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EVALUATION OF *IN VITRO* FREE RADICAL SCAVENGING AND ANTIOXIDANT ACTIVITIES OF *Mimusops elengi* (Bokul) LEAF EXTRACT

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Abstract

In prevention and therapeutics of diseases in which free radicals are implicated antioxidants with free radical scavenging activities may have great potential and hence research on natural antioxidant has received much attention in modern medical science to overcome various harmful effects of synthetic antioxidants on human body. In the present study the methanolic extract of *Mimusops elengi* and its different fractions were evaluated for the total phenolic, total flavonoid content, reducing power capacity, DPPH radical scavenging activity and total antioxidant activity. Dried ground powdered sample of *M. elengi* leaves (25 g) were exhaustively extracted with methanol (MeOH, analytical grade). Four different fractions such as petroleum ether, chloroform, ethyl acetate and dia-ion resin absorbed fractions were evaluated for their phytotherapeutic activities by different described assay method using UV- spectrophotometer. The highest values of total phenolic and flavonoid content were found to be 36.55±0.01 mg GAE/g of dry extract and 18.97±0.11 mg Cat.E /g of dried extract respectively for ethyl acetate fraction compared to the reference antioxidant in a dose dependent manner. On the other hand, the values of iron reducing power (Absorbance 1.107 ± 0.002 at 100 µg/ ml), DPPH radicals scavenging (IC_{50} 31.80µg/ ml) and total antioxidant activity (Absorbance 2.588±0.001 at 100µg/ ml) were found highest in Dia-ion resin absorbed fractions. These results indicate the activities of *Mimusops elengi* leaves extracts on free radical and oxidative damage which may act as natural antioxidant.

Keywords: *Mimusops elengi*; Free Radical; Antioxidant;Total Flavonoid; Total Phenolic Content; Reducing Power Capacity.

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INTRODUCTION

Research on natural antioxidant has received much attention in modern medical science because of various harmful effects of synthetic antioxidants on human body. The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, lever injury and cardiovascular diseases [1]. On the other hand, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body and cause irreversible oxidative damage [2]. Therefore antioxidants with free radical scavenging activities may have great relevance in prevention and therapeutics of diseases in which free radicals are implicated [3].Nowadays, people are very much concerned about the toxic effect of synthetic antioxidant. So the interest in natural antioxidant has greatly increased. This interest is based upon the belief that herbal medicines are safe, inexpensive and have less adverse effect. The world health organization (WHO) estimates that two third of the world population still depends upon traditional medicine for the treatment of various types of diseases [4]. Natural antioxidants are primarily plant phenolic compounds which are existing in *M. elengi* leaf. *Mimusops elengi* Linn, popularly known as Bakul is an important traditional medicinal plant. It is mentioned in all the ancient scriptures of Ayurveda as it is a common tree in many parts of Bangladesh. People also cultivated it as an ornamental plant in their garden for thick shade and fragrant flowers. It has been used in the indigenous system of medicine for the treatment of various ailments for centuries. The bark and fruit of this plant are used in the treatment of diarrhea and dysentery, and a decoction of the bark is used as a gargle [5,6]. Several therapeutic uses such as cardio tonic, alexipharmic, stomachic, anthelmintic and astringent have been ascribed to the bark of *Mimusops elengi* [5,7]. Phytochemical review shows the presence of taraxerol, taraxerone, ursolic acid, [8,9], alkaloid isoretronecyl tigalate [10] and mixture of triterpinoid saponins in the bark of *mimusops elengi*. In Unani medicine it is known as “Mulsari” and most of the parts of the plant are used in various ways to cure a variety of human diseases like toothache, leucorrhoea, ulcers of urethra, fever headache, loosing of teeth, weak and spongy gums, constipation, stomatities [4]. Crude methanolic extracts of *mimusops elengi* leaf exhibited statistically significant antioxidant activity [11]. The presence of high phenolic content in the *M. elengi* extract has been observed by FC reagent test and also from phytochemical test, it was also found as a good source of flavonoids and triterpenes [12]. The methanol extracts of the leaves of *M.*

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elengi showed significant activities in all antioxidant assays compared to the reference antioxidant ascorbic acid in a dose dependent manner [13].

M. elengi leaves extract is having good antioxidant property, as evidenced by increased antioxidant states and decreased lipid peroxidation, which reflect the protective effect of *M. elengi* leaves extract from the risk Diabetic complications [14]. So, an effort has been made to investigate the antioxidant activity of different fractions of methanolic extract of *M. elengi* leaves grown in Bangladesh.

