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Variation of Secondary Metabolites, Chlorophyll Contents, and Antioxidant Activity in Six Medicinally Important Plants in Bangladesh

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ABSTRACT

Plant phenolics and flavonoids function as antioxidants that act as scavengers of free radicals in the human body. This study aimed to determine the total phenolics and flavonoids contents, ferric-reducing antioxidant power (FRAP), free radical scavenging ability, chlorophyll contents, and total amounts of carotenoids of six medicinal plants *viz*. *Anisomeles indica* (L.) Kuntze, *Eclipta prostrata* (L.) L., *Glinus oppositifolius* (L.) Aug.DC., *Litsea glutinosa* (Lour.) C.B.Rob., *Origanum vulgare* L., and *Oxalis debilis* Kunth. The results reveal that *L. glutinosa* has the maximum amount of total phenolic content (TPC) (1.906 mg GAE g^{-1} FW) and total flavonoid content (TFC) (13.933 mg QUE 100g^{-1} FW), while the lowest TPC (0.2803 mg GAE g^{-1} FW) was observed in *O. vulgare* and the least TFC (1.6 mg QUE 100g^{-1} FW), was observed in *A. indica*. The leaves of *L. glutinosa* showed excellent antioxidant properties (IC_{50} = 6.24 mg mL^{-1}), and *G. oppositifolius* showed the least antioxidant potential (IC_{50} =18.423 mg mL^{-1}). Pigment content such as chlorophyll-a was highest in *E. prostrata* (1.5963 mg g^{-1}), while *L. glutinosa* has the highest chlorophyll-b (2.176 mg g^{-1} FW), chlorophyll-(a+b) (3.6157 mg g^{-1} FW), and carotenoids (1.61 µg 100g^{-1} FW) content. A strong linear correlation (DPPH, R^2 = 0.8688, FRAP, R^2 = 0.8595) was found between TPC and antioxidative capability. *L. glutinosa* contains significant amounts of phenols and flavonoids, which have antioxidant qualities, suggesting the possibility of using this species in phytotherapy and pharmacy.

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Introduction

People have been utilizing plants to treat a variety of illnesses/ailments from ancient times; because they contain a number of active chemicals with significant pharmacological effects. Natural antioxidants, like flavonoids and polyphenols, are commonly used in medicine to treat a variety of serious diseases due to their well-known ability to reduce oxidative stress and neutralize free radicals (Yen and Chuang, 2000). High concentrations of phenolics and flavonoids with potent antioxidant qualities found in leafy greens have been linked to a lower incidence of diabetes, cancer, heart disease, and neurological illnesses, according to epidemiological research (Adebooye et al., 2008). In addition, phenolic and flavonoid compounds naturally produced in plants have numerous functions including anti-inflammatory, antimicrobial, antiviral, neuroprotective, antitumor, antipyretic, antimalarial, analgesic, antioxidant activity with free-radical scavenging capacity, anti-proliferative agents, antiangiogenic, etc. (Hakim et al. 2021; Ullah et al., 2020). Consequently, as compared to artificial phenolic antioxidants like butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate, consumers prefer to eat fruits and vegetables because of their high antioxidant content and low toxicity (Jideani et al., 2021).

Bangladesh is home to more than 1,200 medicinal plants (Uddin and Lee, 2020) that have been used to treat human as well as veterinary ailments for a long time (Sarwar, 2020). Among them six important medicinal plants, naturally available and which are traditionally used as therapeutic medicine by the urban and tribal peoples of Bangladesh *viz. Anisomeles indica* (L.) Kuntze, *Eclipta prostrata* (L.) L., *Glinus oppositifolius* (L.) Aug.DC., *Litsea glutinosa* (Lour.) C.B.Rob., *Origanum vulgare* L., and *Oxalis debilis* Kunth, were used for the study. People have been using these medicinal herbs extensively for many years to cure common illnesses like cough, fever,

asthma, diarrhea, allergies, inflammation, skin problems, jaundice, hair loss, abdominal pain, gastrointestinal problems, dyspepsia, healing of cuts and wounds, colic, rheumatic arthritis, etc. throughout the world (Akber et al., 2011; Kadir et al., 2013; Sofowora et al., 2013). Therapeutically and industrially essential chemical compounds like alkaloids, flavonoids, phenolic compounds, and others can be significantly identified as a new source of crude drugs from their phytochemical constituents (Mangoale and Afolayan, 2020; Velu et al., 2018). The experiments were carried out with the aforesaid plant parts to investigate the status of phytochemicals (total phenolics, flavonoids, and carotenoids) along with their overall antioxidant enzyme activity and to quantify the green leaf pigments available in them.

Materials and Methods

Plant Samples

Three different plants harbored at the Bangladesh Agricultural University Botanical Garden, each representing a replicate from each species, were marked (Table 1). The study area is located at 90°26'29.6" E and 24°43'26.8" N, 29 meters above sea level (Sarwar, 2020). A humid subtropical monsoon climate (Köppen *Cwa*) prevailed in this area with an average annual rainfall of about 273.5 cm (https://en.wikipedia.org/wiki/Mymensingh). The temperature falls below 15°C in winter and reaches as high as 40°C in summer.

Their tender twigs were picked up with a pair of scissors and stored in a Ziploc bag before chemical analysis. Tender leaves from each replicate were cut into small pieces, and the chemical analysis was performed with the freshly harvested samples following the standard protocols.

