



## Screening of Bio-active Pigments, Antioxidant Activity, Total Phenolic and Flavonoid Content of Some Economically Important Medicinal Plants for Ethno-botanical Uses

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### ABSTRACT

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An experiment was conducted to determinate the phytochemical properties (chlorophyll-a, chlorophyll-b, carotenoids, antioxidant activity, total phenolic and flavonoid content) in ten medicinal plants namely Syal Kata (*Argemone mexicana* L.), Akanda (*Calotropis gigantea* (L.) Dryand.), Dumur (*Ficus carica* L.), Chalmugra (*Gynocardia odorata* R.Br.), Kata kachu (*Lasia spinosa* (L.) Thwaites), Shetodrone (*Leucas aspera* (Wild.)), Khona (*Oroxylum indicum* (L.) Kurz), Reri (*Ricinus communis* L.), Ghat kachu (*Typhonium trilobatum* (L.) Schott) and Bazna (*Zanthoxylum rhetsa* DC.) for ethno-botanical uses. The young fresh leaves were harvested and were subjected to methanolic (95%) extract. Total phenolic contents were analysed by using Folin - Ciocalteu method where gallic acid was used as standard. Total phenolic content varied from 94.53 mg GAE/100 g FW (*C. gigantea*) to 484.88 mg GAE/100 g FW (*L. aspera*). Total flavonoid contents were performed by using Quercetin as standard. Total flavonoid content varied from 137.25 mg QUE /100 g FW (*C. gigantea*) to 334.27 mg QUE/100 g FW (*G. odorata*). Antioxidant activity of these extracts was performed by using DPPH free radical scavenging assay. Total antioxidant capacity varied from IC<sub>50</sub> value 35.37 mg/mL (*A. mexicana*) to 90.47 mg/mL (*F. carica*) where ascorbic acid is used as standard. Results indicated that among the ten medicinal plants phenolics rich in *L. aspera*, flavonoids rich in *G. odorata*, antioxidant activity rich in *A. mexicana*. *Z. rhetsa* leaf rich in chlorophyll-a content (311.67 mg/100 g FW), *L. aspera* leaf rich in chlorophyll-b content (157 mg/100 g FW), *Z. rhetsa* rich in chlorophyll- (a+b) content as 439 mg/100 g FW, *A. mexicana* leaf rich in carotenoids content as 96 mg/100 g FW.

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## Introduction

Generation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Yagi, 1987; Finkel and Holbrook, 2000; Pham-Huy, He and Pham-Huy, 2008) is the consequence of lipid peroxidation which is mandatory and a normal physiological process in living systems (Hargguchi, 2001; Korkmaz et al., 2018). Free radicals have been associated with DNA damage carcinogenesis, coronary heart disease, and many other health problems related to advancing age, anemia, asthma, arthritis, inflammation, neurodegeneration, mutagenesis, alzheimer's and AIDS as well (Potterat, 1997; Das and Nanda, 1999; Cadenas and Davies, 2000; Marnett, 2000; Uchida, 2000; Devasagayam et al., 2004; Yingming et al., 2004; Sevindik, 2020). In vitro studies and findings strongly advocate that anti-oxidation potential have resilient protective effects against the stated diseases (Steinberg,

1991; Block et al., 1992; Ames et al., 1993; Knekt et al., 1997; Elliot, 1999; Kaur and Kapoor, 2002). Synthetic antioxidants viz. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate which are most widely used being restricted due to their probable health risks and side effects (Caillet et al., 2006; Kumaran and Karunakaran, 2007). Therefore, the exploration for antioxidants from natural resources has gained much consideration, and efforts have been made to identify new natural resources for active antioxidant compounds from plants. Plant secondary metabolite such as phenolic and flavonoids exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden (Amadò et al., 2002; Oberoi et al., 2015; Sevindik et al., 2017; Mohammed et al., 2019)

