Original Research Article

Phytochemical Study and Antioxidant Properties of Aqueous Extracts of *Murraya paniculata* Leaf

ABSTRACT:

Aims: To investigate the chemical groups present, total phenolic compound, total flavonoid and to evaluate the possible antioxidant activity of aqueous extracts of *Murraya paniculata* leaf.

Place and Duration of Study: Department of Applied Chemistry and Chemical Engineering Department, Noakhali Science and Technology University, Bangladesh, in 2017

Methodology: The dry leaves were boiled and extracted with water. The extracts were concentrated and then investigated for their total phenolic compound, total flavonoid, evaluated the antioxidant activity. The preliminary screening of the various extracts was carried out using standard methods. Total phenolic content (TPC) was determined by the modified Folin-Ciocalteu method, total antioxidant capacity (TAC) and total flavonoid content were measured according to the Phosphomolybdate-method and Dowd-method respectively.

Results: The aqueous extract showed good antioxidant activity with good phenolic and flavonoid contents (400±0.44 mg ascorbic acid equivalent/g dried weight, 263±0.62 mg gallic acid equivalent/g dried weight, 63±0.19 mg quercetin equivalent/g dried weight respectively).

Conclusion: The research work revealed the presence of various bioactive phytochemical compounds which show the medicinal importance of leaves *Murraya paniculata* through a facile extracting method.

Keywords: *Murraya paniculata, Phytochemicals, Antioxidant activity, Phenolic content, Flavonoid content.*

1. INTRODUCTION

Numerous diseases including neurodegenerative, cancer, aging, cellular injury, cardiovascular and renal disorders are related to the antioxidant damage due to lone unpaired electron containing highly reactive oxygen species (ROS) [1-5] . In the medical science various antioxidant drugs are developed to treat the patients suffering from these diseases due to oxidative stress, however, their long term therapeutic can cause harmful side effects for human body. Phytochemicals can act as alternative to these synthetic products [6]. A medicinal plant consists of various bioactive substances which can be used as raw elements for chemotherapeutical semi synthesis and they have useful uses for therapeutic purposes [7-

9]. Many people in the Indian subcontinent use traditional medicine from medicinal plants. Various medicinal plants are grown in Bangladesh. As medicinal plants have no side effects on human health and they have medicinal values, these plants materials should be investigated and characterized to use them in human health treatment [10]

M. paniculata is a common plant in Indian subcontinent also known as orange jasmine or Honey bush or Kamini belonging to the family *Rutace* [11]. This tree is found throughout India, Bangladesh, tropical Sri Lanka to Myanmar, southern China and Taiwan, Thailand and throughout the Malesian region to Northeastern Australia and Caledonia. This tree is small in size with a spreading crown and short trunk, leaves alternate, impercipient, 10-17 cm long; leaflets usually 3-5, mostly 3-7 cm long, ovate or elliptic-lanceolate or rhomboid, glossy and darker above, gland-doted, base cuneate or rounded. The leaves are stimulant and astringent. They have successful uses to treat diarrhea and dysentery in many countries[11]. It is reported that these leaves are used to heal joint pain as paste with mustered oil. The leaves have anti-diabetic [12], anti-nociceptive and anti-inflammatory [13], anti-diarrheal, oxytocic, anti-fertility and in-vitro antioxidant properties [14].

It is found that many researches were conducted to explore antioxidant activity from petroleum ether, chloroform, methanol, ethanol, hexene extracts of various parts of *M. paniculata* plant [11, 15], however in this study the leaves of *M. paniculata* were only boiled with water to get extracts which is an easy, early, and cost-effective method to investigate phytochemicals present in leaves of *M. paniculata*.

The aim and objective of the current study were to investigate the chemical groups present, total phenolic compound, total flavonoid and to evaluate the possible antioxidant activity of *M. paniculata* leaves by using a very simple method of boiling by water to justify its use in traditional treatments.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The leaves of *M. paniculata* were collected from the Botanical Garden, Dhaka, Bangladesh and identified by a botanical expert. The leaves were separated from undesirable materials, washed with tap water.The leaves were boiled with water for 4-5 hours to extract the polar compounds. After that, the extracted sample was collected, filtered and then dried and stored for further investigation [16]..