Chemical Reagents

Analytical-grade chemical reagents and solvents were used, including HCl, $FeCl_3.6H_2O$, Quercetin, Aluminium Chloride, Sodium Acetate Trihydrate, Gallic Acid (GA), Acetic Acid, TPTZ (2,4,6-tripyridyl-s-triazine), Folin-Ciocalteu Reagent, Potassium Acetate, and DPPH (2,2-diphenyl-1-picrylhydrazyl).

Determination of Total Phenolic Contents (TPC)

TPC was determined by the Folin-Ciocalteu technique slightly modified from the protocols described by Adebooye et al., (2008). 2.5 g of leaf sample and 50 mL of methanol were combined in a 250 mL beaker. The samples were then homogenized using an OV-5 homogenizer (VELP, Italy) for two to three minutes. The mixture was centrifuged for 5 minutes at 5000 rpm to remove the supernatant for phenol analysis after being left in the dark for 60 minutes. Gallic acid (GA) solutions were used as the standard. A 50 mL test tube was filled with precisely 0.5 mL of various Gallic acid solutions or plant extract concentrations. Then 2.5 mL of the Na_2CO_3 (7.5%) solution and 2.5 mL of the Folin-Ciocalteu reagent were added. The mixture was kept at 25°C in dark condition for half an hour. At 760 nm, absorbance was then measured.

Total Flavonoid Content (TFC) Determination

To determine TFC a modified version of Kumaran and Karunakaran's (2007) protocol was followed. Quercetin was used as standard. One milliliter of Quercetin solutions or plant extracts at different concentrations was added to 50 ml test tubes separately followed by the addition of 5.6 mL of distilled water, 200 mL of 1M Potassium Acetate, 200 µl of 10% $AlCl_3$, and 3 mL of Methanol in each tube. After 30 minutes of dark keeping at 25°C, the mixture's absorbance at 420 nm was assessed using a spectrophotometer. By plotting the concentration against the respective absorbance values, a standard curve was established for calculating the total flavonoid content as QUE per 100 g FW (Figure 1b).

Assessment of Ferric Reducing Antioxidant Power (FRAP Values)

The method outlined by Kumari and Padmaja (2012) was used to compute the FRAP value. The absorbance value was recorded at 593 nm. A standard curve was constructed after the FRAP values were calculated for various standard antioxidants. The outcomes were determined using the following equation and expressed as µg AAE per mg of the fresh plant extract.

AAE = [(Absorbance at 593 nm/0.002) – 0.004].

FRAP value of sample (µM) = (Change in the absorption of sample from 0 to 4 minute/change in absorbance of standard from 0 to 4 minute) × FRAP value of the standard (100 µM) and expressed as µ mol Fe(II)/g (Benzie and Strain, 1999).

DPPH Radical Scavenging Capacity Assay

A stable free radical DPPH was used to measure the extracts' capacity to scavenge free radicals. 50 mL of methanol and 2.5 g of samples were combined, and the mixture was then exposed to darkness for 30 minutes. The mixture was centrifuged at 5000 rpm for 5 minutes to separate the extract. To make the assay up to 5 mL of volume, 2 ml of plant extracts and 3 ml of 40 g ml^{-1} DPPH in methanol were employed. After the mixture was well combined, it was left in the dark at 25°C for 30 minutes.

Table 1. List of the plants used in this study.

Common Name	Scientific Name	Family
Gobura	*Anisomeles indica* (L.) Kuntze	Lamiaceae
Kalokeshi	*Eclipta prostrata* (L.) L.	Asteraceae
Gima	*Glinus oppositifolius* (L.) Aug.DC	Mollugniaceae
Kharajura	*Litsea glutinosa* (Lour.) C.B.Rob.	Lauraceae
Oregano	*Origanum vulgare* L.	Lamiaceae
Amrul	*Oxalis debilis* Kunth	Oxalidaceae

Table 2. Chlorophyll a, chlorophyll b, and total chlorophyll content in leaves.

SL No.	Medicinal plant species	Chl. a	Chl. b	Total Chl.
1	*Anisomeles indica*	1.5663 [a]	1.9493 [b]	3.5157 [a]
2	*Eclipta prostrata*	1.5963 [a]	1.7923 [b]	3.3950 [ab]
3	*Glinus oppositifolius*	1.5153 [a]	1.5047 [c]	3.0200 [b]
4	*Litsea glutinosa*	1.4957 [a]	2.1760 [a]	3.6517 [a]
5	*Oreganum vulgare*	0.3290 [b]	0.1897 [e]	0.5187 [d]
6	*Oxalis debilis*	0.0750 [c]	1.2170 [d]	1.2920 [c]
$LSD_{0.05}$		0.2478	0.2021	0.3905