MATERIALS AND METHODS

The main chemicals used in the study were – Folin- Ciocalteu reagent (FCR), gallic acid (reagent grade), 7.5 % sodium carbonate, aluminium chloride($AlCl_3$), potassium acetate, methanol (analytical grade), catechin, potassium ferricianide [$K_3Fe(CN)_6$], trichloro acetic acid, ferric chloride, ascorbic acid (reagent grade) ,concentrated sulfuric acid (98%), sodium phosphate (Na_3PO_4), ammonium molybdate, DPPH-(1,1-diphenyl-2-picrylhydrazyl), butylated hydroxyl toluene (BHT-reagent grade). In all cases, the absorbance of the solution was measured by UV-spectrophotometer (Shimadzu).

PLANT MATERIALS

Fresh leaves of *Mimusops elengi* Linn (Bakul) was collected from the campus of BCSIR Laboratories, Rajshahi. The leaves were washed thoroughly, dried under shade for 14 days at 35-40^{o}C, cut into small pieces and then slashed to coarse powder with the help of mechanical grinder.

EXTRACTION

Dried ground powdered sample of *M. elengi* leaves (25 g) were exhaustively extracted with methanol (MeOH, analytical grade) by a soxhlet apparatus at 45 ^{o}C. The resulting juicy extract was filtered and concentrated to obtain a crude residue (23.5%) by using Buchi Rotavapor R-200. The process was repeated for three times to increase the crude extract.

Then sufficient water was added to the crude residue and water triturate part was collected from crude extract. The water triturate fraction was passed through a previously well packed dia-ion resin column which had selectively to collect only the phenolic group containing compound. Then the materials which were found in resin column, collected by passing methanol solvent. Then petroleum ether, ethyl acetate and chloroform solvents were passed through the residue respectively. Finally, petroleum ether, ethyl acetate and chloroform triturate were collected.

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DETERMINATION OF TOTAL PHENOLIC CONTENT

The content of total phenolic compounds of different fractions of *Mimusops elengi* leaves were determined employing the method as described by Singleton and Rossi [15] using Folin-Ciocalteu reagent(FCR) as oxidizing agent and gallic acid as standard. The absorbance of the solution was measured at 760 nm using a UV spectrophotometer against blank.

DETERMINATION OF TOTAL FLAVANOIDS

Total flavonoid content of different extractives of *M. elengi* leaves was determined by aluminium chloride colorimetric method using catechin as reference compound [16]. A volume of 125µL of extract is added to 75 µL of a 5% NaNO2 solution. The mixture was allowed to stand for 6 min, then 150 µL of aluminium trichloride (10%) was added and incubated for 5 min, followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was adjusted to 2500 µL with distilled water. After 15 min of incubation the mixture turned to pink and the absorbance was measured at 510 nm. The total flavonoids content was expressed as g E catechin/g DM.

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

The 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants [17]. With this method antiradical power of antioxidant activity of different extracts of *Mimusops elengi* were determined by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule.

DETERMINATION OF REDUCING POWER CAPACITY

The reducing power of methanol extract and different fractions of *M. elengi* leaves were evaluated by the method of Oyaizu (1986) [18] using potassium ferricyanide [$K_3Fe(CN)_6$] (1%) solution. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The iron reducing power of different extracts of *M. elengi* were estimated by measuring the formulation of Perl's Prussium blue at 700 nm.

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY

Total antioxidant activity of different extracts of *M. elengi* leaves were determined by the method of Prieto et a*l*. [19] with some modifications. The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol and carotinoids.

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This method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound. 3 ml of reaction mixture containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 1 % ammonium molybdate was added into the experimental samples and incubated at 95^{o}C for 90 minutes to complete the reaction and measured the absorbance of the solution at 695 nm.

RESULTS AND DISCUSSION

Total phenolic, total flavonoid content, reducing power capacity, DPPH free radical scavenging activity and total antioxidant activity of methanolic extract and its different fractions of *M. elengi* were determined according to the methods described in material and method section.

