Evaluation of *in vitro* Antioxidant Activity and Total Phenolic Content of Methanol Bark Extract of *Mimusops elengi*

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ABSTRACT

Introduction: Some researchers suggest that two-thirds of the world's plant species have medicinal value; in particular, many medicinal plants have great antioxidant potential. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. **Methods:** The present study was projected to evaluate the *in vitro* potential of methanol extract of *Mimusops elengi* bark in correlation to the ascorbic acid which was used as a reference standard. The antioxidant properties of methanolic extract that probably involve free radical mechanisms were evaluated by the methods namely DPPH, ABTS, Hydroxy radical and Nitric oxide radical Scavenging assays. **Results:** The IC_{50} values of methanol extract in DPPH radical, nitric oxide, ABTS radical and hydroxyl radical were obtained to be 2.2 µg/ml, 158.49 µg/ml, 7.69 µg/ml and 3 µg/ml respectively. However, the IC_{50} values for the standard ascorbic acid were noted to be 0.66 µg/ml, 200 µg/ml, 0.7 µg/ml and 1 µg/ml respectively. Measurement of total phenolic content of the methanol extract of *M. elengi* was achieved using Folin–Ciocalteau reagent containing 698.7 ± 0.93 mg/g of phenolic content, which was found signicantly higher when compared to reference standard gallic acid. **Conclusions:** The observed results clearly indicate that *M. elengi* has a significant anti-oxidant activity and the results correlate positively with total phenol content strongly plead in favour of the use of this plant as potential food additives in replacement of synthetic compounds.

Key Words: *Mimusops elengi,* Anti-oxidant activity, Total Phenolic content, Methanol bark extract.

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INTRODUCTION

Oxidation is a basic part of the aerobic life and our metabolism. During oxidation, many free radicals are produced which have an unpaired nascent electron. Atoms of oxygen or nitrogen having central unpaired electron are called reactive oxygen or nitrogen species.[1-4] These are harmful to the body and may cause peroxidation of membrane lipids, aggression of tissue membranes and proteins or damage to DNA and enzyme.[5] These can be the cause of some pathology related to arthritis, hemorrhagic shock, coronary artery diseases, cancer as well as age related degenerative brain diseases.[6] Antioxidants are the vital substances which possess the ability to protect the body from damage caused by the free radical induced oxidative stress.[7] There is an increasing interest in the study of antioxidant substances mainly due to the findings of the therapeutic effects of free radical scavengers on the organism. A great number of plants worldwide showed a strong antioxidant activity[8-9] and a powerful scavenger activity against free radicals.[10-11]

Human body posse's defense mechanisms against free radical induced oxidative stress which involves preventive mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses such as superoxide dismutase (SOD), glutathione peroxidase (GP_X), catalase (CAT) etc. Non enzymatic antioxidants are ascorbic acid (vitamin C), α tocopherol (vitamin E), glutathione (GSH), carotenoids, flavanoids etc. All these act by one or more of the mechanisms like reducing activity, free radical Scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic

disease and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants.[12] This is one of the reasons for the interest in the study of the antioxidant activity of the medicinal plants, since the synthetic antioxidants like butylated hydroxyl toluene (BHT) and butylated hydroxyl Anisole (BHA) commonly used have side effects and are also carcinogenic and even toxic. [13-14]

The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body.[15] The beneficial health effects of plants are attributed to flavanoids, a class of secondary metabolites which protect the plant against ultraviolet light and even herbivores.[16] The protective effects of flavanoids are due to their capacity to transfer electrons to free radicals and to chelate metal catalysts[17] activate antioxidant enzymes,[18] reduce α tocopherol radical[19] and inhibit known free radical producing enzymes, such as myeloperoxidase and NADPH oxidase[20] and xanthine oxidase.[21] Further flavanoids have demonstrated exceptional cardioprotective effects, essentially because of their capacity to inhibit LDL peroxidation.[22]