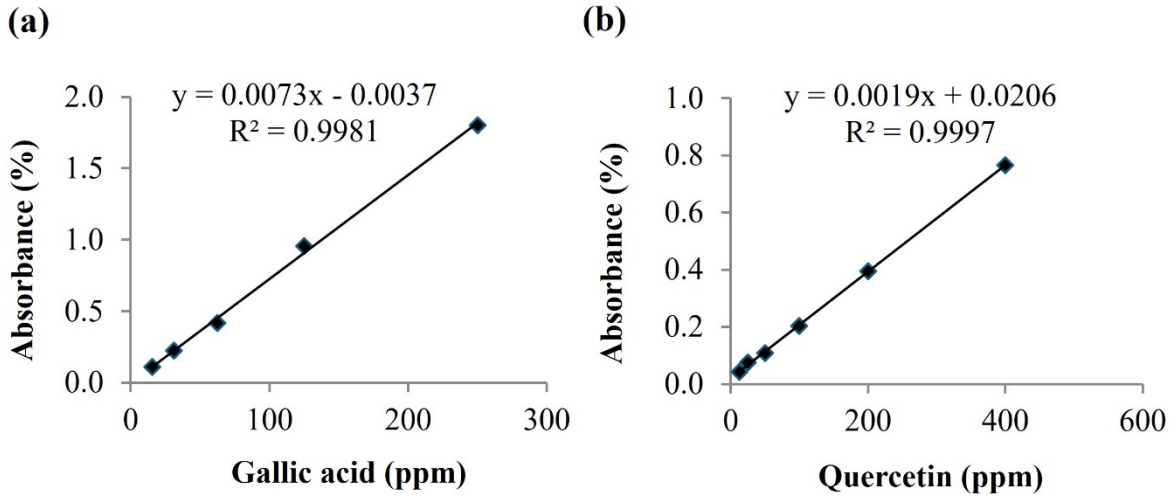

Figure 1. (a) Gallic acid and (b) Quercetin standard curve concentrations using ascorbic acid.

Methanol served as the negative control, ascorbic acid served as the positive control, and an extract devoid of DPPH served as the blank. The absorbance measurement was made at 517 nm, and the % of inhibitory activity was calculated as follows:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Here,
A_0 = absorbance of the blank sample,
A_1= absorbance of the plant sample.

By plotting the percentage inhibition against the concentration of the corresponding extract, the IC_{50} value. The calibration curve and linear regression were used to determine the IC_{50} value, which is the amount of antioxidants required to reduce DPPH by 50%. The results are expressed as a percentage decrease in DPPH absorption. Antioxidant activity is inversely proportional to the IC_{50} values.

Pigments Determination

The pigments were extracted according to Abugri et al. (2013). 1.0 g grinded samples and 25 mL of methanol (99.9%) were agitated and kept in the dark for seven days. Water was eliminated using Whatman No. 1 filter paper and anhydrous Sodium Sulfate (Na_2SO_4). Absorbance was measured with a spectrophotometer (DR-6000, Hach, USA) at 470 nm, 649 nm, 664 nm, and 760 nm. The amount of chlorophyll (a and b), total chlorophylls (a+b), and carotenoids were calculated following the formulas:

Chlorophyll a (Chl a) = (13.36 A664 - 5.19 A649) × 25/FW

Chlorophyll b (Chl b) = (27.43 A649 - 8.12 A664) × 25/FW

Total Chlorophyll (Chl a+b) = Chl a+ Chl b

Carotenoids (CX+C)=(4.785 A470+3.657 A664-12.76 A649)×25/FW

Where,
A649 = absorbance at 649 nm,
A664 = absorbance at 664 nm,
A470 = absorbance at 470 runs,
FW = Fresh weight of plant leaves (mg)

Statistical Analyses

One-way ANOVA analysis was employed to assess whether the difference between the mean values of each parameter studied was statistically significant or not using the free software Statistix 10. Additionally, Microsoft Excel was utilized to prepare graphs and process data.

Results

Total Phenolic Content

The TPC of the leaves of six different plants varied significantly, ranging from 0.4073 to 1.739 mg GAE $100g^{-1}$ fresh weight (Figure 3a). *Litsea glutinosa* had the highest total soluble phenolic concentration (1.739 mg GAE $100g^{-1}$ FW), followed by *A. indica* (1.222 mg GAE $100g^{-1}$ FW). The lowest phenol was detected in *O. vulgare* (0.4073 mg GAE $100g^{-1}$ FW) leaves.

Assuming the phenolic content of *L. glutinosa* (1.739 mg GAE $100g^{-1}$ FW) to be 100 per cent, the relative phenolic contents in *O. vulgare*, *O. debilis*, *E. prostrata, G. oppositifolius,* and *A. indica* were as 23.421%, 55.664%, 49.896%, 33.105%, and 70.270%, respectively.

Total Flavonoid Content

The amount of flavonoids varied considerably from 1.6 to 13.933 mg QUE $100g^{-1}$ fresh weight (Figure 3b). The highest flavonoid was found in *L. glutinosa* (13.933 mg QUE $100g^{-1}$ FW), while the least was found in the leaves of *A. indica* (0.2873 mg QUE $100g^{-1}$ FW). Assuming the phenolic content of *L. glutinosa* (13.933 mg QUE $100g^{-1}$ FW) as 100 per cent, the relative phenolic contents in *O. vulgare*, *O. debilis*, *E. prostrata*, *G. oppositifolius,* and *A. indica* were as 76.08%, 54.55%, 83.86%, 63.16%, and 11.48%, respectively.

Leaf pigments

The chlorophyll-a content of leaves varied considerably, ranging from 0.075 to 1.5963 mg g^{-1} FW. The leaf of *E. prostrata* contained the highest chlorophyll-a (1.5963 mg g^{-1} FW), followed by *A. indica* (1.5663 mg g^{-1} FW). The lowest chlorophyll-a concentration was in the leaf of *O. debilis* (0.075 mg g^{-1} FW).