The leaves were washed properly and were taken in about 800 mL water in a 1000 mL beaker. The leaves were boiled with water by heating with burner. After 4-5 hours the leaves were boiled and the extract was collected and filtered. The extract was dried and used for different investigation

2.2 Preliminary phytochemical screening

Preliminary phytochemical screening of the aqueous extract of *M. paniculata* was carried out according to the methods described in Dev et al. 2015 [17], to investigate the presence of alkaloid, reducing sugar, flavonoids, saponins, phenolic compounds, tannins, amino acids and proteins, glycosides.

2.3 Antioxidant Activity

In order to investigate the antioxidant properties of the examined extract, reducing power assay, total antioxidant capacity, total phenolic content, total flavonoids and reduction of ferric ions by ortho-phenanthroline color method were performed [18]

2.4 Reducing Power Assay

Reducing Power Assay of the extract was measured based on the conversion from Fe (III) to Fe (II) which was indicated by the formation of Perl's Prussian blue and it was observed at 700 nm [19]. 2 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of potassium ferricyanide (1%) were added to 2 mL of various concentration of sample. After incubation at 50 °C for 20 min, the addition of 2 mL of trichloroacetic acid (10%) was followed. Then the mixture was centrifuged at 3000 rpm for 10 min and upper portion of the solution was collected. 2 mL of distilled water and 0.4 mL of 0.1% (w/v) fresh ferric chloride was added with 2 mL of sample mixture. After 10 min reaction, the absorbance was measured at 700 nm. In this method, higher the absorbance shows higher the reducing power and BHT was used as reference standard. Increased absorbance of the reaction mixture showed enhanced reducing power.

2.5 Total Antioxidant Capacity

The total antioxidant capacity of the extracts was determined by phosphomolybdate method where ascorbic acid was used as a standard [20]. 1 mL of reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mm ammonium molydate) was added with 0.1 mL of sample solution. The mixture in capped tubes was incubated in a water bath at 35 °C for 90 min. Then the mixture was cooled at room temperature and finally the absorbance of the mixture was measured at 765 nm against a blank. Ascorbic acid was used as the standard and Total Antioxidant Capacity was measured as equivalents of Ascorbic acid. The total antioxidant capacity is concentration-dependent.

2.6 Reduction of Ferric Ions by *ortho*-phenanthroline Color Method

2 mL of extracts of various concentration as mixed with a reaction mixture containing 1 mL *ortho*-phenanthroline (5 mg in 10 mL methanol), 2 mL ferric chloride 0.2 mm (3.24 mg in 100 mL distilled water). Then the mixture was kept in room temperature for 10 minutes. Finally, the absorbance was measured at 510 nm. Gallic acid was used as reference standards [18, 21].

2.7 Total Phenolic Content

The total phenolic content of the extracts was measured by following Folin-Ciocalteu method [18]. A reaction mixture was prepared by adding 0.5 mL of each extract (1 mg/mL), 5 mL Folin-Ciocalteu reagent (1:10 v/v in distilled water) and 4 mL of 7.5% sodium carbonate with each other. The mixture was vortexed for 15 s and kept for 30 min at 40 °C for color development. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. The standard curve was prepared using a various concentration of solutions of gallic acid in 50% methanol. The result was expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

2.8 Flavonoid Contents

The flavonoid contents of extracts were measured according to Dowd-method [22]. 0.5 mL of extract solution in methanol was added with 0.1 mL of 10% (w/v) $AlCl_3$ solution, 0.1 mL (1 M) potassium acetate and 2.8 mL distilled water. The mixture was allowed to stand for 30 min at room temperature and measured at absorbance at 415 nm against the blank. Quercetin was used as standard. The result was expressed as quercetin equivalents in milligrams per gram (QE/g) of dry extract.

2.9 Statistical Analysis

All data are presented as mean ± standard deviation (SD) for at least three replications for each experiment. All statistical analysis and graphs were drawn using MS-Excel.

3. RESULTS

Phytochemical screening of the extract stated the presence of alkaloids, flavonoids, saponins, phenolic compounds and tannins, glycosides however reducing sugar and amino acid and protein were absent.