Medicinal plants-derived phenol, flavonoids and antioxidants which are in the form of raw extracts and/or chemical constituents are very proficient to inhibit the process of oxidation by neutralizing the free radicals (Zengin et al., 2011; Sevindik, 2018; Akgül et al., 2020). Among the 7000 species of medicinal plants recognized all over the world, more than 500 types of precious medicinal plants are said to be found in Bangladesh. We have selected ten most important and commonly used medicinal plants for our study. The selected medicinal plants were: Syal Kata (*A. mexicana*) indigenous in Mexico use to control malarial fever, leprosy, cold sores, wound healing, skin diseases and jaundice (Dash and Murthy 2011), Akanda (*C. gigantea*) cures intermittent fever, cold, cardio tonic, asthma, scabies etc. (Singh et al., 2014), Dumur (*F. carica*) is used in cancer treatment, hypoglycemic, and antimicrobial activities (Veberic et al., 2008), Chalmugra (*G. odorata*) use in skin disease, Kata kachu (*L. spinosa*) helpful for skin, Shetodrone (*L. aspera*) controls chronic skin eruptions and chronic rheumatism (Dash et al., 2014), Khona (*O. indicum*) is curing to inflammation, asthma, dysentery, vomiting etc (Deka et al., 2013), Reri (*R. communis*) is used to treat diarrhea, dysentery and skin infections (Pandhure, 2014), Ghat kachu (*T. trilobatum*) is traditionally prescribed for gastric ulcer, headache, swelling, chronic bronchitis etc. (Ali et al., 2012) and Bazna (*Z. rhetsa*) is known for curing bronchitis, heart troubles, piles and toothache (Ghani, 2003).

The main purpose of this study was to evaluate the bio active pigments, free radical scavenging activity, total phenolic and flavonoid content of ten commercially important medicinal plants for free radicals scavenging purposes.

## Materials and Methods

### Sample Collection

Plant samples *A. mexicana*, *C. gigantea*, *F. carica*, *G. odorata*, *L. spinosa*, *L. aspera*, *O. indicum*, *R. communis*, *T. trilobatum*, *Z. rhetsa* were collected from Bangladesh Agricultural University Botanical Garden in the ziploc bag and brought to the plant physiology laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh and stored for further chemical analyses.

### Reagents

Folin-Ciocalteu reagent, Sodium carbonate, DPPH (2, 2-diphenyl-2-picrylhydrazyl), Quercetin, potassium acetate, Aluminium Chloride.

### Parameter Determination

#### Spectrophotometric Pigments Determination

Chlorophyll a, b and total carotenoids were determined according to the modification of the procedure stated by (Lichtenthaler, 1987). In brief, 1gm fresh leaf or fruit samples were taken in glass bottles and 25 mL acetone added and shaken properly and the content kept in the dark condition for overnight. Absorbance reading was taken in the next day in spectrophotometer (DR-6000, Hach, USA) at 470, 649, 666 and 750 nm wave lengths. Afterward, amount of chlorophyll a, chlorophyll b, chlorophyll (a+b) and total carotenoids (sum of carotene and xanthophyll) were calculated using the following formulae and expressed as mg/100g fresh weight.

$$\text{Chlorophyll a (C}_a\text{)} = (13.36 A_{666} - 5.19 A_{649}) \times 25/\text{FW}$$

$$\text{Chlorophyll b (C}_b\text{)} = (27.43 A_{649} - 8.12 A_{666}) \times 25/\text{FW}$$

$$\text{Total Chlorophyll (C}_{a+b}\text{)} = (5.24 A_{666} + 22.24 A_{649}) \times 25/\text{FW}$$

$$\text{Carotenoids (C}_{X+C}\text{)} = (4.785 A_{470} + 3.657 A_{666} - 12.76 A_{649}) \times 25/\text{FW}$$

Where;

$A_{649}$  = Absorbance at 649 nm

$A_{664}$  = Absorbance at 664 nm

$A_{470}$  = Absorbance at 470 nm

FW = Fresh weight of plant tissue extracted (mg)

### DPPH Radical Scavenging Capacity Assay

The antioxidant activity of extracts, based on the scavenging activity of the stable DPPH free radical, was determined by the modified method described by Lee et al. (Lee et al., 2004). Exactly 2.5 g of composite leaf samples were weighing out to a 250 mL beaker and 50 mL methanol added and then homogenized plant samples for 2-3 minutes using OV-5 Homogenizer, VELP, Italy. The mixture was kept for 30 minutes in the dark condition. Immediately after darkness the content centrifuged for 5 minutes at 5000 rpm and then extract was separated for determining DPPH radical scavenging activity. The assay contained 3 mL of 40 µg/mL DPPH in methanol and made up to 5 mL with 2ml plant extracts. The contents were mixed well immediately and then incubated for 30 min at room temperature (25-27°C) at dark condition. The degree of reduction of absorbance was recorded at 517 nm using DR 6000 UV-Spectrophotometer.

