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A Comparative Evaluation of the Antioxidant Activity of Some Medicinal Plants Popularly Used in Bangladesh

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ABSTRACT

Background and Objective: The people of the rural area of Bangladesh have a great history of putting into use many indigenous medicinal plants to cure diseases. The attention has focused on phytochemicals as sources of natural antioxidants. Therefore, the study was conducted to investigate phenol, total flavonoid content and antioxidant activities in five medicinal plants namely Neem (*Azadirachta indica*), Tulsi (*Ocimum tenuiflorum*), Pudina (*Mentha spicata*), Ulat kambal (*Abroma augusta*) and Lemon grass (*Cymbopogon citratus*). **Materials and Methods:** Five medicinal plants were collected from the medicinal garden of BIRTAN regional station, Noakhali, Bangladesh. The High-Performance Liquid Chromatography (HPLC) method assessed the phenolic compound. The antioxidant activity (DPPH radical scavenging activity) and total flavonoid content were assessed by the UV spectrophotometer method. **Results:** Significant differences in natural antioxidants among the species were studied. Antioxidant activity was found to range from 40.483-78.373%. The highest antioxidant activity was observed in Neem (78.373% inhibition), followed by Lemongrass (74.717%) and Pudina (62.483%). The phenolic compound and total flavonoid content ranged from 1.13-1.953 mg GAE/g extract and 97.6-145.203 mg QE/g extract, respectively. Neem leaf extract showed the highest in both flavonoid compounds and antioxidant activity suggesting the good potential of this plant could be considered responsible for conferring antioxidant ability and use for traditional medicine. A positive significant correlation was observed between antioxidant activity with total flavonoid content (0.528*) and an insignificant positive correlation with phenolic compound (0.225). **Conclusion:** It is possible to suggest that the plant extracts' total phenolic and flavonoid content is the reason for the antioxidant activity. Medicinal plants with good antioxidant activity could serve as potential sources of natural antioxidants.

KEYWORDS

Neem leaf, lemon grass, antioxidant activity, phenolic content, flavonoids, medicinal plants, medicinal value

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INTRODUCTION

Reactive oxygen species (ROS), such as Hydrogen Peroxide (H_2O_2) and Hypochlorous Acid (HOCl) and free radicals, such as the Hydroxyl Radical (OH^-) and Superoxide Anion (O_2^-), are produced as normal products of cellular metabolism. Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases and premature aging[1]. Many medicinal plants contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids, which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[2]. Moreover, plant secondary metabolites such as flavonoids and terpenoids play an important role in defense against free radicals[3]. Therefore, consumers should increase their intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above diseases[4]. Traditional herbal medicines have been attracting great attention as alternative and supplemental therapies[5]. Devil's cotton is used in diabetes mellitus, as an antioxidant, uterine tonic and an emmenagogue, dysmenorrhea, amenorrhoea, sterility and other menstrual disorders, rheumatic pains of joints and headaches with sinusitis. The seed oil of Ulat kambal has been used for the treatment of pain[6].

Data from various studies indicate that medicinal plants contain a wide variety of natural antioxidants, such as phenolics, flavonoids and tannins, which possess more potent antioxidant activity than common dietary plants. Compounds responsible for such antioxidant activity can be isolated and used for the prevention and treatment of free radical-related disorders[7]. Therefore, recent attention has increased to finding naturally occurring antioxidants for use in food or medicine to substitute artificial antioxidants. Synthetic antioxidants are being limited due to their carcinogenicity[8]. Rural individual's existence in diverse belts depends on indigenous plants and plant produce to fulfil their daily food intake, fodder, medicinal remedies, etc[9]. Indigenous herbaceous and sharp plants resources around the rural sites have been the main source of natural medicine for treating diseases since ancient times. Even though the extensive use of wild plants as medicines in hilly residents and remote areas of Bangladesh, a miniature is known about the antioxidant potential and chemical composition of these mentioned plants.

The antioxidant properties of medicinal plants are the key reasons for their pharmacological movements. Phenolic compounds in medical plants e.g. tulsi (*Ocimum tenuiflorum*) extracts including orientin, vicenin[10], eugenol, cirsilineol, isothymusin, isothymonin and rosmarinic acid[11], have been showing as noble antioxidant elements. Mineral antioxidants like zinc have been shown significantly high in tulsi (*Ocimum tenuiflorum*)[12]. There is an abundant genetic variability in the composition of tulsi (holy basil) cultured in diverse environments[13].