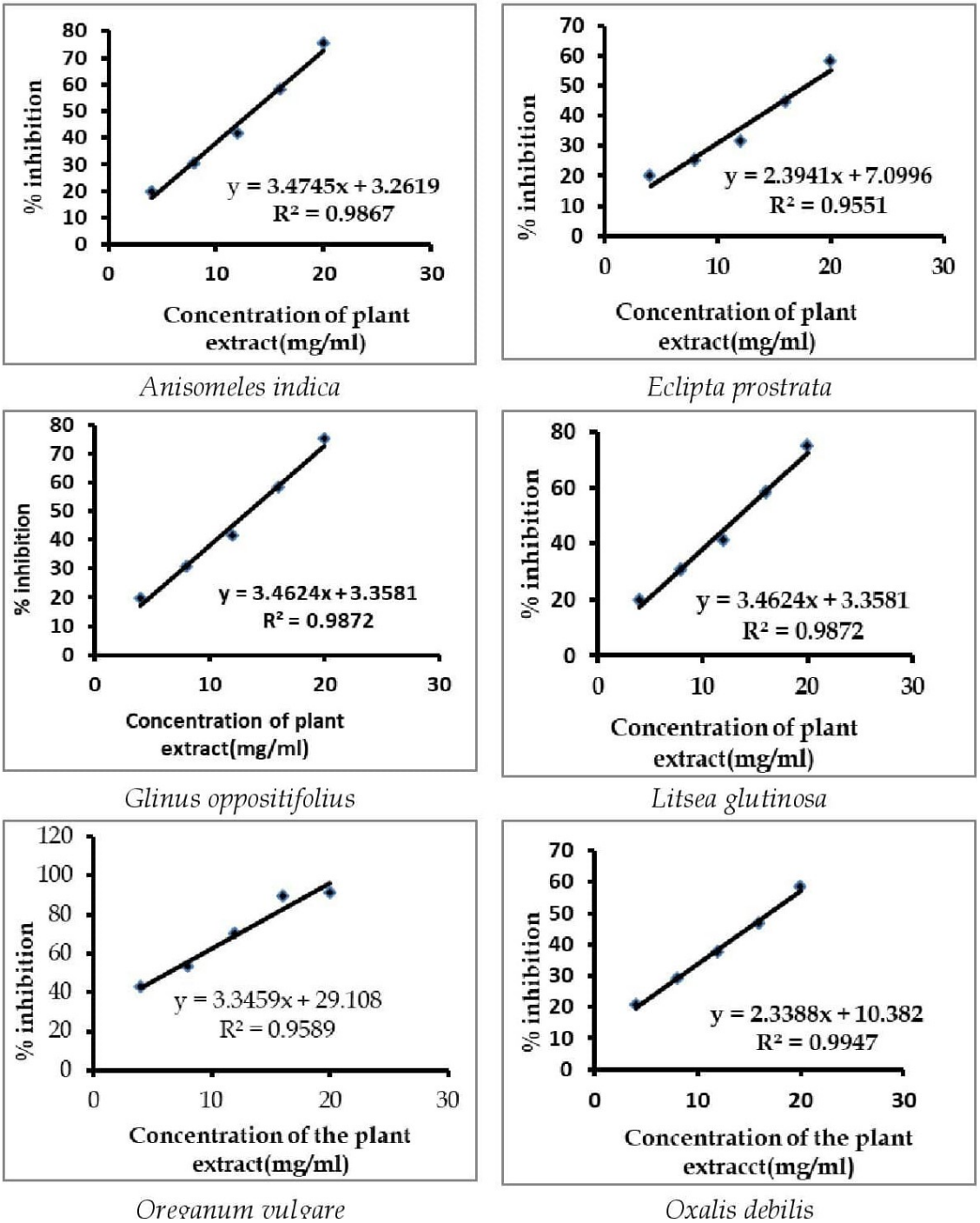

Figure 2. Construction of linear regression equation (y=mx+c) by plotting different concentrations of the plant extracts on the X-axis and the % inhibition on the Y-axis