The percentage of scavenging activity/inhibition activity was calculated and expressed as mg/mL.

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

Where;

$A_0$  = Is the absorbance of control (without extract)

$A_1$  = Is the absorbance of sample. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain  $IC_{50}$  value.

$IC_{50}$  = Is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system. The  $IC_{50}$  values are inversely proportional to the antioxidant activity.

### Determination of Total Phenolic Content (TPC)

Total phenolic compound assayed with a method modified after according to Li et al., (2008). Exactly 2.5 g of composite leaf samples were weighing out to a 250 mL beaker and 50 mL methanol added and then homogenized plant samples for 2-3 minutes using OV-5 Homogenizer, VELP, and Italy. The mixture was kept for 60 minutes in the dark condition and then the content centrifuged for 5 minutes at 5000 rpm, supernatant extract was taken for phenol determination.

Gallic acid was used here as standard. Exactly, 0.5 mL different concentrations of Gallic acid solutions or plant extracts were taken into a 50 mL test tube. Then 2.5 mL of

Folin-Ciocalteu reagent and 2.5 mL of Na<sub>2</sub>CO<sub>3</sub> (7.5%) solution was added. The mixture was kept in dark condition for half an hour at room temperature. Then absorbance was measured at 760 nm. The absorbance value is the reflection of the total phenolics content of the compound. After plotting the absorbance in ordinate against the concentration a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples and expressed as mg gallic acid equivalent (GAE)/100 g of leaf fresh weight (FW).

#### Determination of Total Flavonoids Content (TFC)

Flavonoid content assayed with a method modified after Kumaran and Karunakaran (2007). Exactly 2.5 g of composite leaf samples were weighing out to a 250 mL beaker and 50 mL methanol added and then homogenized plant samples for 2-3 minutes using OV-5 Homogenizer, VELP, and Italy. The mixture was kept for 60 minutes in the dark condition and then the content centrifuged for 5 minutes at 5000 rpm, supernatant extract was taken for flavonoid determination.

Quercetin was used here as standard. Exactly, 1 mL different concentrations of Quercetin solutions or plant extracts were taken into a 50 mL test tube. Then 3 mL of methanol, 200 µL of 10% AlCl<sub>3</sub>, 200 µL of 1 M potassium acetate and 5.6 ml distilled water was added. The mixture was kept in dark condition for half an hour at room temperature. Then absorbance was measured at 420 nm. The absorbance value is the reflection of the total flavonoid content of the compound. After plotting the absorbance in ordinate against the concentration a linear relationship was obtained which was used as a standard curve for the determination of the total flavonoid content of the test samples and expressed as mg Quercetin acid equivalent (QUE)/100 g of leaf fresh weight (FW).

#### Statistical Analyses

The collected data were statistically analyzed by using Minitab 17. Tukey's LSD test was applied to compare the treatments means at 0.05 level of confidence. Significant differences among treatments means were determined using the Duncan Multiple Range Test (DMRT).

## Result and Discussion

### Chlorophyll-a Content in Leaves of Ten Different Medicinal Plants

Chlorophyll-a content in leaves varied significantly among the tested six different medicinal plants and ranged from 311.67 to 67.33 mg/100 gm FW. *Z. rhetsa* leaf showed the highest amount of chlorophyll-a content (311.67 mg/100 gm FW) followed by the second highest in *O. indicum* leaf (299 mg/100 gm FW). The lowest content of chlorophyll-a was recorded in, *T. trilobatum* leaf (67.33 mg/100 gm FW). *A. mexicana*, *C. gigantea*, *F. carica*, *G. odorata*, *L. spinosa*, *L. aspera* and *R. communis*, contained 101.33, 107.33, 112.33, 171.67, 176.33, 199 and 111.33 mg/100 gm FW respectively (Figure 1).

### Chlorophyll-B Content in Leaves of Ten Different Medicinal Plants

Chlorophyll-b content in leaves varied widely among the tested ten plants and ranged from 157 to 41.88 mg/100

gm FW. *Leucas aspera* leaf showed the highest amount of chlorophyll-b content (mg/100 gm FW) followed by the second highest in *Z. rhetsa* leaf (127.66 mg/100 gm FW). The lowest content of chlorophyll-b was found in *A. mexicana* leaf (mg/100 gm FW). *C. gigantea*, *F. carica*, *G. odorata*, *L. spinosa*, *O. indicum*, *R. communis* and *T. trilobatum* contained 68.67, 85.67, 121, 124.67, 120.67, 64.33 and 71.33 mg/100 gm FW respectively (Figure 2).