3.1 Reducing Power Assay

The reducing capacity of a compound may act as an important property which reflects its potential antioxidant activity. Figure.1 shows the dose response curves for the reducing powers of all concentrations of extracts. The aqueous extract of leaves of *M. paniculata* showed significant reducing power (0.836±2) comparing to standard BHT (1.099±1.4) at 500 µg /mL.

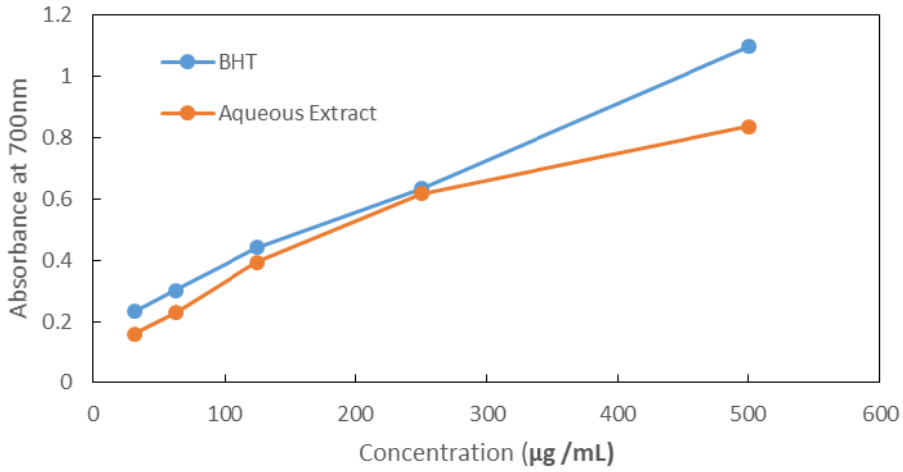

Figure. 1. Comparative Analysis of aqueous extract of *M. paniculata* leaves with standard BHT for reducing power assay

3.2 Total antioxidant capacity

Total antioxidant capacity of the aqueous extract of *M. paniculata* leaves was determined by phosphomolybdate method [17-18]. Figure.2 shows the total antioxidant capacity for all concentrations of aqueous extract of *M. paniculata* leaves. The total antioxidant capacity of aqueous extract of leaves of *M. paniculata* was found (400±0.44 AAE/g) which was closed to standard ascorbic acid that proved the strong antioxidants in this extract.

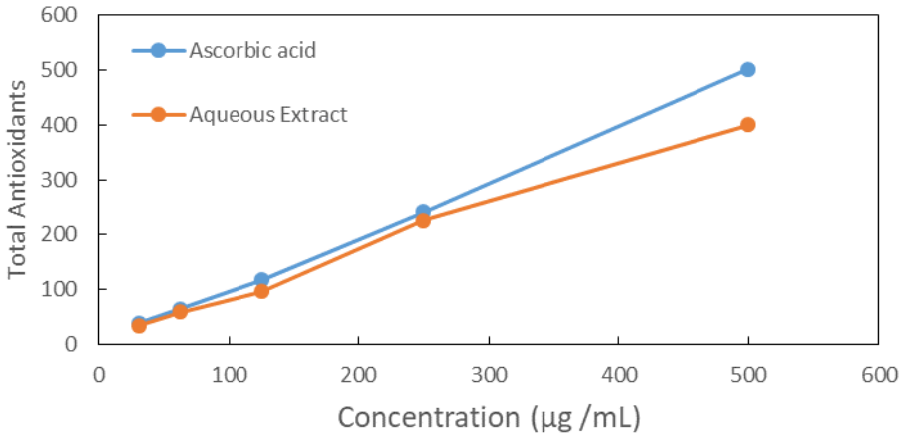

Figure. 2. Comparative analysis of aqueous extract of *M. paniculata* leaves with standard ascorbic acid for total antioxidant capacity

3.3 Ferric Reducing Antioxidant Potential

The ferric ion reduction is broadly utilized to assess antioxidant activity. In this strategy, Fe^{2+} responds quickly with *o*-phenanthroline and form a red colour complex which is outstandingly stable. This complex is visible in absorption at wavelength of 510 nm. The aqueous extract of *M. paniculata* leaves showed significantly antioxidant activity and the values (1.337±0.42) which is higher than standard antioxidants gallic acid (1.140±0.23) at 500 μg/mL (Figure. 3).

