344mg/g FW).

Lippia alba had the highest total chlorophyll content (4.219 mg/100gFW), suggesting its robust photosynthetic ability and potential medicinal potency due to the presence of these pigments. *Acmella paniculata* and *Cynodon dactylon* also showed high total chlorophyll content (3.966 mg/100gFW and 3.892 mg/100gFW, respectively). *Portulaca oleracea* had the lowest total chlorophyll content (0.782 mg/100gFW), which may be attributed to its succulent nature and adaptation to low-light environments, which typically require less chlorophyll.

Lippia alba showed the highest carotenoid content (1.856 mg/gFW), followed by *Acmella paniculata* (1.669 mg/gFW). *Vitex trifolia* and *Codariocalyx motorius* had moderate levels (1.572 mg/gFW and 1.528 mg/gFW, respectively), while *Portulaca oleracea* had the lowest (0.461 mg/gFW).

The present research revealed that the leaves of the selected medicinal plants possessed a good amount of chlorophyll and carotenoids that might have contributed to total antioxidant activity. The variations in chlorophyll and carotenoid content among medicinal plants can be attributed to their differing habitats, physiological needs, and adaptation strategies. *Lippia alba* and *Acmella paniculata* both of which thrive in well-lit environments, exhibit high chlorophyll and carotenoid levels, supporting their robust photosynthetic activity and medicinal properties.

Vitex trifolia and *Codariocalyx motorius* also show significant levels of these pigments, aligning with its known medicinal benefits. Our results are in line with the previous results both for chlorophyll and carotenoid contents (Muthukrishnan *et al*. 2015).

Table 1. Chlorophyll and carotenoids content of selected medicinal plants.

Plant species	Chl. a (mg/100 g FW)	Chl. b (mg/100 g FW)	Total Chl. (mg/100 g FW)	Carotenoids (µg/g FW)
Acmella paniculata	1.680 a	2.286ab	3.966 ab	1.669a
Andrographis paniculata	1.609 a	1.307 cde	2.916 c	1.383 ab
Blumea lacera	1.024 b	0.641 ef	1.665 d	1.140 b
Caesalpinia bonduc	0.980 b	0.344 f	1.323 d	1.468 ab
Centella asiatica	1.532 a	1.791 bcd	3.323 bc	1.507 ab
Codariocalyx motorius	1.890 a	1.985 abc	3.876 ab	1.528 ab
Cynodon dactylon	1.524 a	2.368 ab	3.892 ab	1.483 ab
Hyptis capitata	1.669 a	1.499 cd	3.168 bc	1.409 ab
Hyptis suaveolens	1.750 a	1.200 de	2.950 c	1.123 b
Lippia alba	1.668 a	2.551 a	4.219 a	1.856 a
Portulaca oleracea	0.594 b	0.188 f	0.782 d	0.461 c
Vitex trifolia	1.790 a	1.851 abcd	3.641 abc	1.572 ab
$LSD_{0.05}$	0.452	0.722	0.884	0.509

N.B.: Chl. = Chlorophyll, Total Chl. = (Chl. a+ Chl. b), FW= Fresh weight.

Significant variations in total phenolics and flavonoid contents were observed ($P \leq 0.05$) among the medicinal plants and ranged from 118.98-288.62 mg GAE/100 of fresh leaves for phenolics and 95.19-198.51 mg QE/100 of fresh leaves for flavonoids (Fig. 1). *Hyptis capitata* and *Hyptis suaveolens* showed the highest contents of phenolics (288.62 mg GAE/100 of fresh leaves) and flavonoids (198.51 mg QE/100 of fresh leaves). The rest of the plant species also showed a good amount of phenolics and flavonoids (ranging from 191.94-206.41 mg GAE/100 g FW for phenolics and 110.17-180.13 mg QE/100 g FW for flavonoids). However, *P. oleracea*, *A. paniculata*, and *C. motorious* possessed the lower amount of phenolics and *V. trifolia*, *A. paniculata*, and *C. motorious* were assessed as the lower flavonoids contained. In the same way, the flavonoid contents in different medicinal plants as reported earlier were also in coordination with the current research (Upadhyaya *et al.* 2011).