The purpose of the current investigation was to estimate the antioxidant activity, phenolic compound and flavonoid content of indigenous medicinal species collected from the Noakhali District, Bangladesh. Furthermore, the experiment sought to define the association between the DPPH antioxidant activity, phenolic compound and flavonoid content of five plant extracts that might be favourable sources of natural antioxidants and functional foods. The main objectives of this study were to estimate the antioxidant capacity of five medicinal plants through DPPH free radical scavenging activity. The phenolic compound and flavonoid content of extracts were also estimated.

MATERIALS AND METHODS

Study area: The field experiment was conducted in medicinal garden of Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN) regional station, Noakhali, Bangladesh. The study was continuing from April to June 2022. The plant samples were collected from the medicinal research field on April 7, 2022. The collected samples were packed in plastic bags and transported to the Waffen

Table 1: Medicinal plants sample for analysis

Local name	English name	Scientific Name	Family	Materials used
Neem	Indian lilac	*Azadirachta indica* L.	Meliaceae	Leaf
Sabuj tulsi	Holy basil	*Ocimum sanctum* L.	Lamiaceae	Leaf
Kalo tulsi	Holy basil	*Ocimum sanctum* L.	Lamiaceae	Leaf
Podina (dwarf)	Spearmint	*Mentha spicata* L.	Lamiaceae	Leaf
Lemongrass	Malabar grass	*Cymbopogon citratus*	Poaceae	Leaf
Ulat Kambal	Devil's cotton	*Abroma augusta* L.	Malvaceae	Leaf petiole

Table 2: Indigenous use of six medicinal plants

Plant material	Local name	Indigenous uses
Azadirachta indica A. Juss (Meliaceae)	Neem	Leprosy, skin infections[14]
Mentha arvensis L. (Labiatae)	Pudina	Leaves extracts are useful in internal heat and fever[15]
Ocimum sanctum L. (Labiatae)	Tulsi	To cure bronchitis, dysentery, dyspepsia, skin diseases and chronic fever[16]
Abroma augusta L. (Malvaceae)	Ulat Kambal	Anti-diabetic, analgesic, anti-inflammatory, thrombolytic, antioxidant, hypolipidemic, etc. To treat various diseases like diabetes mellitus, as a uterine tonic in emmenagogue, dysmenorrhea, amenorrhoea, sterility and other menstrual disorders, rheumatic pains of joints and headaches with sinusitis[17]
Cymbopogon citratus (Poaceae)	Lemon grass	*Cymbopogon citratus* is used as a sedative in Mexico[18]. The leaves are also consumed for sedative and analgesic purposes in Brazil[19,20] have found that *C. citratus* is widely used traditionally as medicine for treating nervous disturbances. Similarly, the tea prepared from leaves is broadly used as an antiseptic, antifever, antidyspeptic, carminative, tranquilizer and stomachic agent[21]. Several studies reveal that it is also used as an anti-inflammatory, antiseptic, diuretic, neurobehavioral, antimicrobial and fungistatic[22]

Research Laboratories, Dhaka, Bangladesh for antioxidant analysis. Flavonoid standards, quercetin and DPPH, gallic acid, BHA (tertbutyl-4-hydroxy-anisol), α-tocopherol and Folin–Ciocalteu reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Graded methanol and ethanol were purchased from Daejung Chemical and Metals Co. Ltd., Korea.

Plant sample: The plants samples are presented in Table 1. Data on the selected medicinal plants' uses, plant parts used, preparation and administration were collected from indigenous people (Table 2).

Preparation of plant extracts: The collected plant sample was washed, dried and powered at room temperature. The dried powdered samples 2 g for each were suspended and extracted in 25 mL of 80% methanol (v/v) and remained for one day in a shaker. After that, the extracts were filtered Advantec 4B (Tokyo Roshi Kaisha Ltd., Japan). The extraction procedure was repeated twice under the same conditions. Then the extract was dried using a vacuumed rotary evaporator (N-1000; EYLA, Tokyo, Japan) in a water bath at 40°C. Then the dried samples were weighed and kept at 4°C until further use.