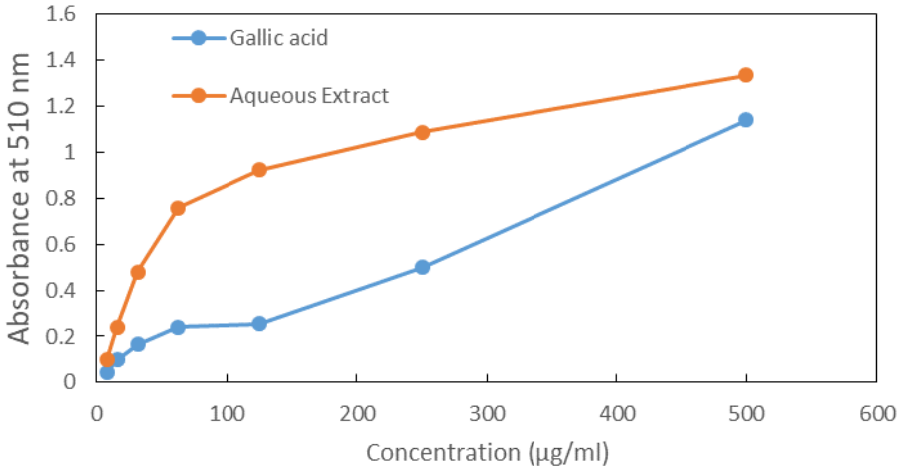

Figure. 3. Comparative Analysis of aqueous extract of *M. paniculata* leaves with standard Gallic acid for Reduction of Ferric Ions by Ortho-phenanthroline Color method

3.4 Total phenolic and flavonoids contents

Figure. 4 shows the total phenolic content and total flavonoids of aqueous extract of *M. paniculata* leaves at 500 μg/mL. The phenolic content was measured spectrophotometrically at 765 nm. Flavonoid contents in selected plant extracts were determined using aluminum chloride calorimetric method. The results were derived from the calibration curve ($y = 0.0124 + 0.1013$, $R^2 = 0.9963$) of quercetin (QE) (0-500μg/mL) The total phenolic content of the aqueous extract of *M. paniculata* leaves were measured in terms of gallic acid equivalent (GAE) (mg/g). The total phenolic content of the aqueous extract was 263±0.62 (mg GAE/g). The total flavinoid content of the aqueous extract was 63±0.19 (mg QE /g).

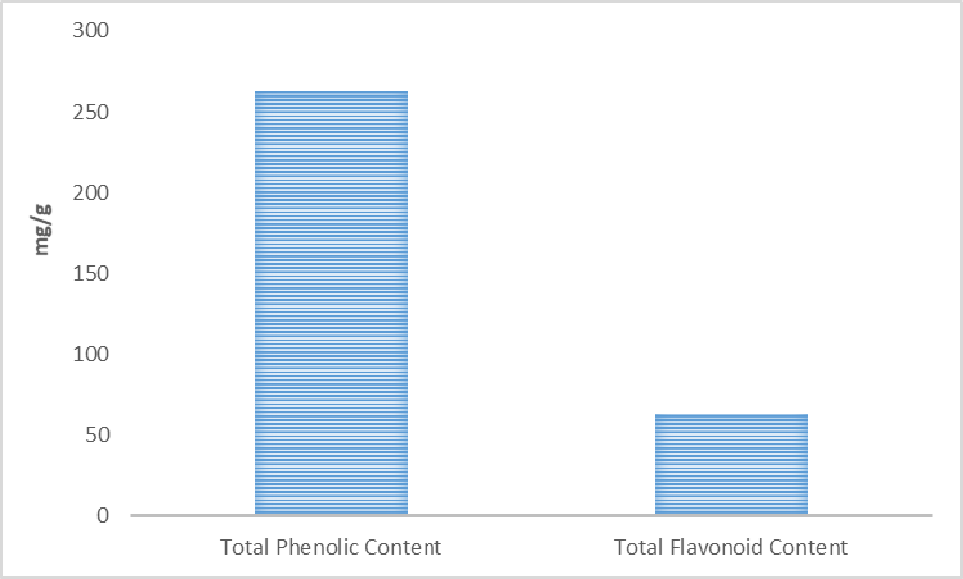

Figure. 4. Total phenolic compound and flavonoid aqueous extract of *M. paniculata.*