The antioxidant activity of the selected medicinal plants was expressed as the Ferric Reducing Antioxidant Power (FRAP) and DPPH radical Scavenging Activity. *Hyptis suaveolens* showed the highest FRAP values (3.4390 ± 0.091 µmol Fe^{2+}/g FW) indicating a high antioxidant ability. Besides this, IC_{50} values indicate the amount of plant extract required to scavenge the DPPH radical. So, the lower IC_{50} values indicate a high DPPH scavenging ability. Our results revealed that the IC_{50} values of the selected medicinal plant extract lie between 3.376 to 38.56 mg/ml. Higher IC_{50} and lower antioxidant activity were observed in *C. motorius* whereas the lower $IC_{50,}$ as well as higher antioxidant activity, were observed in *Hyptis suaveolens* and *H. capitata.* These two plants belong to the Lamiaceae family, very much known for high antioxidant activity as reported earlier (Murshed *et al.* 2021). Many more previous results also supported our findings. In *L. alba*, IC_{50} values were reported as 107.09 µg/ml (Oliveira *et al.* 2017). Moreover, 100.00 µg/ml (Roy *et al.* 2016) were reported in *C. dactylon* L. In addition, IC_{50} values were also reported as 0.97 mg/ml in *C. asiatica* (Zhang *et al.* 2011) and 3.22 mg/ml (Oscar *et al.* 2020) in *H. suaveolens*. Dehsheikh *et al. (*2019) reported 4.70 mg/ml in *V. trifolia*. According to the previous research and with the findings of the current research, it may be revealed that the selected medicinal plants possessed a high amount of antioxidant materials due to having high DPPH radical scavenging

activity of plant extract (Maimulyanti and Prihadi 2016). *A. paniculata* also showed good antioxidant activity. All of these medicinal plants were assessed as potential antioxidant-enriched plants (Table 2).

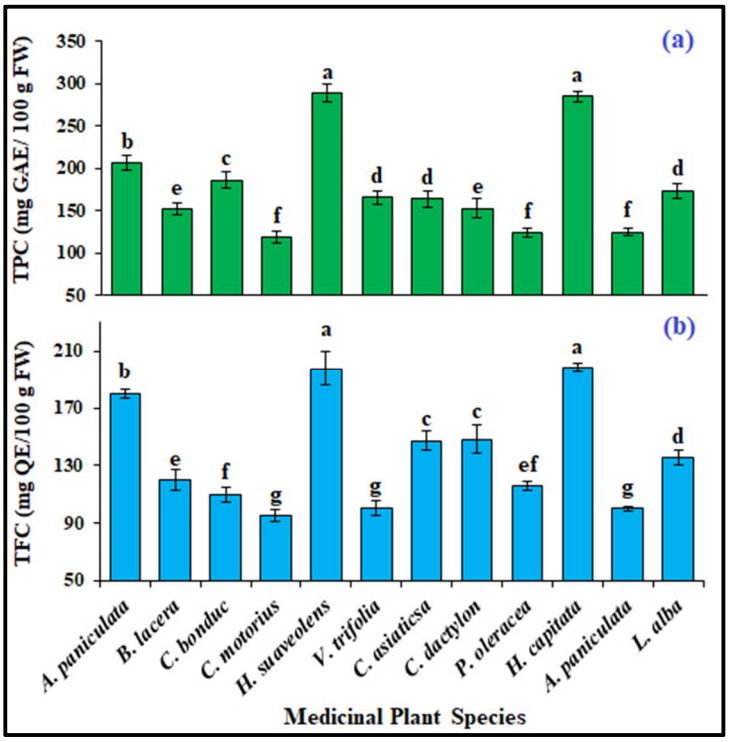

Fig. 1 (a) Total phenolics and (b) Flavonoid contents of the medicinal plants.