Four different fractions such as petroleum ether (PEF), chloroform (CLF), ethyl acetate (EAF) and dia-ion resin (DRF) fraction of crude methanol extract of *M. elengi* were taken to determine the total phenolic content. Among the crude extracts and all fractions the highest phenolic content was found in ethyl acetate fraction 36.55 mg gallic acid equivalent/g of dried sample (Table 1, Figure 1). Gallic acid was used as standard in this assay. Other fractions also showed the activity but those are somewhat less than that of ethyl acetate fraction. From the results of table I the order of the activity of total phenolic content was; EAF> DRF >CLF >PEF.

Total flavonoid content of the crude extract and different fractions of *M. elengi* were determined by aluminium chloride colorometric method. Catechin was used as standard in this assay and the results are also presented in Table 1 and Figure 2. The highest value was found in EAF (18.97±0.11) mg catechin equivalent/ g of dried sample. This result is in good agreement with the reported information [20, 21] where, they reported that phenolic compounds and flavonoids are associated with anti oxidative action in biological system.

The reducing power of *M. elengi* was determined according to the method previously described [18] and the results were shown in Figure 3. . Among the four different fractions and crude extract, the dia-ion resin fraction (DRF) showed the highest absorbance value 1.107±0.002 at 100µg/ml concentration, followed by chloroform fraction (CLF) with absorbance value 0.182±0.014 while petroleum ether fraction (PEF) showed absorbance value 0.065±0.007 and ethyl acetate fraction (EAF) showed absorbance value 0.696±0.003 (Figure 3). Tanaka et al. [22] observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. Saha et al. [23] reported that the Fe^{3+} to Fe^{2+} transformation occurred in the presence of extract samples.

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The results of DPPH radical scavenging activity of four different fractionsand thecrude extracts are shown in Figure 4. Butylated hydroxy toluene (BHT) was used as standard in this assay. Among the extracts, the lowest value of IC_{50} was found in dia-ion resin absorbed (DRAF) fraction (31.80 µg/ml). On the other hand chloroform, petroleum ether and ethyl acetate fractions showed the value 32.21, 45.00 and 38.60 µg/ml respectively while IC_{50} value of BHT was found 31.23µg/ml. The activity of DRAF (31.80 µg/ml) was the best (Figure 4) for DPPH radical scavenging.

Total antioxidant activity of different fractions of methanolic extract of *M. elengi* was determined by phosphomolybdanum method in which the antioxidant compound and the formation of green phosphate Mo(VI) to Mo(V) was occurred by the antioxidant compound and the formation of green phosphate Mo(V) complex with a maximum absorption at 695 nm. Among the fractions, DRAF showed the highest total antioxidant activity with absorbance 2.588±0.001 at 320 µg/ml whereas the chloroform, petroleum ether and ethyl acetate showed the absorbance 2.142, 1.595, 2.533 at 320µg/ml of extract respectively (Figure 5).

Methanol fraction showed the absorbance value 2.168±0.001 at the same concentration. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [24]. The antioxidant effect is mainly due to phenolic compounds, such as phenolic acids and phenolic diterpenes [25].

Table 1: Total phenolic and total flavonoid content in methanolic extracts of *Mimusops elengi* and its different fractions

Name of Extracts	Concentration (µg/mL)	Total Phenolic content (mg GAE/g) of dried extract (Mean ± STD)	Total Flavonoid content (mg Cat.E/g) of dried extract (Mean ± STD)
Crude Methanol extract	100	22.17 ± 0.001	4.48 ± 0.05
Petroleum ether fraction	100	6.49 ± 0.000	1.86 ± 0.13
Chloroform fraction	100	11.34 ± 0.001	2.71 ± 0.10
Ethyl acetate fraction	100	36.55 ± 0.001	18.97 ± 0.11
Dia-ion resin adsorbed fraction	100	34.78 ± 0.001	13.55 ± 0.13