4. DISCUSSION:

Phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, saponins, phenolic compounds and tannins. Secondary metabolites are very important for the plant [23, 18]. The presence of alkaloids proves the antimalarial, antiseptic, antibacterial properties of the plant materials [24] whereas the presence of flavinoids have the potential of anti-inflammatory, anti-microbial, anti-cancerous, anti-allergic activity [25]. Phenolic constituents and saponins, present in the plant materials, exert immunomodulatory activity and antibacterial and antifungal properties [26]. The presence of tannins in the extracts may be utilized for accelerating wound and inflamed mucous membrane healing [27].Finally, the presence of these phytochemicals shows the medicinal efficacy of the leaves extracts of *M. paniculata*.

In the Reducing Power Assay, total antioxidant action is an indicator of the entire capacity to withstand the negative impact of stretch actuated by free radical formation [28]. The reduction of Fe (III) is often used as an indicator of electron donor activity. the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form caused due to the presence of the antioxidants [23,29]. Fe^{2+} can be examined by absorbance measurement at 700 nm. In case of known amount of sample, the higher absorbance indicates the better reducing power. The antioxidants present in the extracts of *M. paniculata* caused their reduction of Fe^{3+} / ferricyanide complex to the ferrous form, and thus proved the reducing power [30].

Total antioxidant capacity of the aqueous extract of *M. paniculata* leaves was determined by phosphomolybdate method. In this method, formation of a green phosphomolybdate (V) complex is the

reduction of molybdenum (VI) to molybdenum (V) in the presence of antioxidant, which can be measured spectrophotometrically at 765 nm. Total antioxidant activity focus on the thermodynamic conversion and measures the number of electrons or radicals donated or quenched by a given antioxidant molecule and measure the capacity of biological samples under defined conditions. High antioxidant capacity of the extract might be caused for the polarity of water as solvent [18, 31]. The polar solvents have a much stronger ability to dissolve and hence extract polar phytochemicals [18].

Ortho-substituted phenolic compounds interact with iron and may assert pro-oxidant effects. *o*-phenanthroline quantitatively forms complexes with ferric ion and the complexes get smashed in the presence of chelating agents [13]. The extracts interfered with the formation of ferrous-*o*-phenanthroline complex, which may give the information about the metal chelating activity of extracts [17, 32-33]. Here ferric ion is reduced to ferrus ion. The Ferric Reducing Antioxidant Potential is linearly concentration-dependent [22]. Phenolic compounds are plant constituents having antioxidant activity due to redox properties [33]. Under the basic reaction conditions, a phenol loses an H^+ ion to produce a phenolate ion, which reduces Folic-Ciocalteu reagent [34, 35]. The high level of phenolic compounds is the main reason for the enhanced antioxidant activity of polar extract. The elevated level of phenolic compound is the most reason for the enhanced antioxidant activity of polar extracts [36]. The main role of phenolic compounds as free radical scavengers has been detailed in many papers [37-38] The phenolic contents act as hydrogen donars thus as an efficient antioxidant activity [34]. Flavonoids are secondary metabolites with antioxidant activity, the capacity of which depends on the number and position of free OH groups [22, 39].

5. CONCLUSION:

The research work revealed the presence of various bioactive phytochemical compounds which showed the medicinal importance of leaves of *M. paniculata* through a very simple and cost-effective method. Like other solvent extraction, water boiling method also provides antioxidant rich extracts though there are no uses of chemical solvent or Soxhlet. Moreover, this method can be practicable in home and be an easy method to use the leaves of *M. paniculata* as herbal medicine in healing of various diseases. The aqueous extract of leaves of *M. paniculata* is a good source of antioxidant and phenolic compounds which are very adjuvant for medicinal purpose. Further investigation to study the mechanism of action of antioxidants is necessary to better understand their ability to control diseases that have a significant impact on human health.