Vitamin C content also varied significantly and ranged from 10.418 mg/100 g FW in *A. paniculata* to 41.763 mg/100 g FW in *C. bonduc*. The highest vitamin C was found in *C. bonduc* leaves (41.763 ± 2.108 mg/100 g FW) although the remaining plants also possessed a good amount of Vitamin- C (Table 2). Our findings were in support of the previous reports (Oliveira *et al.* 2013, Zhang *et al.* 2011, Rey *et al.* 2020).

A strongly positive correlation was found between the total phenolics content and antioxidant activity of the selected medicinal plants. Fig. 2 showed that, with increasing phenolics and flavonoid content, the Ferric Reducing Antioxidant Power also increased indicating a strong positive correlation (R^2 = 0.911 for phenolics and 0.7927 for flavonoids). In addition, IC_{50} values were decreased with increased phenolics and flavonoid content indicating a strong correlation between the antioxidant activity and phenolics and flavonoids content (R^2= 0.742 for phenolics and 0.5809 for flavonoids). The lower the IC_{50} values, the higher the antioxidant activity, and here it can be said that with increasing phenolics and flavonoids content the DPPH radical scavenging activity was increased which represents the higher antioxidant activity. Furthermore, a strong correlation was found between the FRAP values and DPPH scavenging activity (R^2= 0.5758, Fig. 2). Reduced IC_{50} values with higher FRAP values indicate high antioxidant activity. The correlation between the phenolics content and antioxidant activities expresses the relationship between the antioxidant activity and the phenolics content. A strong positive correlation indicates

higher antioxidant activity with increased phenolics. Phenolic compounds contribute to antioxidant activity as reported by several researchers (Aryal *et al.* 2019, Dehsheikh *et al.* 2019). A similar correlation was found between the flavonoids and antioxidant activity. In addition, FRAP values and DPPH radical scavenging activity also strong correlation among the medicinal plant species.