### Total Chlorophyll Content in Leaves of Ten Different Medicinal Plants

Chlorophyll content in leaves of ten different medicinal plants varied widely (Figure 3). Among the tested ten plants it ranged from 439 to 138 mg/100 gm FW. *Z. rhetsa* leaf showed the highest amount of chlorophyll-(a+b) content as 439 mg/100 gm FW followed by the second highest in *O. indicum* leaf (420 mg/100 gm FW). The lowest value of chlorophyll-(a+b) content was found in *T. trilobatum* leaf (138 mg/100 gm FW). Chlorophyll-(a+b) contents of *A. mexicana*, *C. gigantea*, *F. carica*, *G. odorata*, *L. spinosa*, *L. aspera*, and *R. communis* were 143.21, 178, 198, 293, 301, 356 and 176 mg/100 gm FW, respectively (Figure 3)

### Total Carotenoids Content in Leaves of Ten Different Medicinal Plants

Total carotenoids content in leaves varied widely among the tested ten plants and ranged from 96 to 52.67 mg/100 gm FW (Figure 4). *A. mexicana* leaf showed the highest amount of carotenoids content as 96 mg/100 gm FW followed by the second highest in *F. carica* leaf (52.67 mg/100 gm FW). The lowest value of carotenoids content was found in *T. trilobatum* leaf (7.77 mg/100 gm FW). The leaves of other species showed the carotenoids content as: *C. gigantean* (18.33 mg/100 gm FW), *G. odorata* (19.60 mg/100 gm FW), *L. spinosa* (28 mg/100 gm FW), *L. aspera* (34.33 mg/100 gm FW) *O. indicum* (39 mg/100 gm FW) *R. communis* (33.33 mg/100 gm FW) and *Z. rhetsa* (22 mg/100 gm FW) (Figure 4).

### Radicals Scavenging Activity By DPPH

The IC<sub>50</sub> value (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of leaf extract to scavenge DPPH radical varied significantly among the tested ten medicinal plants and ranged from 35.37 mg/mL (*A. mexicana*) to 90.47 mg/mL (*F. carica*) (Figure 5). The IC<sub>50</sub> values were to be 76.15, 71.15, 66.06, 66.71, 55.98, 78.13, 75.57, 50.97 mg/mL for *C. gigantean*, *G. odorata*, *L. spinosa*, *L. aspera*, *O. indicum*, *R. communis*, *T. trilobatum*, and *Z. rhetsa*, respectively. The *A. mexicana* leaf extract demonstrated the most potent activity (Figure 5).

### Total Phenolic Content (TPC)

Variation in TPC was very large, ranged from 94.53 to 484.88 mg GAE/100 g fresh weight. *L. aspera* had total soluble phenolic content of 484.88 mg GAE/100 g FW that was the highest among all the study samples. The lowest TPC content was detected in *C. gigantea* (94.53 mg GAE/100 g FW) leaves. The TPC content in the extracts of analyzed portions of the assayed plants was found in the order as *L. aspera* > *R. comunis* > *Z. rhetsa* > *L. spinosa* > *G. odorata* > *F. carica* > *O. indicum* > *A. mexicana* > *T. trilobatum* > *Calotropis gigantean* (Figure 6).