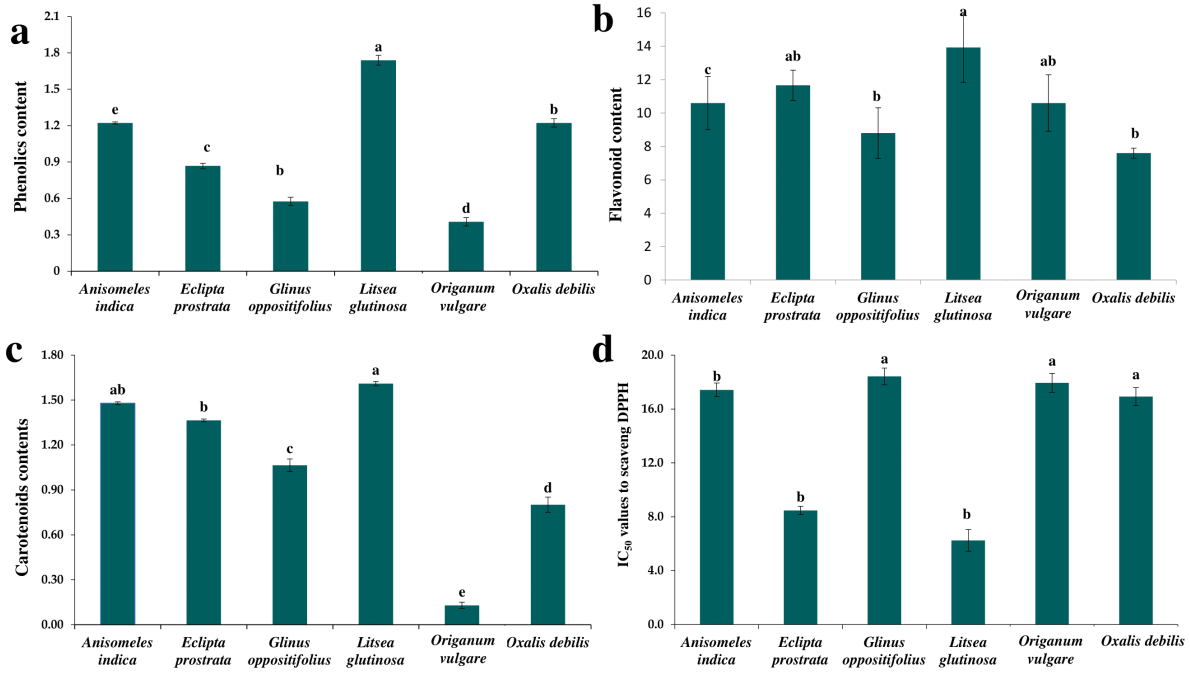

Figure 3. (a) Total soluble phenolic content, (b) Total soluble flavonoid content, (c) Amount of carotenoids, (d) IC_{50} values for methanolic extracts. The data points are the mean of 3 replicates ± SEM. Bars with different alphabets are significantly different at $P \leq 0.05$.

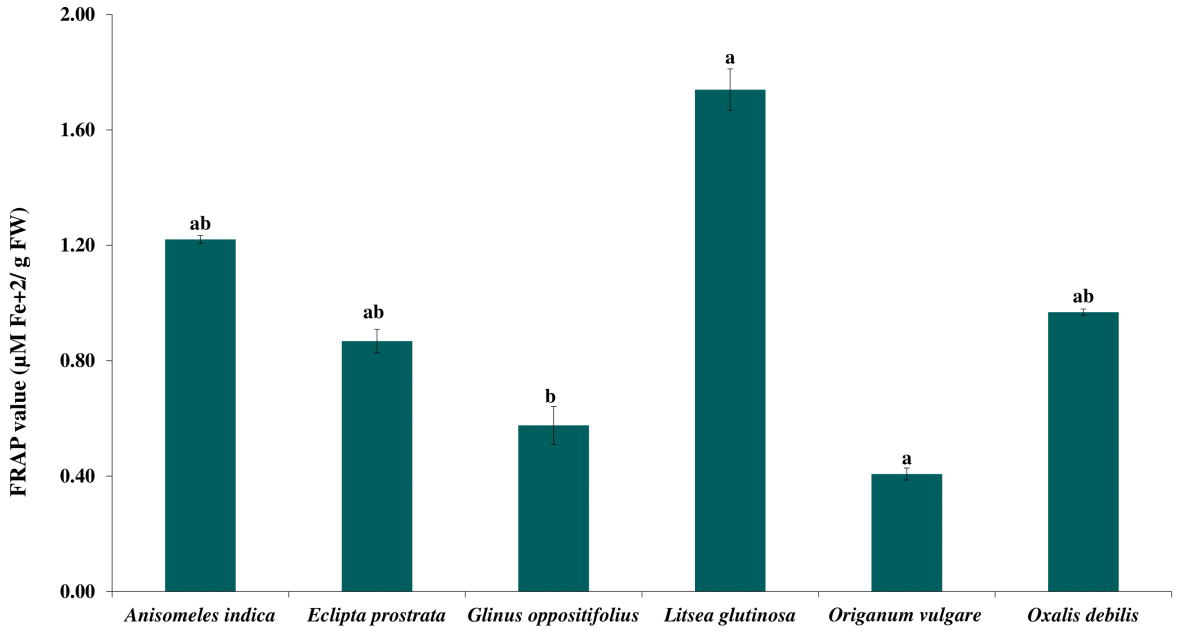

Figure 4. The FRAP values of leaf extracts from six different medicinal plants for DPPH radical scavenging. The data points are the mean of 3 replicates ± SEM. Bars with different alphabets are significantly different at $P \leq 0.05$.

Assuming *E. prostrata* 's chlorophyll-a content (1.5963 mg g^{-1} FW) as 100 percent, the relative chlorophyll-a contents in *A. indica, G. oppositifolius, O. debilis*, *L. glutinosa,* and *O. vulgare* were 98.12%, 94.925%, 4.7%, 44.164%, 93.697%, and 20.610%, respectively. The chlorophyll-b content of leaves ranged from 0.1897 to 2.1760 mg g^{-1} FW. The leaf of *L. glutinosa* contained the highest chlorophyll b (2.1760 mg g^{-1} FW), followed by *A. indica* (1.9493 mg g^{-1} FW). The lowest level of chlorophyll-b was encountered in the leaf of *O. vulgare* (0.1897 mg g^{-1} FW). Assuming the chlorophyll-b content of *L. glutinosa* leaf as 100 percent, the chlorophyll-b contents of *O. vulgare*, *O. debilis* var. *corymbosa*, *L. glutinosa*, *G. oppositifolius*, and *A. indica* were 8.718%, 55.93%, 82.37%, 69.14%, and 89.58%, respectively. The chlorophyll (a+b) values ranged from 0.5187 to 3.65 mg g^{-1} FW. The leaf of *L. glutinosa* had the highest chlorophyll (a+b) content (3.65 mg g^{-1} FW), followed by *A. indica* (3.51 mg g^{-1} FW). *Origanum vulgare* leaf chlorophyll (a+b) content is the lowest amount (0.5187 mg g^{-1} FW). Others such as *O. vulgare* (0.5187 mg g^{-1} FW), *O. debilis* (1.2920 mg g^{-1} FW), and *E. prostrata* contained chlorophyll (a+b) (3.395 mg g^{-1} FW).