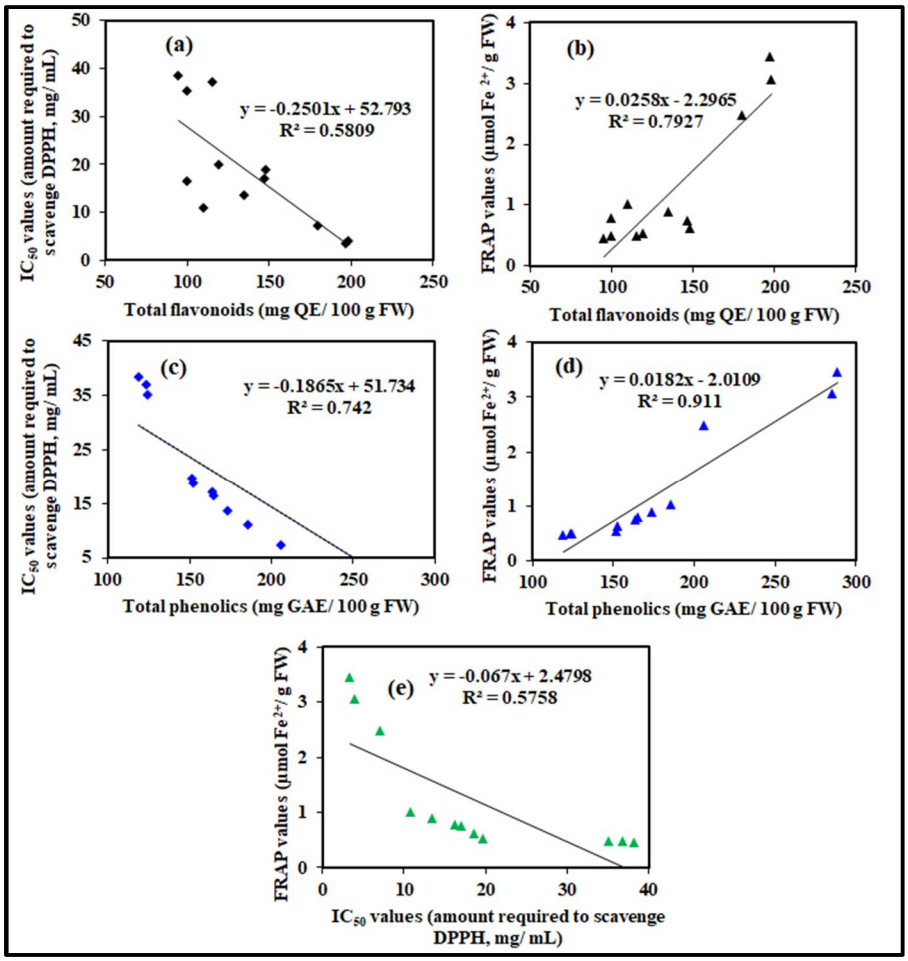

Fig. 2. Correlation between (a) DPPH scavenging activity and the total flavonoids (b) FRAP and the total flavonoids (c) DPPH scavenging activity and the total phenolics (d) FRAP and the total phenolics and (e) FRAP and the DPPH scavenging activity.

The plant species and the traits are clustered using the quantitative data of biochemical parameters of 12 plant species. In the cluster analysis of the plant species, the species were divided into three clusters (Fig. 3a). Clusters 1, 2, and 3 contained 4, 3, and 5 plant species respectively. Plant species *H. capitata*, *H. suaveolens*, *V. trifolia* and *C. bonduc* belonged to cluster 1, *C. motorius*, *A. paniculata* and *P. oleracea* belonged to cluster 2 while *B. lacera*, *L. alba*, *C. asiatica*, *C. dactylon*, and *A. paniculata* belonged to cluster 3. Plant species were separated into various

Table 2. Antioxidant activity expressed by FRAP values, the DPPH radical scavenging activity and Vitamin-C content of the medicinal plants

Plant species	FRAP (µmol Fe^{2+}/ g FW)	IC_{50} values*	Vit- C (mg/ 100 g FW)
A. paniculata	2.4667 ± 0.065 c	7.145 ± 0.051 f	10.418 ± 0.723 h
B. lacera	0.5276 ± 0.041g	19.708 ± 1.021c	33.118 ± 0.948 de
C. bonduc	1.0108 ± 0.071 d	10.940 ± 1.132 e	41.763 ± 2.108 a
C. motorius	0.4523 ± 0.064 g	38.560 ± 1.157 a	20.194 ± 0.970 g
H. suaveolens	3.4390 ± 0.091 a	3.376 ± 0.384 g	37.451 ± 1.423 b
V. trifolia	0.7755 ± 0.313 ef	16.544 ± 0.773 d	35.395 ± 1.177 bcd
C. asiaticsa	0.7467 ± 0.212 ef	18.080 ± 0.931 cd	25.498 ± 1.382 f
C. dactylon	0.6133 ± 0.082 fg	19.371 ± 0.574 c	37.352 ±2.784 b
P. oleracea	0.4794 ± 0.029 g	36.770 ± 1.309 ab	36.911 ± 0.519 bc
H. capitata	3.3405 ± 0.131 b	4.079 ± 0.101 g	36.164 ± 1.591 bcd
A. paniculata	0.4826 ± 0.04 g	35.387 ± 1.252 b	31.117 ± 0.947 e
L. alba	0.8810 ± 0.043 de	13.703 ± 1.059 e	33.783 ± 0.580 cde
$LSD_{0.05}$	0.1966	2.7967	3.4795

*Amount required to scavenge DPPH radical, mg/ml.