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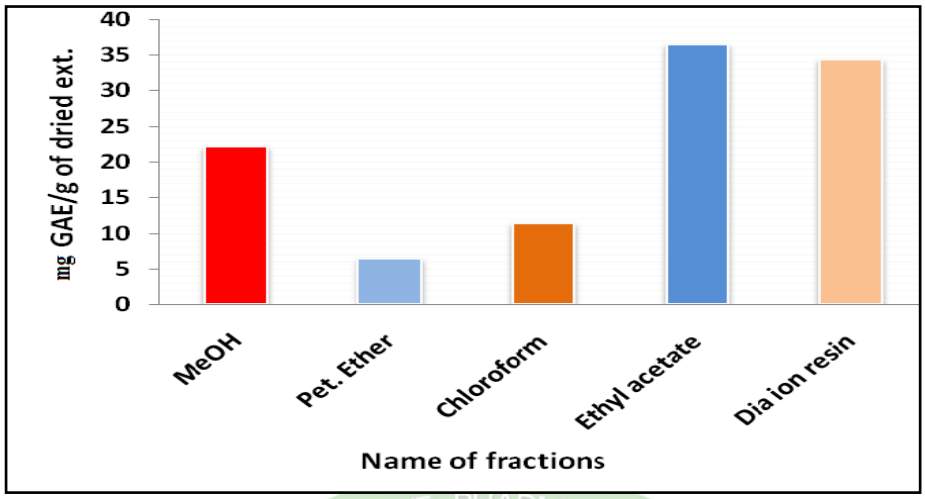

Fig.1: Total phenolic content in methanolic extracts of *M. elengi* and its different fractions.

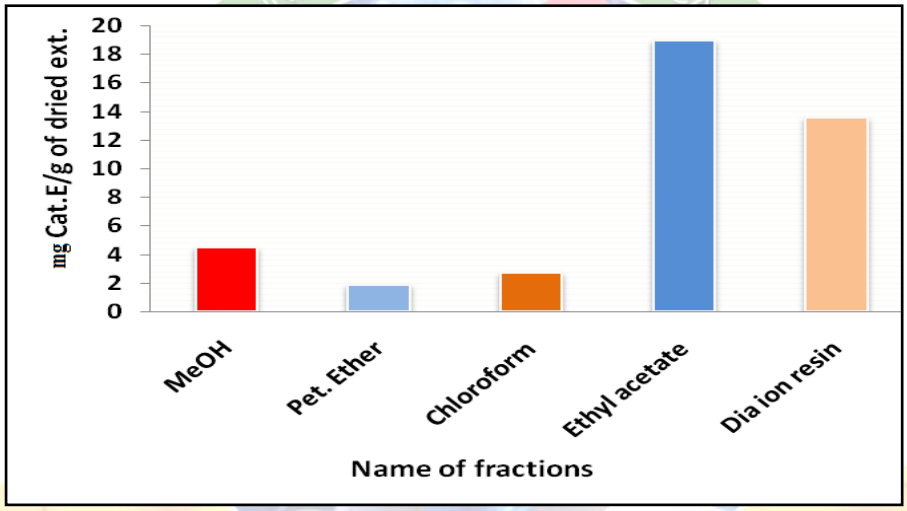

Fig. 2: Total flavanoid content in methanolic extracts of *M. elengi* and its different fractions.

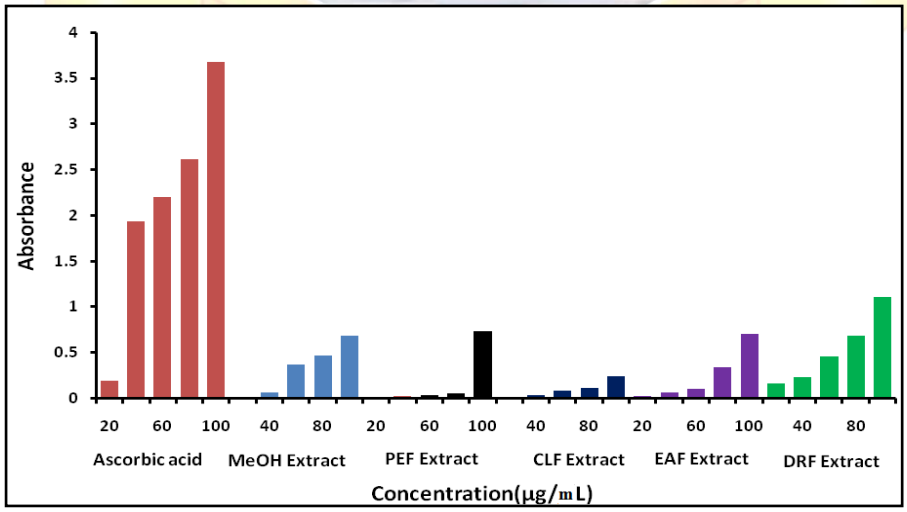

Fig. 3: Comparative iron reducing power assay of methanolic extracts of *M. elengi* and its different fractions with standard (Ascorbic acid).