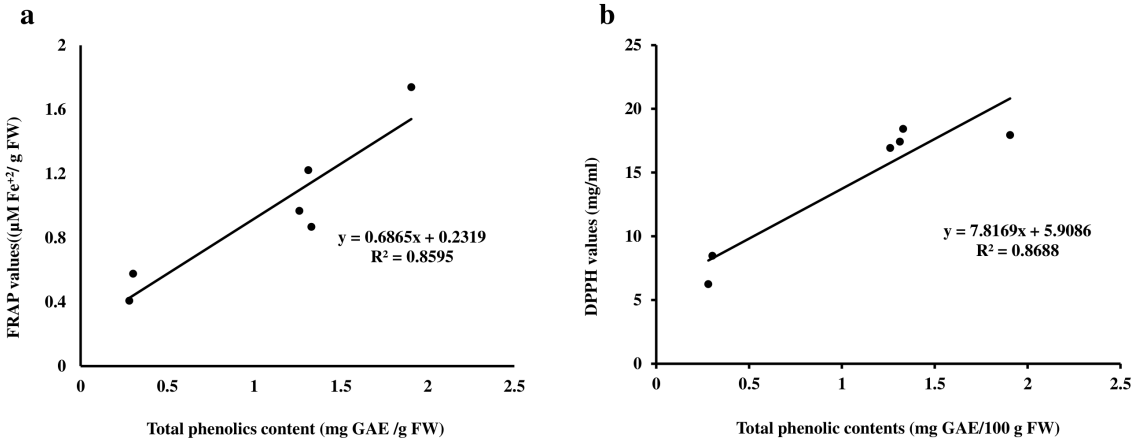

Figure 5. (a) Correlation between the FRAP values and the total phenolics contents. (b) Relationship between DPPH scavenging assay and total phenolic compounds in six medicinal plants.

The chlorophyll (a+b) content of *O. vulgare*, *O. debilis*, *E. prostrata*, *G. oppositifolius*, and *A. indica* was determined to be 14.204%, 35.380%, 92.970%, 82.701%, and 96.275%, respectively, when compared to the chlorophyll (a+b) content of *L. glutinosa* leaf, assumed at 100%.

Carotenoid Content

Total carotenoid content ranged from 0.1290 to 1.610 µg g^{-1} FW (Figure 3c). *Litsea glutinosa* leaf showed the highest carotenoid content (1.610 µg g^{-1} FW), followed by *A. indica* (1.4787 µg g^{-1} FW). The lowest value was detected in *O. debilis*, while *G. oppositifolius* (1.0647 µg g^{-1} FW) and *E. prostrata*. (1.3643 µg g^{-1} FW). Assuming the carotenoids content of *L. glutinosa*'s leaf as 100%, and the relative carotenoids in *O. vulgare*, *O. debilis, G. oppositifolius, E. prostrata*, and *A. indica* were 8.012%, 50.329%, 66.130%, 84.739%, and 91.844%, respectively.

DPPH Radical Scavenging Assay

The DPPH scavenging abilities of the chosen medicinal plants were employed to investigate their total antioxidant activities. The linear regression equation (y=mx+c) was constructed using % inhibition. Concentrations and the IC_{50} values were calculated using the linear regression equation (Figure 2). The IC_{50} values of leaf extract varied significantly and ranged from 6.24 to 18.423 mg mL^{-1} leaf methanolic extract (Figure 3d). *Oxalis debilis*, *E. prostrata*, and *L. glutinosa* had IC_{50} values for leaf extracts to scavenge DPPH that are statistically identical (Figure 3d). Assuming IC_{50} values of leaf methanolic extract of *L. glutinosa* leaves (6.24 mg mL^{-1}) to scavenge DPPH as 100%, the relative IC_{50}, *O. debilis*, *E. prostrata*, *G. oppositifolius,* and *A. indica* were as 287.5%, 271.66%, 135.68%, 295.24%, and 279.17%, respectively.

Ferric Reducing Antioxidant Power (FRAP)

The testing method and the chemical makeup of the extract both have a significant impact on the plant extract's antioxidant capability. It cannot be fully assessed by a single approach because it can be influenced by a multitude of things. To assess the diverse antioxidant action pathways, numerous measures of antioxidant characteristics are necessary. So, the antioxidant potential of six medicinal plants was also assessed using the FRAP test. Ferric (III) ions are changed into ferrous (II) ions in this test to measure antioxidant capacity. A simple technique, the FRAP assay could be used with both alcoholic and aqueous plant extracts. The antioxidant capabilities of these plants ranged from 0.40733 to 1.7392 M Fe^{2+}/g, as illustrated in Figure 4. With 1.73930 µM Fe^{2+}/g, *L. glutinosa* had the maximum antioxidant capacity, whereas *O. vulgare* had the lowest value (0.40733 µM Fe^{2+}/g). Other species' leaves displayed FRAP contents of 0.5757 µM Fe^{2+}/g for *G. oppositifolius* and 0.9680 µM Fe^{2+}/g for *O. debilis*. The relative FRAP content of *O. vulgare*, *O. debilis*, *E. prostrata*, *G. oppositifolius*, and *A. indica* were 23.421%, 56.354%, 49.896%, 33.105%, and 70.270%, respectively, when the value of the FRAP content in *L. glutinosa* leaf was taken into consideration.