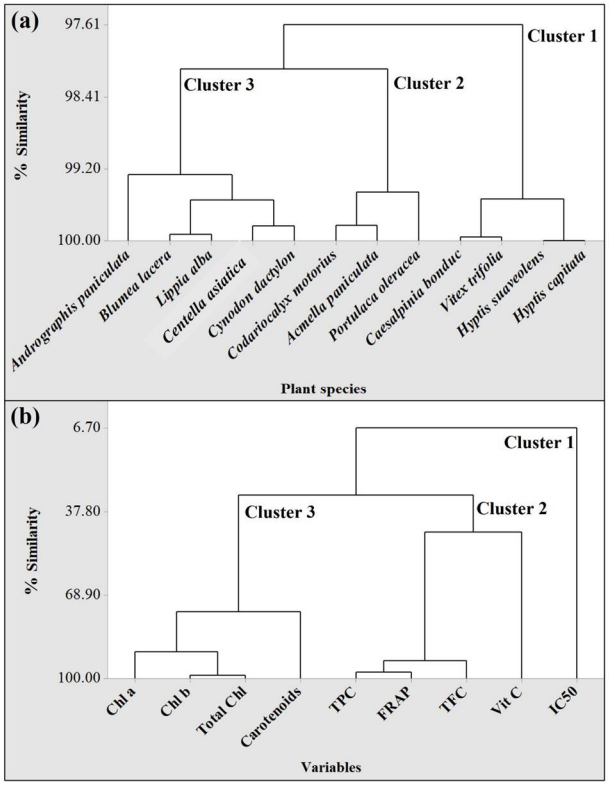

Fig. 3. Dendrogram of the studied plant species based on the quantitative data of different chemical compositions (a) and the relationship of different chemical variables of the studied plant species (b)

clusters due to variations in quantitative values of different biochemical descriptors, which might be influenced by their genetic makeup (Chanda *et al*. 2018). Additionally, multivariate analysis of biochemical traits of different plant species produced different clusters in the dendrogram. The *H. capitata*, *H. suaveolens*, *V. trifolia*, and *C. bonduc* may have similar antioxidant activities; the plant species *C. motorius*, *A. paniculata*, and *P. oleracea* may show another biochemical activity and the rest of the plant species may have similar phytochemical properties and antioxidant activities ((Fig. 3a). Again, in the cluster analysis of the phytochemical and antioxidant traits, the traits were divided into three (3) clusters (Fig. 3b). Cluster 1 contained only one (1) species while clusters 2 and 3 contained 4 traits each. The IC_{50} values belonged to cluster 1, TPC, FRAP, TFC, and Vit C belonged to cluster 2 while traits related to pigments (Chl a, Chl b, Total Chl, and Carotenoids) belonged to cluster 3. Traits were separated into various clusters due to variations in quantitative values of different plant species, which might be influenced by their modes of function (Chanda *et al.*, 2018). Again, the dendrogram of the traits revealed that the dissimilarity of IC_{50} with TPC is 91.01%, that with TFC is 86.72%, and that with FRAP is 91.01% (Fig. 3b). This implies that the correlation of IC_{50} with TPC, TFC, and FRAP is inversely correlated which further confirms the result obtained from the correlation study (Fig. 2). Again, fig. 3b shows that the dissimilarity of FRAP with TPC is 0% while with TFC is 4.29% which implies that the correlation of FRAP with TPC and TFC is directly correlated. This result also further confirms the correlation study results (Fig. 2).

Fresh leaf extracts of twelve traditionally important medicinal plants were investigated to determine their phytochemical constituents and antioxidant capacity. Among these plants, *Hyptis suaveolens* and *H. capitata* showed higher levels of phenolics, flavonoids, and antioxidant activities, as assessed by DPPH scavenging capacity and FRAP values while other plants also performed well with good amounts of phytochemicals. A strong positive correlation between the phenolics, flavonoids, and antioxidant capacity indicated that phenolics and flavonoid content contributed largely to the antioxidant activities of the plant extract.

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