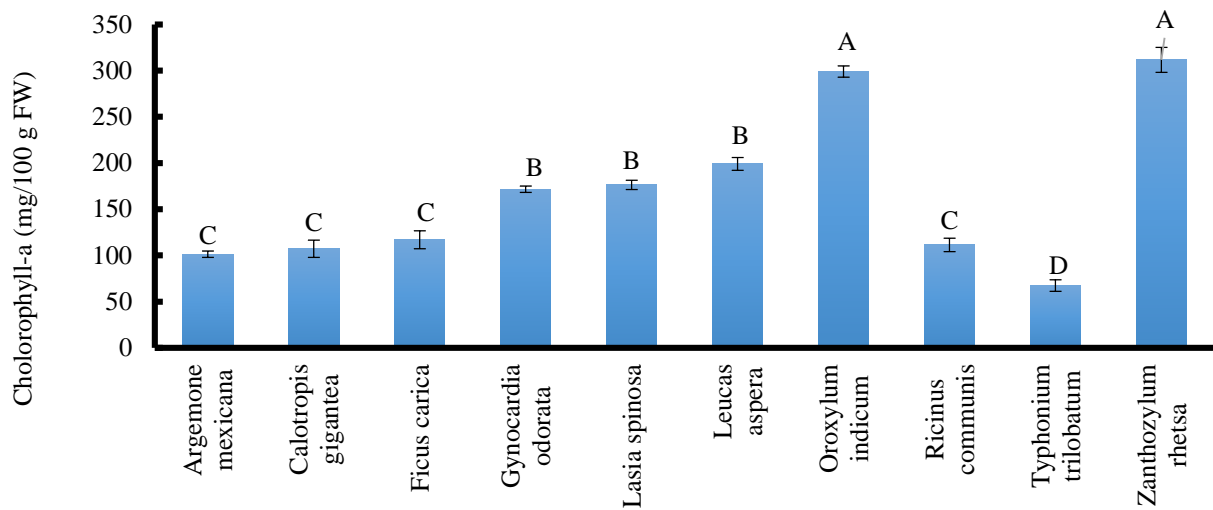


Figure 1. Chlorophyll-a content in leaves of ten different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing different letters are significantly different from each other at  $P \leq 0.05$ .

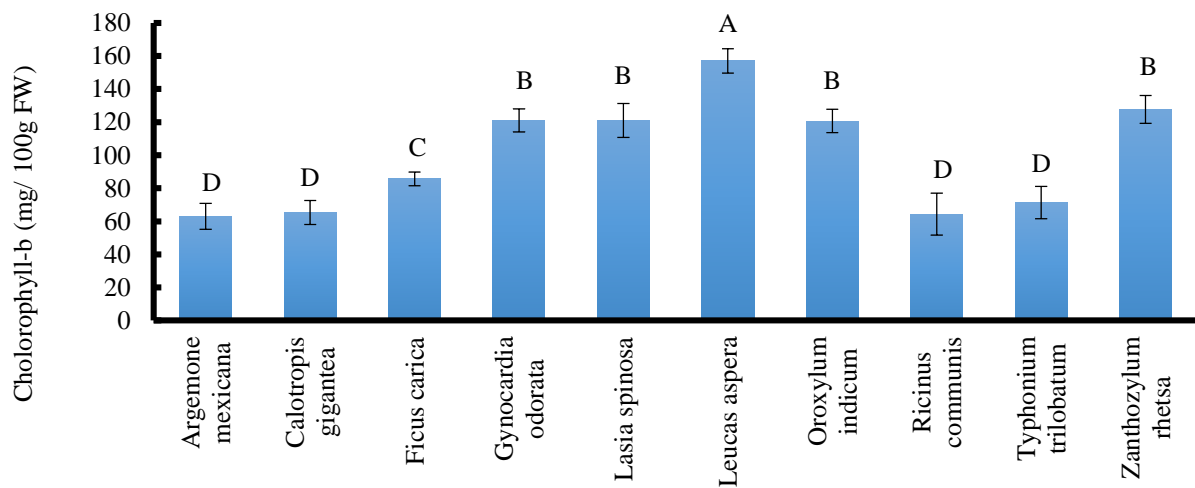


Figure 2. Chlorophyll-b content in leaves of ten different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing different letters are significantly different from each other at  $P \leq 0.05$ .