Relationship between Antioxidant Capacity and TPC

Despite notable variations in the total antioxidant capacities and phenolic contents of the chosen plants, Figure 5a shows that there are linear-positive relationships (R^2= 0.8595) between the FRAP values and the TPC. The results of the two methods used to measure total phenolic content and antioxidant capacity showed strong correlations, suggesting that phenol molecules play a significant role in these plants' antioxidant activities and may therefore be important to their beneficial properties (Feng et al., 2016). A comparable robust connection (R^2= 0.8688, Figure 5b) was seen between the medicinal plants' chosen phenolic content and their capacity to scavenge DPPH. The findings demonstrated that the DPPH scavenging capacity of medicinal plants is positively correlated with their phenolic content, indicating the plants' efficacy in harnessing the phytochemicals they contain.

Discussion

Plant extracts contain phenolic compounds, which have been demonstrated to significantly increase a system's antioxidant capacity and to be important in preventing several oxidative stress-related diseases, including cancer (Shahidi and Ambigaipalan, 2015). Recent *in vitro* studies have shown that phenolics are more efficient than carotenoids, vitamins C and E, and other nutrients (Rodríguez-Roque et al., 2013). By scavenging lipid free

radicals or preventing hydrogen peroxides from turning into free radicals, they demonstrate antioxidant function. In this study, the phytochemical content of each plant is screened, and the variations in phenolics, flavonoids, chlorophyll contents, and antioxidant activity are also evaluated. Among six medicinal plants studied, total phenolics content was found in the order of *O. vulgare* < *G. oppositifolius* < *E. prostrata* < *O. debilis* < *A. indica* < *L. glutinosa* (Figure 3a). The plant with the highest phenolic content was *L. glutinosa,* a member of the Lamiaceae family, which is applied to treat diabetes, edema, colds, arthritis, asthma, and traumatic injuries (Wang et al., 2016). Phenolic compounds are characterized by multiple phenol groups; some of them chelate metal ions and donate hydrogen atoms or electrons to neutralize free radicals in aqueous solutions (Petti and Scully, 2009). Furthermore, phenolic compounds have a range of biological properties that may be related to their antioxidant action, including antibacterial, anticancer, and antimutagenic properties (Shui and Leong, 2002).

Flavonoids, which comprise flavones, flavonols, and condensed tannins, are typical secondary plant metabolites. Epidemiological studies suggest that eating foods rich in flavonoids may protect individuals against illnesses linked to oxidative stress (Li et al., 2023). Numerous plant sources of flavonoids have demonstrated their anti-oxidant stress and anti-free radical capabilities *in* vitro (Shen et al., 2022). The amount of flavonoid in the leaf's methanolic extracts was found in the order of *A. indica* < *O. debilis* < *G. oppositifolius* < *O. vulgare* < *E. prostrata* < *L. glutinosa* (Figure 3b). The plant *E. prostrata* had the second-highest amount of phenolics. Studies have shown that *E. prostrata* (syn. *Eclipta alba*) is an excellent cancer fighter, antioxidant, anti-mycotoxin, anti-hyperglycemic, and immune modulator (Ayyakkannu et al., 2020).

According to Yen and Duh (1994), antioxidants are chemicals that, in the presence of ambient oxygen or reactive oxygen species, can either block or slow down the oxidation processes. They serve as a protective barrier against diseases caused by free radicals (Kumari and Padmaja, 2012). Endogenous antioxidants comprise non-enzymatic substances such as uric acid, bilirubin, albumin, and metallothionein as well as enzymes like glutathione peroxidase, catalase, and superoxide dismutase (Mirończuk-Chodakowska et al., 2018). Antioxidants from outside the body, such as dietary supplements or medications containing the active component of an antioxidant chemical, are necessary when the body's defenses against reactive oxygen species (ROS) are insufficient to fully protect it (Pisoschi and Negulescu, 2011). Recent studies reported that synthetic antioxidants harm human health (Lourenço et al., 2019). Thus, scientists have been searching more actively in recent years for safe, effective natural chemicals that combat free radicals. In combination with the body's antioxidant defenses, consuming antioxidant-rich foods and plants seems like a smart idea. Antioxidants come primarily from the food we eat and other parts of plants (Lobo et al., 2010). Natural antioxidants can be found in abundance in traditional medicinal herbs. Many medicinal plants comprise antioxidants that protect cells from the cellular damage induced by ROS, superoxide, and peroxyl and hydroxyl ions, among other free radicals (Ramesh and Ilyas, 2017). The theory underlying oxidative stress holds that imbalances between the generation ROS and antioxidant defenses create oxidative stress which is responsible for a variety of illnesses, including rheumatism, diabetes, carcinogenesis, aging, arthritis, asthma, and various neuroscience disorders (Xu et al., 2017).