REFERENCES:

1. Losada-Barreiro S, Bravo-Díaz C. Free radicals and polyphenols: The redox chemistry of neurodegenerative diseases. Eur. J. Med. Chem. 2017; 133: 379–402.

2. Ngoua-Meye-Misso R, Ndong JD, Sima-Obiang C. Phytochemical studies, antiangiogenic, anti-inflammatory and antioxidant activities of Scyphocephalium ochocoa Warb. (Myristicaceae), medicinal plant from Gabon. Clin Phytosci . 2018; 4:15-28.

3. Agrawal S, Kulkarni GT, Sharma VN. A comparative study on the antioxidant activity of methanolic extracts of Terminalia paniculata and Madhuca longifolia. Free Rad. Antiox. 2011; 1: 62–68.

4. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr. J. 2016; 15:71-81.

5. Spengler G, Gajdács M, Marc MA, Domínguez-Álvarez E, Sanmartín C. Organoselenium Compounds as Novel Adjuvants of Chemotherapy Drugs—A Promising Approach to Fight Cancer Drug Resistance. *Molecules* 2019; *24*:336-351.

6. Hasler CM, Blumberg JB. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition. 1999; 129: 756-57.

7. Himal PC, Nisha S, Jyoti S, Anupa KC, Mansoor S. Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. Kathmandu Univ. J Sci Eng & Technol, 2008; 1: 49-54.

8. Noumedem JA, Tamokou JdD, Teke GN. Phytochemical analysis, antimicrobial and radical-scavenging properties of Acalypha manniana leaves. Springer Plus. 2013; 2: 503-06.

9. Ricardo J. Ferreira RJ, Kincses A, Gajdacs M, Spengler G, Santos DJVAd, Molnar J, Ferrira MJ. Terpenoids from Euphorbia pedroi as Multidrug-Resistance Reversers. J. Nat. Prod. 2018; 81: 2032-40.

10. Gajdacs M, Urban E.The relevance of anaerobic bacteria in brain abscesses: a ten-year retrospective analysis (2008–2017). Infectious Diseases 2019; 51: 779-81.

11. Sharma S, Sharma S. Pharmaceutical activities of Phytochemicals in Murraya spp. - a review. Journal of Pharmacy Research. 2015; 9:217-36.

12. Wu L, Li P, Wang X, Zhuang Z, Farzaneh F, Xu R. Evaluation of anti-inflammatory and antinociceptive activities of Murraya exotica. Pharmaceutical Biology. 2010; 48: 1344-53.

13. Rahman MA, Hasanuzzaman M,Nazimuddin Shahid IZ. Anti-diarrhoeal and anti-inflammatory activities of Murraya paniculata (L.) jack. Pharmacologyonline. 2010; 3:768-76.

14. Kong YC, Ng KH., But PP, Li Q, Yu SX, Zhang HT, Cheng KF, Soejarto DD, Kan WS, Waterman PG. Sources of the antiimplantation alkaloid yuehchukene in the genus Murraya. J Ethnopharmacol. 1986; 15: 195-200.

15. Gautam MK, Goel RK. Exploration of preliminary phytochemical studies of leaves of Murraya paniculata (L.). Int. J. of Pharm. & Life Sci. 2012; 3: 1871-74.

16. Gomathi1 SAC, Rajarathinam SRX, Sadiq AM. Phytochemical screening of aqueous extract of Tamarind (Tamarindus indica L.). International journal of basic and applied research.2017;7: 65-70.

17. Dev UK, Hossain MT, Islam MZ. Phytochemical investigation, antioxidant activity and anthelmintic activity of Mikania micrantha leaves. World J Pharm Res. 2015;4 :121–33.

18. Islam MZ, Hossain MT, Hossain F, Mukharjee SK, Sultana N, Paul SC. Evaluation of antioxidant and antibacterial activities of Crotalaria pallida stem extract. Clinical Phytoscience. 2018; 4:8 -15.