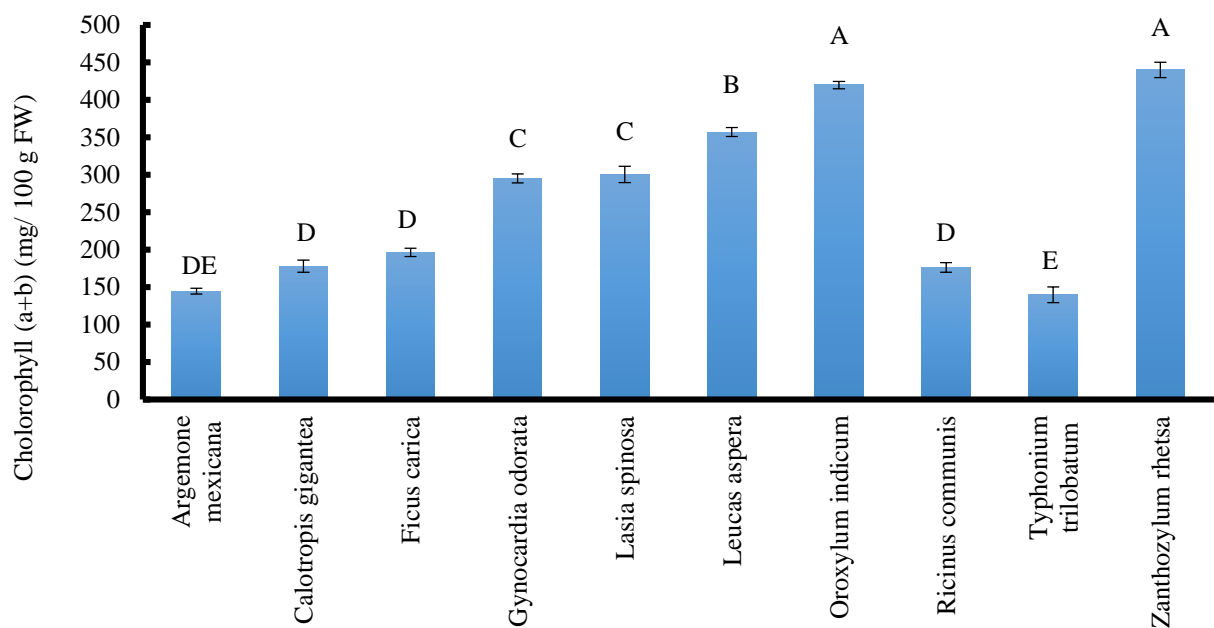


Figure 3. Chlorophyll (a+b) content in leaves of ten different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing different letters are significantly different from each other at  $P \leq 0.05$ .

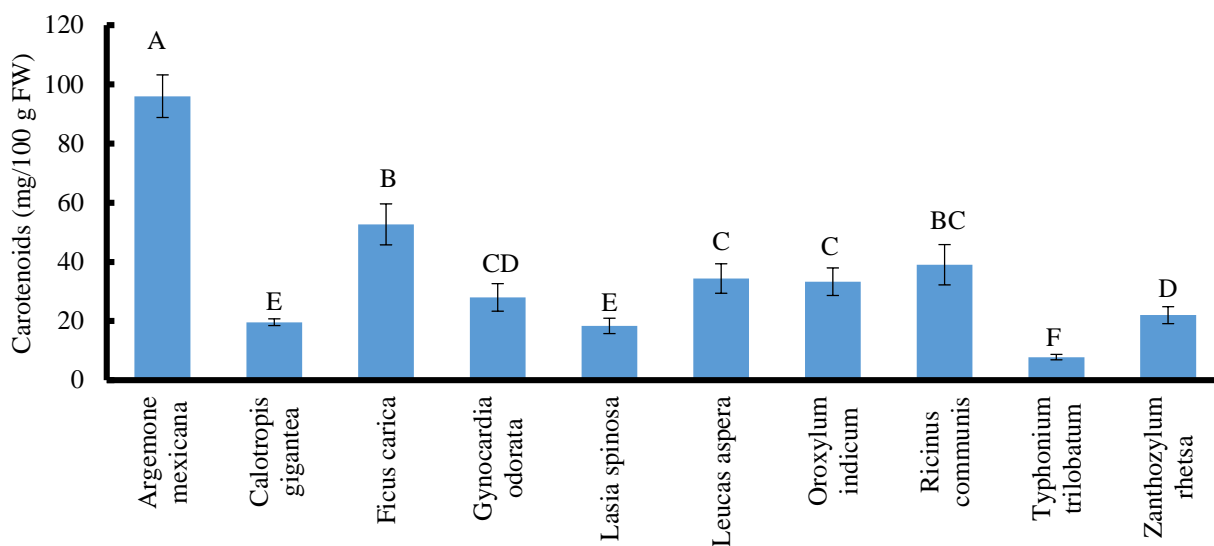


Figure 4. Amount of carotenoids in leaves of ten different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing different letters are significantly different from each other at  $P \leq 0.05$ .