In the DPPH test, antioxidants convert the DPPH radical to a yellow compound, diphenyl-picryl hydrazine, with the extent of the reaction depending on the antioxidants' hydrogen-donating capacity (Frezzini et al., 2019). According to Chohra et al. (2020), The plant extract's antioxidant activity and its IC_{50} values are inversely correlated. A reliable and consistent technique for evaluating antioxidants' capacity to scavenge free radicals is the DPPH assay. (Zaman et al., 2020). A higher IC_{50} value implies a poorer scavenging activity of the scavengers, whereas a lower IC_{50} value shows that the extract is more successful as a DPPH scavenger since more scavengers were needed to achieve a 50% scavenging reaction (Olugbami et al., 2014). Of the six plants examined, *G. oppositifolius* displayed the lowest level of antioxidative ability due to its greatest IC_{50} value, while *L. glutinosa* had the highest level of antioxidative activity and the lowest IC_{50} value (6.24 mg mL^{-1}). The body's regular biochemical activities produce free radicals that are linked to several conditions, including diabetes, edema, traumatic damage, arthritis, asthma, and gastrointestinal problems such as dyspepsia, diarrhea, and dyspepsia (Wang et al., 2016).

Carotenoids defend against the sun and may prevent sunburns, photosensitivity, and even some types of skin cancer by altering how fibroblasts move through their cell cycle and aiding in the process of epithelization (Adumanya, 2016). According to this investigation, the leaf extract of *L. glutinosa* exhibited a high concentration of carotenoids, 1.61 mg/100 g FW. The findings of the analysis show that *L. glutinosa* leaf is a source of carotenoids and may hold promise for the creation of pharmaceuticals with anti-oxidant qualities. This study implies that *A. indica*, which has the second-highest carotenoid content, has potential applications in the therapy of rheumatism, AIDS, *Helicobacter pylori*, and cancer.

Photosynthetic pigments, such as porphyrin, are made of substances with very different chemical structures. The green pigment known as chlorophyll is made up of a magnesium ion-containing tetrapyrrole ring. The phytol chain, a long hydrophobic chain, is present in it (Kumari and Padmaja, 2018). Carotenoids and chlorophyll-a and -b make up the entirety of a leaf's pigment and are necessary for photosynthesis. Different species have different foliar pigment concentrations. The productivity of green plants is dependent on photosynthesis efficiency (Venkatesh et al., 2022). Chlorophyll and carotenoid variation in leaves, as well as their relationship, can be influenced by both internal and external factors. Ivanov et al. (2020) reported that chlorophyll and carotenoid content varied as per microclimatic conditions. The response to light and shade conditions of terrestrial plants has been measured by the ratio of chlorophyll-a and chlorophyll-b. Pigments can be analyzed both qualitatively and quantitatively by the absorbance of light (Teng et al., 2020). The amount of chlorophyll-a is expected to be higher than chlorophyll-b.

In this study, Chlorophyll-a content is higher in *E. prostrata* and Chlorophyll-b content in *L. glutinosa.* Comparing the total chlorophyll content of six plants, *L. glutinosa* showed the highest total chlorophyll content. Chlorophyll or its derivatives can be used as photodynamic agents to treat tumors or cancer (Brandis et al., 2006).

The FRAP assay indicates the antioxidant capacity of foods, drinks, and supplements that contain polyphenols. It interacts with a potential antioxidant (Spiegel et al., 2020). The study indicated that *L. glutinosa* contains many phytoconstituents and excellent antioxidant activity by effectively scavenging various free radicals. In this study, *L. glutinosa* shows a high quantity of FRAP values was found to be 1.739 μM Fe^{+2}/g FW in the leaf extract. The second highest FRAP value contains *A. indica.*

The results of the correlation analysis show that there was a significant correlation between these techniques, suggesting that all three assays were appropriate and trustworthy for estimating the total antioxidant capabilities of plant extracts. Other correlations between DPPH and phenolic compounds are also substantially associated because of the strong correlation that exists between the FRAP values and phenolic compounds.

Conclusions

The study examined the overall antioxidant capacity, phenolic content, and flavonoid content of extracts derived from six therapeutic plants. Science backs the traditional use of *L. glutinosa* as a readily accessible source of naturally occurring antioxidants with major health advantages. This is especially true given the plant's high level of antioxidant activity. This study does not clarify the specific functions of a plant species to a particular disease rather it generalizes the presence of live-saving essential secondary metabolites in those species. A significant amount of research is required to separate and identify the active components and their efficacy. Furthermore, future studies are recommended to explore the *in vivo* antioxidant capabilities of *L. glutinosa* and other medicinal plants under investigation.

Declarations

Authors Contributions

MMK, AKMGS, and MA designed the experiment. FF experimented and analyzed the data. All authors checked and validated data and results. FF and MJHJ prepared the draft manuscript. MMK, AKMGS, and MA reviewed and revised the draft. After studying the work, all authors gave their permission for submission.

Data Availability Statement

The data supporting the findings of this work are publicly accessible at https://www.doi.org/10.57760/sciencedb.06991 in the Science Data Bank.

Declaration of Competing Interest

The researchers stated that none of their known conflicts of interest or interpersonal connections could have impacted the published results.

Ethical Approval

We did not use any human or animal participants in our study. Upheld the highest standards of personal decorum, acting honorably in all of our interactions and undertakings in the workplace. In both our words and deeds, we were being honest. Our actions and decisions were motivated by the University's overall welfare rather than self-interest.

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