Free radical scavenging activity: Antioxidant activity was measured using the stable radical DPPH based on method elaborated by Moreno *et al.*[23] with some changes. Concentration of extracts was added to 4 mL of 0.004% methanol solution of DPPH. About 30 min shaken and left the mixture in a dark place. The absorbance was calculated with a spectrophotometer (EMCLAB, Kulturstrasse, Duisburg) at 517 nm. All observations were performed in triplicate. The antioxidant activity was determined as the percent inhibition caused by the hydrogen donor activity of each sample according to the following:

$$\text{Inhibition}(\%) = \frac{\text{1- Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100$$

Phenolic compound: As 20 µL of each extract (1 mg/mL) was taken in 100 mL volumetric flax then 100 mL of 80% deionized water and 20% acetonitrile were added to the solution and allowed to stand for 10 min after vortexing. Then methanol (9:1%, v/v) was added. The samples were degassed by placing them in an ultrasonicate bath at 25°C. The extracted sample was filtered using a 0.22 µm hydrophobic PTFE syringe filter. The filtrate was taken into a 1.5 mL glass vial and 20 µL was injected into HPLC for analysis. The phenolic compound values are expressed in terms of gallic acid equivalents (GAE) in milligrams per gram of plant extract. All determinations were performed in triplicate.

Total flavonoid content (TFC): The flavonoid content was estimated by Ghasemnezhad *et al.*[24] total of 5 mL volume was made by using 0.5 mL of sample mixed with 0.1 mL of 10% $Al(NO_3)_3$, 0.1 mL of potassium acetate (1 M) and 4.3 mL 80% ethanol. The mixture was vortexed and kept in the solution for 40 min for proper reaction at room temperature. A spectrophotometer was used to measure absorbance at 415 nm. It was filtered all solutions with Whatman filter paper before measurement. Three times were performed all determinations. Quercetin equivalent (mg QE/g of sample) is expressed unit of total flavonoid content.

Statistical analysis: Antioxidant activity, phenolic compound and total flavonoid content are reported as the Mean±Standard Deviation (SD). Significance differences for multiple comparisons were determined using One-way Analysis of Variance (ANOVA). Mean comparisons were performed by Duncan's new multiple range test (DMRT) at 5% level of probability. The bivariate correlations between all antioxidant capacity assays and total phenolic contents were analyzed.

RESULTS AND DISCUSSION

Antioxidant activity: The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols and flavonoids. In this study, the antioxidant capacity of six medicinal plants collected sample from the field of BIRTAN regional station, Noakhali, Bangladesh, was evaluated. In Table 3 DPPH savanging activity was summarized. Significant difference ($p<0.01$) was observed between the six medicinal plants examined for DPPH antioxidant activity ranging from 40.438 to 78.73%. This antioxidant activity range was wide. It means that a wide variety of bioactive compounds, such as phenolics, flavonols, carotenoids and tannins, are estimated in the medicinal plants investigated. More antioxidant activity was found in Neem (78.373%) which was statistically similar to Lemongrass (74.717%) at $p<0.05$ for DMRT mean performance test. It was followed by Pudina (dwarf) (62.483%) and Ulat kambal (58.543%).

The highest antioxidant activity of Neem could be due to the high concentration of total flavonoid content and higher phenolic compound in the plants. Any natural drug that is used as a remedy for skin diseases is assumed to possess antioxidant properties. Phenolic and flavonoid content has been shown to contribute significantly to antioxidant activity[25]. Antioxidant activity was found lower than the present studied result in Lemongrass (62.47%) reported by Woidylo *et al.*[26]. The lowest antioxidant activity was performed by the medicinal plant Kalo tulsi (40.483%). The DPPH values of white holy basil were higher than those of black holy basil. Reactive oxygen species (ROS) and free radicals are implicated in DNA damage, cancer and accelerated cell aging. Leaf extracts of Neem were reported to contain phenolic acid and a complex mixture of 20 known flavonoids, predominantly eriodictyol and luteolin[27]. Higher antioxidant activity (74.717%) was also observed in the leaves of lemongrass for higher phenolic compound and total flavonoid content. The lowest levels of DPPH scavenging activity were in the leaves of Kalo tulsi, with an estimated 40.483% of the DPPH radical quenched ($p<0.05$).