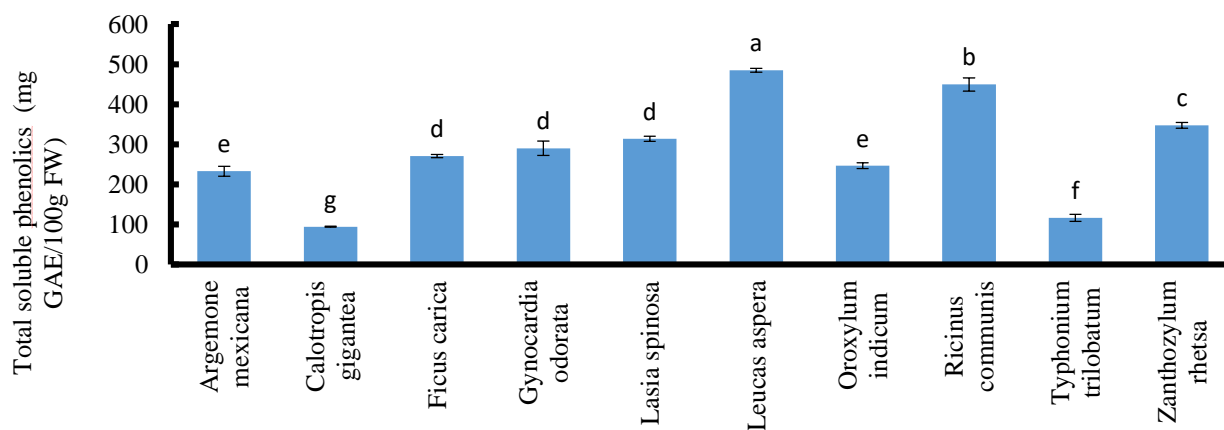


Figure 5. Total soluble phenolic content in the leaves of ten different medicinal plants. It was expressed as mg gallic acid equivalent (GAE)/100 g of leaf fresh weight (FW). Each data point is the average of three replicates  $\pm$  SEM. Bars sharing different letters are significantly different from each other at  $P \leq 0.05$ .

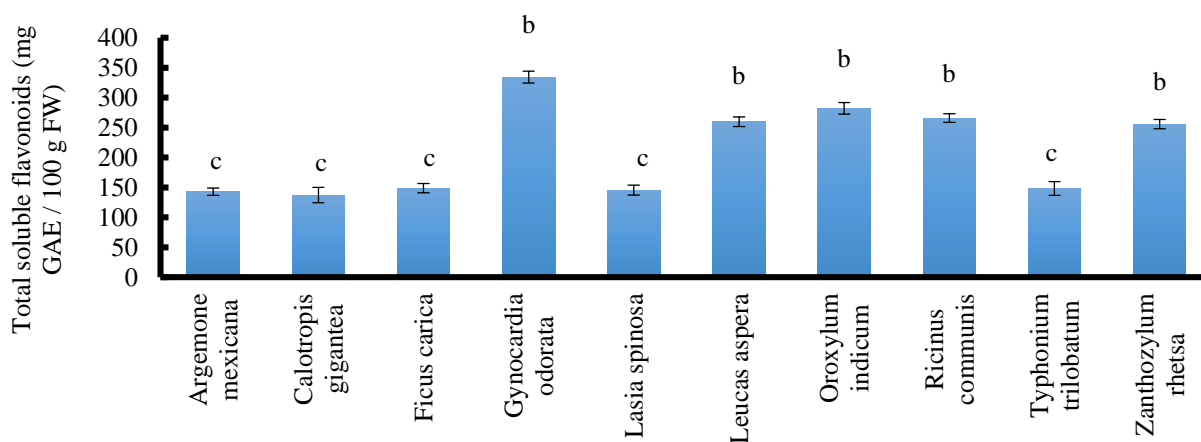


Figure 6. Total soluble flavonoid content in the leaves of ten different medicinal plants. It was expressed as mg Quercetin acid equivalent (QUE)/100 g of leaf fresh weight (FW). Each data point is the average of three replicates  $\pm$  SEM. Bars sharing different letters are significantly different from each other at  $P \leq 0.05$ .