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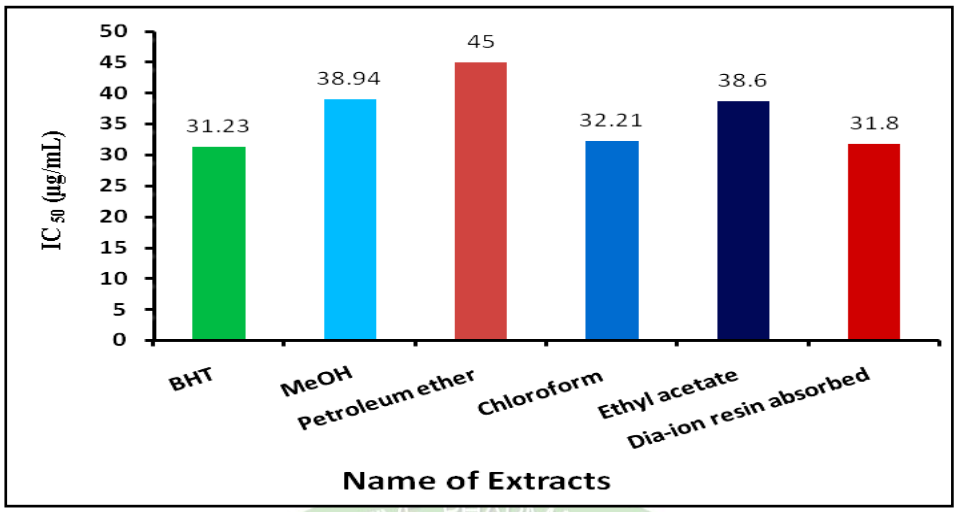

Fig. 4: Comparative IC_{50} values of DPPH assay on methanolic extracts of *M. elengi* and its different fractions with standard (BHT).

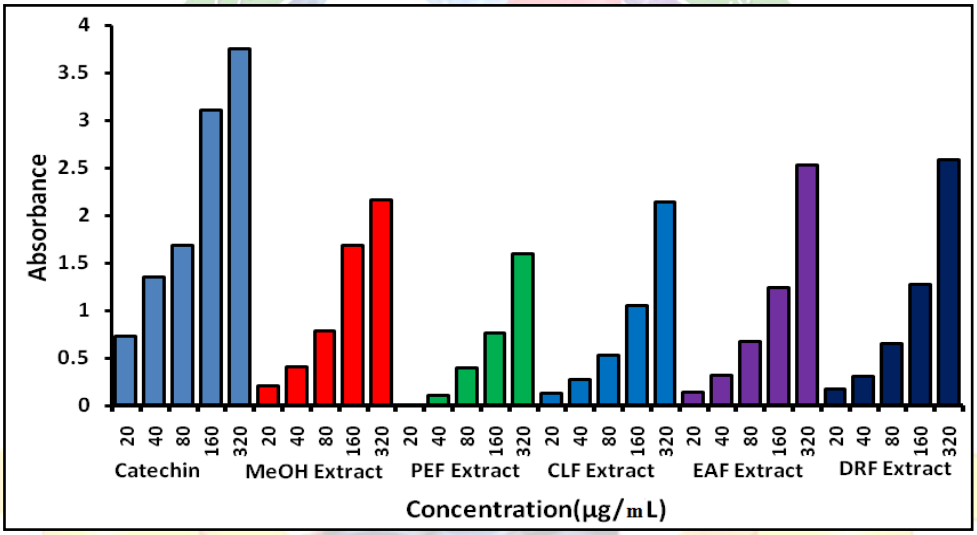

Fig. 5: Comparative antioxidant activity of methanolic extracts of *M. elengi* and its different fractions with standard (Catechin).

CONCLUSION

The study revealed that among the four different fractions of methanollic extract of *M. elengi*, ethyl acetate fraction possesses the highest values of total phenolic and flavonoid content. On the other hand the values of reducing power capacity, DPPH free radical scavenging and total antioxidant activity of the extracts were found highest for Dia ion resin fraction. Based upon the results of this study it may be concluded that *M. elengi* leaves grown in Bangladesh having polyphenols, flavonoids and other antioxidant properties might be helpful in preventing various oxidative stress related diseases and it may also be used as a potential source of pharmacological application.