Phenolic compound: The TPC ranges from 1.13±0.052-1.953±0.049 mg gallic acid equivalent per gram of sample, in Kalo tulsi and Ulat kambal, respectively. The highest value of phenolic compound in the study in Ulat kambal (1.953±0.049) mg gallic acid equivalent per gram. The lowest phenolic compound

Table 3: Mean performance of different antioxidant activity

Medicinal plants	Mean±SD		
	Phenolic compound (mg GAE/g)	Antioxidant activity (DPPH scavenging activity) (%)	Total flavonoid content (mg QE/g)
Neem	1.403±0.058[b]	78.373±1.637[a]	145.203±1.526[a]
Sabuj tulsi	1.367±0.074[b]	50.77±2.455[c]	115.8±1.931[d]
Kalo tulsi	1.13±0.073[c]	40.483±2.607[d]	125.872±1.857[c]
Podina (dwarf)	1.343±0.090[b]	62.483±2.642[b]	127.603±2.319[c]
Lemon grass	1.417±0.065[b]	74.717±1.515[a]	135.432±2.412[b]
Ulat Kambal	1.953±0.069[a]	58.543±1.658[b]	97.6±2.486[e]

Same alphabets(s) in a column did not differ significantly at p≤0.05 by DMRT and all analyses are mean of triplicate measurements±standard deviation

was observed in Kalo tulsi (1.13±0.052 mg GAE/g). Earlier findings that 51.1 mg GAE/g as a dry weight basis were reported by Amiot *et al*.[28]. These results were not doubtful because phenolic compounds in plant foods are largely influenced by genetic factors and environmental conditions[29]. The difference in phenolic compound content could affect the antioxidant capacity of plants because many phenolic compounds in plants are good sources of natural antioxidants[30]. The phenolic content of both white and red holy basil was estimated and significant differences between them ($p<0.05$). The phenolic compound of white and red holy basil was 1.367±0.052 and 1.13±0.052 mg gallic acid equivalent per gram of sample, respectively.

Many plant species have a remarkably high total phenolic content. The high level of phenolics in Neem (*A. vulgaris*) could be due to known phenolic compounds, such as caffeic acid, neochlorogenic acid and ferulic acid[31].

Total flavonoid content: Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties, which protect against free radicals that damage cells and tissues[32]. Therefore, the total content of flavonoids was evaluated from the regression equation of the calibration curve, expressed in QE as milligrams per gram of extract sample (mg QE/g). The content of flavonoids varied from 97.6±1.758-145.203±1.080 mg QE/g extract sample (Table 3). The highest amount of flavonoids was found in the leaf extracts of Neem (145.203±1.080 mg QE/g extract), followed by Lemon grass (135.432±1.705 mg QE/g extract), Pudina (127.603±1.640 mg QE/g extract) and Kalo tulsi (125.872±1.313 mg QE/g extract), revealed that this type of plant chemicals are responsible for free radical scavenging activity. Flavonoids represent the antioxidant activity of plants[33] with their scavenging activity[34]. The current study was mainly focused on understanding the effect of Neem leaves on curing various skin diseases. High flavonoid content indicates the probability of significant antioxidant potential of the Neem leaves. The total flavonoid content of Neem falls by earlier estimations done by Bhatnagar and McCormick[35].

Some people also apply Neem leaves on the skin to treat wounds and burns. In an earlier study, Mahmoud *et al*.[36] reported that Neem leaf extract had a characteristic effect on dermatophytes, especially for lower polar extracts over high polar ones. The author suggested that one possible explanation for this is the flavonoid quercetin contained in the extract. Researchers explain this activity by the presence of active ingredients like triterpenes or limonoids such as meliantriol, azadirachtin, desactylimbin, quercetin, sitosterol, nimbin, nimbidin, nimbinin, nimbosterol and margisine[37] and/or to different bitter substances such as alkaloids, phenols, resins, glycosides, terpenes and gums[38]. Lamson and Brignall[39] attributed the antifungal properties of Neem extract to the inhibition of protease activity of dermatophytes induced by Neem organic extract. It should also be mentioned here that in a study conducted by Verschoyle *et al*.[40]. It was found that the antibacterial activity of the combinations of quercetin and quercitrin, quercetin and morin and quercetin and rutin were much more active than either flavonoid alone. Quercetin was not