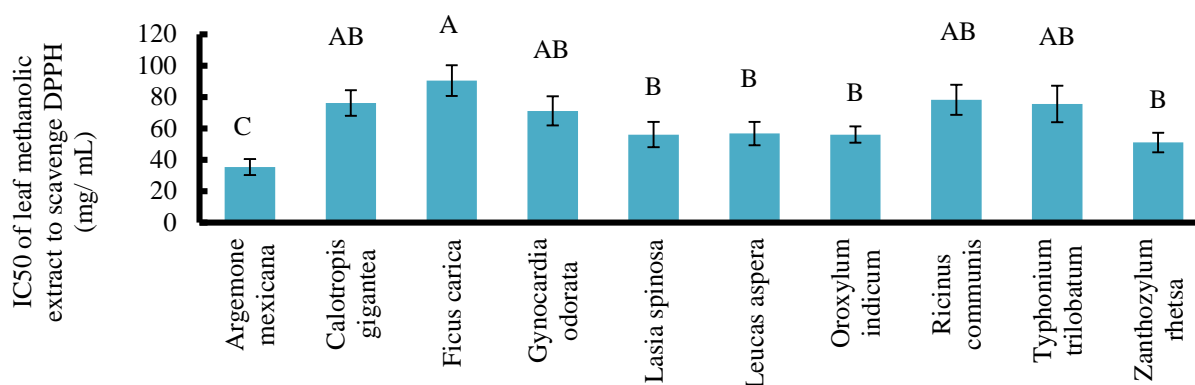


Figure 7. IC50 values for methanolic extracts of leaves from ten different medicinal plants to scavenge DPPH radical. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing different letters are significantly different from each other at  $P \leq 0.05$ .

#### Total Flavonoids Content (TFC)

The total flavonoid content in leaves of ten different medicinal plants varied considerably from 137.25 to 334.27 mg QUE/100 g fresh weight (Fig. 2). *G. odorata* showed the highest amount of total soluble flavonoid (334.27 mg QUE/100 g FW) followed by in *O. indicum* (282.16 mg QUE/100 g FW). The lowest amount of flavonoid was detected in *C. gigantea* (137.25 mg QUE/100 g FW) leaves. The total flavonoid content of the other plant extracts was *O. indicum* > *R. communis* > *L. aspera* > *Z. rhetsa* > *F. carica* > *T. trilobatum* > *A. mexicana* (Figure 7).

Free radicals which are mandatorily produced in normal biochemical reactions (Yen and Duh, 1994) are thought to play a critical role in many diseases such as cancer, atherosclerosis, aging, immunosuppression, inflammation, coronary heart diseases, diabetes, hair loss, and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Lobo et al., 2010). Dietary and other components of medicinal plants are rich sources of natural antioxidants (Lobo et al., 2010) which protects cells against degenerative effects of free radicals and thus provide healthy longer life (Ramesh and Ilyas, 2017; Polash et al 2019; Sultana et al., 2019).

The strong free radical scavenging activity exhibited by the ten plants could be attributed to high phenols, flavonoids as well as to bioactive pigments. Therefore, they can be considered as a potential source of natural antioxidants. The vital role of phenolic and flavonoids compounds as scavengers of free radical is accentuated in several reports (Madsen et al., 1996; Moller et al., 1999; Murshed et al., 2021). This thought to provide a very good antioxidant capacity due to their redox properties against free radicals, quenching singlet and triplet oxygen or decomposing peroxides as well as inhibitory effect on carcinogenesis (Dixon and Pasinetti, 2010; Sravanthi and Rao, 2015). Besides, the phenolic and flavonoids compounds possess several biological properties such as antitumor, antimutagenic and antibacterial properties, and these activities might be related to their antioxidant activity (Shui and Leong, 2002).

Photosynthetic pigments such as chlorophyll has anti-inflammatory, antioxidant, and wound-healing properties. Chlorophyll is a good source of antioxidant nutrients (Lanfer-Marquez et al., 2005; Polash et al., 2018; Polash et

al., 2020) Chlorophyll is an efficient deliverer of magnesium and helps the blood carry oxygen to the cells and tissue. Chlorophyll assists in the chelation of heavy metals (Hosikian et al., 2010) Chlorophyll has been studied for its potential in stimulating tissue growth and in stimulating red blood cells in connection with oxygen supply. Chlorophyll may reduce the binding of carcinogens to DNA in the liver and other organs (Ferruzzi and Blakeslee, 2007). It was proved that carotenoids have a positive role on the epithelization process and influence the cell cycle progression of the fibroblasts (Stivala et al., 1996). Carotenoids act as photoprotective agents and may reduce the risk of sunburns, photo-allergy and even some types of skin cancer (Adumanya, 2016).

#### Conclusion

The tested medicinal plants are the most viable and safer compared to synthetic products to protect against diseases because they have no health risks and side effects.

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