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REFERENCES

1. Liao KL and Yin M C. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. Journal of agricultural and food chemistry,2000, 48: 2266-2270.
2. Tseng TH, Kao ES, Chu CY, Chou FP, Lin Wu HW and Wang CJ. Protective Effects of Dried Flower Extracts of *Hibiscus sabdariffa* L. against Oxidative Stress in Rat Primary Hepatocytes. Food Chemistry and Toxicology , 1997, 35:1159-1164.
3. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant Activities of some Extracts of *Thymnus zygis*. Free Radical Research, 1997, 26:469-478
4. Roqaiya M, Begum W, Majeedi SF, Saiyed A. A Review on traditional uses and phytochemical properties of *Mimusops elengi Linn.*IJHM, 2015, 2(6): 20-23.
5. Ghani A. Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka, 2003, pp. 303
6. Jahan N, Ahmed W and Malik A (1995). New steroidal glycosides from *Mimusops elengi.* Journal of Natural Products, 1995, 8 (8):1244-1247.
7. Kirtikar KR and Basu BD. Indian Medicinal Plants; M/s. Bishensingh Mahendra Palsingh, Dehradun, 1935, pp.1494-96.
8. Misra G and Mitra CR. Constituents of fruit and seeds of *Mimusops elengi*. Phytochemistry , 1967, 6 (3): 453.
9. Misra G and Mitra CR (1968). Constituents of leaves, hard wood and root of *Mimusops elengi*. Phytochemistry ,1968, 7 (3): 501-502
10. Hart NK, Johns SR and Lamberton JA. Alkaloids of *Mimusops elengi* bark. Australian Journal of Chemistry, 1968, 21: 1393-95.

DOI: 10.21276/IJIPSR.2018.06.09.729

11. Kadam PV, Yadav KN, Deoda RS, Shivatare RS, Patil MJ. *Mimusops elengi*: A Review on Ethnology, Phytochemical and Pharmacological Profile. Journal of Pharmacognosy and Phytochemistry, 2012, 3(1):64-74.

12. Kar B, SureshRB, KarmakarI, DolaiN, BalaA, Mazumder UK, Halder PK. Antioxidant and in vitro anti-inflammatory activities of *Mimusops elengi* leaves. Asian Pacific Journal of Tropical Biomedicine, (2012),S976-S980.

13. Saha MR, Alam MA, Akter R and Jahangir R. In-vitro free radical scavenging activity of *Ixora coccinea L*,Bangladesh Journal of Pharmacology , 2008, 3(2): 90-96.

14. Shaik J, Kashim SM, Naidu PB. Protective activity of ethanolic leaf extract of *Mimusops elengi Linn* on lipid peroxidation and antioxidant enzymes in experimental diabetic rats. An int . J. Advances in Pharm. Sci, 2011, 2(2-3):264-275.

15. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Viticult* , 1965, 16: 144-158.

16. Zhishen J, Mengcheng T and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry,1999, 64, pp.555-559.

17. Choi YJ, Kim SK,Kim SH, Lee KS, Choi KY (2000) *Saccharomyces cerevisiae* Ste5 is important for induction and substrate specificity of Fus3 MAP kinase in the pheromonesignaling pathway. Mol Cells, 2000, 10(3): 301-8

18. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition 1986, 44:307-315.

19. Prieto P, Pineda M and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. Analytical Biochemistry, 1999, 269, 337-341.

20. Rice-Evans C, Sampson J, Bramley PM and Holloway DE. Why do we expect carotenoids to be antioxidants in vivo. Free Radical Research, 1997, 26: 381–398.

21. Jorgensen LV, Madsen HL, Thomsen MK and Dragsted L. Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy, Free Radical Research, 1999, 30: 207- 220.

22. Tanaka M, Kuie CW, Nagashima Y and Taguchi T. Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. Nippon.Suisan Gakkaishi , 1988, 54: 1409–1414

DOI: 10.21276/IJIPSR.2018.06.09.729

23. Saha MR, Hasan MR, Akter R, Hossain MM, Alam MS, Alam MA and Mazumder MEH. In vitro free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi*. Bangladesh Journal of Vet. Med, 2008, 6(2): 197-202.

24. Osawa T. Novel natural antioxidants for utilization in food and biological systems. *In*: Uritani I, Garcia VV, Mendoza EM (Eds) Post harvest biochemistry of plant food-materials in the tropics. Japan Scientific Societies Press, Japan. 1964, pp. 241-251.

25. Shahidi F, Janitha PK and Wanasundara PD. Phenolic antioxidants. CRC *Critical Rev.* Food Science andNutrition. 1992, 32 (1): 67-103.