Table 4: Correlation coefficient among different antioxidant activity

Traits	Phenolic compound (mg GAE/g)	Antioxidant activity (DPPH scavenging activity (%)	Total flavonoid content (mg QE /g)
Phenolic compound (mg GAE/g)	1	-	-
Antioxidant activity (DPPH scavenging activity) (%)	0.225	1	-
Total flavonoid content (mg QE/g)	-0.635**	0.528*	1

*,**Significance at 1 and 5% levels, respectively

detected in this experiment but quercetin belongs to under flavonoids category that has tremendous antioxidant activity[41]. Neem has natural anti-histamine and anti-inflammatory properties. Research revealed that quercetin may help to prevent prostate cancer[42]. Low amount of flavonoid content was investigated in Ulat kambal (97.6±1.758) (Table 3), which has the highest amount of phenolic compound in the studied sample.

Correlation between antioxidants and total phenolics and total flavonoids: Correlation is the mutual relationship between two variables. The total phenolic content showed an insignificant positive correlation with antioxidant activity (0.225) (Table 4). This insignificant positive correlation suggested that the major antioxidant components might not be phenolics and could be sterols, tocopherols, ascorbic acid and carotenoids. This type of relation has other factors that act as proton donors from its hydroxyl group and show radical scavenging activity[43]. The association of correlation between total flavonoid content and phenolic compound was significantly negative (-0.635**) (Table 4). A significant positive correlation was estimated between flavonoid content and antioxidant activity (0.528*). The antioxidant activity is not limited to flavonoids but also includes vitamins C and vitamin E, carotenoids and chlorophylls. Many earlier studies revealed a high correlation coefficient between phenolic content and antioxidant activity.

CONCLUSION

More antioxidant activity was found in Neem similar to Lemongrass and followed by Pudina and Ulat Kambal. The highest antioxidant activity of Neem is due to the high concentration of total flavonoid content and higher phenolic compound in the plants. Any natural drug that is used as a remedy for skin diseases is assumed to possess antioxidant properties. The DPPH values of Sabuj Tulsi (white holy basil) were higher than those of Kalo tulsi (black holy basil). Higher antioxidant activity was also observed in the leaves of Lemongrass for higher phenolic compound and total flavonoid content. The lowest antioxidant activity was in the leaves of Kalo tulsi. The highest value of phenolic compound in the study was in Ulat kambal followed by Lemon grass and Neem. The phenolic compound was high in Sabuj Tulsi compared to Kalo tulsi. The highest flavonoids in Neem followed by Lemon grass, Pudina and Kalo tulsi, indicating that these phytochemicals are likely to be responsible for the free radical scavenging activity. High flavonoid content indicates the probability of significant antioxidant potential of the Neem leaves. It is also a natural antihistamine and anti-inflammatory and cures various skin diseases. However, a relatively low amount of flavonoids was detected in Ulat Kambal, which had the highest phenolic compound in the studied sample. The total phenolic content of the investigated plant extracts showed an insignificant positive correlation with antioxidant activity and a significant correlation of antioxidant activity was observed with total flavonoid content. In this study, a good relationship between antioxidant activity with phenolic compounds and flavonoid compounds was estimated, thus indicating that the high DPPH activity may be related to the phenolic compounds and flavonoid compounds in these plants.

SIGNIFICANCE STATEMENT

Plants are a source of food and medicines that are used in pharmaceuticals, neutraceuticals and food supplements and contribute to modern medicine. Herbal medicines have less or no side effects compared to allopathic drugs. About eighty percent people of marginal communities around the world are using medicinal plants and rely entirely on traditional medicines. Therefore, there is an urgent need to compare

the antioxidant properties of usable medicinal plants. The antioxidant activity of Neem for high flavonoid content and phenolic compound that remedy skin diseases. Flavonoids are responsible for free radical scavenging activity and act as a natural antihistamine and anti-inflammatory and cure skin diseases. A positive and significant correlation was found between antioxidant activity with phenolic compounds and flavonoid contents.

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