19. Fejes S, Blazovics A, Lugasi A, Lemberkovics E, Petri G, Kery A. In vitro antioxidant activity of Anthriscus cerefolium L.(Hoffm.) extracts. J Ethnopharmacol, 2000; 69: 259–

65.

20. Abdel -Aleem ER, Attia EZ, Farag FF, Samy MN, Desoukey SY. Total phenolic and flavonoid contents and antioxidant, anti-inflammatory, analgesic, antipyretic and antidiabetic activities of Cordia myxa L. leaves. Clinical Phytoscience. 2019; 5:29-38.

21. Akter S, Islam T, Hossain MT. Proximate Analysis, Phytochemical Screening and Antioxidant Acitvity of Tagetes Erecta Leaves. World J Pharm Res. 2015; 4: 1856-66.

22. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants. 2019;8: 96-108.

23. Mondal M, Hossain MS, Das N. Phytochemical screening and evaluation of pharmacological activity of leaf Methanolic extract of Colocasia affinis Schott. Clin Phytosci. 2019;5:8-19.

24. Islam MZ, Hossain MT, Hossen F, Akter MS, Mokammel MA. In- vitro Antioxidant and Antimicrobial Activity of Bougainvillea glabra Flower. Res. J. Med. Plant, 2016;10: 228-36.

25. Maduka HCC, Ugwu CE, Okpogba AN, Ogueche PN, Dike CC, Okonkwo CO, Nwanyanwu CA. Phytochemical Analysis and Antioxidant Properties of the Ethanolic Extract from Tetracarpidium conophorum (African Walnut) and Pterocarpus soyauxii (oha) Leaf. Journal of Applied Life Sciences International, 2018;18(2): 1-7.

26. Maurya RG, Yadav PP. Antiosteoporotic agents from natural sources. Nat. Prod. Chem. 2008; 35: 517-45.

27. Njoku PC, Akumefula MI, Phytochemical and nutrient evaluation of Spondias mombin leaves. Pak. J. Nutr., 2007;6: 613-15.

28. Senhaji S, Lamchouri F, Toufik H.Phytochemical Content, Antibacterial and Antioxidant Potential of Endemic Plant Anabasis aretioïdes Coss. & Moq. (Chenopodiaceae) BioMed REsearch International.2020;2020:1-16.

29. Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem. 1995, 43, 1813–19.

30. Amel B, Seddik K, Shtaywy A, Saliha D, Mussa AZ, Assia B, Saliha D, Abderahmane B, Smain A. Phytochemical analysis, antioxidant activity and hypotensive effect of algerian azarole Crataegus azarolus L.) leaves extracts. Res J Pharm Biol Chem Sci. 2014;5:286–305.

31. Hossain MT. Antioxidant, cytotoxicity, membrane stabilization and anthelmintic activity of ethanolic extract of Sarcochlamys pulcherrima leaves. Int J Green Herb Chem. 2015;4:274–83.

32. Ahmed D, Khan MM, Saeed R. Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from Adiantum caudatum Leaves. Ntioxidants. 2015; 4:394-409.

33. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun, T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. Res.-Fund. Mol. Mutagen. 2005; 579: 200–13.

34. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J. Agri. Food Chem. 2005; 53:1841–56. 34.

35. Fernandes AJ, Ferreira MR, Randau KP, de Souza TP, Soares LA.Total flavonoids content in the raw material and aqueous extractives from Bauhinia monandra Kurz (Caesalpiniaceae). Sci. World J. 2012;2012:1-7

36.Savadi S,Vazifedoost M , Didar Z, Nematshahi MM,Jahed E. Phytochemical Analysis and Antimicrobial/Antioxidant Activity of Cynodon dactylon (L.) Pers. Rhizome Methanolic Extract. Journal of Food Quality 2020;2020:1-10

37. Katalinic V, Milos M, Kulisic M, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry.2006;94: 550–57.

38. Thériault M, Caillet S, Kermasha S, Lacroix M, Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products.Food Chemistry, 2006;98:490–501.

39. Panche, AN. Diwan AD, Chandra SR. Flavonoids: An overview. J. Nutr. Sci. 2016; 29:5-47.