Mimusops elengi Linn commonly known as Bakul belongs to the family *Sapotaceae* and is a small to large evergreen tree found all over the different parts of Bangladesh, Pakistan and India.[23] It is cultivated in gardens as an ornamental tree for sweet-scented flowers. It has been used in the indigenous system of medicine for the treatment of various ailments. Several therapeutic uses such as cardiotonic, alexipharmic, stomachic, anthelmintic and astringent have been ascribed to the bark of *Mimusops elengi*.[24] 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.[25] The pounded seeds pasted with oil are used for the treatment of obstinate constipation. Pillow stuffing made from the dried flowers induces nasal discharge and relieves headache.[25] Several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this species.[25-26] The phytochemical review indicated the presence of taraxerol, taraxerone, ursolic acid,[27-28] betulinic acid, V-spinosterol, W-sitosterol, lupeol,[27-28] alkaloid isoretronecyl tiglate[29] and mixture of triterpenoid saponins in the bark of *Mimusops elengi*. *In vitro* free radical scavenging activity of methanol extract of the leaves of *M. elengi* was reported[30] in the literature. Antioxidant capacity and phenolic content of *Mimusops elengi* fruit extract[31] was also been reported. However no reports are available on the antioxidant activity of the bark of *Mimusops elengi,* therefore present investigation was undertaken to examine the total phenolic content and antioxidant activities of the methanol extract of *Mimusops elengi* bark through various *in vitro* models and possible relationship between phenolic content and antioxidant activity was also seen.

MATERIALS AND METHODS

Chemicals

Folin ciocalteu Reagent, Sodium carbonate, Ammonium peroxysulphate, Ascorbic acid, Sodium Nitroprusside, Sodium dihydrogen phosphate, Orthophosphoric acid, Methanol L.R. grade, Ferric chloride, Thiobarbituric acid, Acetic acid, EDTA were purchased from Merck Pvt Limited, Mumbai. Gallic acid, DPPH, 2 deoxyribose , ABTS were purchased from Himedia, Mumbai. Sulfanilamide, Naphthyl ethylenediamine dihydrochloride were purchased from Loba Cheme Pvt. Limited, Mumbai.

Plant Material

The bark of *Mimmusops elengi* was collected during October-November 2009 from Berhampur, Ganjam District, Orissa, India. Further taxonomic identification was conducted by Dr. Malaya Kumar Mishra, Professor, Department of Botany, Berhampur University, Berhampur, Orissa, India. A voucher specimen of the plant (RIPS/H/0109) has been deposited in the herbarium at the department of botany, Berhampur University.

Preparation of the extract

The sun-dried bark of *Mimusops elengi* (350 g) was powdered and then extracted with 1500 ml of petroleum ether in a soxhlet apparatus, to remove the lipids and other resinous matter from the bark. The crude petroleum ether extract was filtered and evaporated under reduced pressure, using rotary evaporator which was a viscous dark mass with a percentage yield of 4.49 % (w/w). The residue obtained after the extraction with petroleum ether was further extracted with 1800 ml of chloroform by using soxhlet apparatus. The crude chloroform extract was filtered and evaporated under reduced pressure, using rotary evaporator which was a light coloured mass with a percentage yield of 2.37 % (w/w). The residue obtained after the extraction of chloroform extract was further extracted with 1800 ml of methanol by using soxhlet apparatus. The crude methanol extract was

filtered and evaporated under reduced pressure using rotary evaporator which was a viscous dark mass with a percentage yield of 32.74 % (w/w). The methanolic extract thus obtained was dissolved in methanol solvent and used for the assessment of antioxidant activity.

Determination of Total Phenol content

Total phenolic content was determined by Folin-ciocalteau reagent method in which gallic acid was used as a standard phenolic compound.[32] 5 ml of the reagent was mixed with 1 ml of Gallic acid at different concentrations and 3 minutes later 4 ml of 2% Sodium carbonate was added to each of the solutions. Thirty minutes later the blue colour that was developed was read at 760 nm.

Similarly 50 µg/ml and 100 µg/ml concentrations of methanolic extract were treated in the similar manner as that of the standard Gallic acid. The concentrations of total phenols were expressed as mg/g of dry extract.[33] All the determinations were performed in triplicate. Total content of phenolic compounds were expressed as Gallic acid equivalents (GAE) calculated by the following formula

$$C = c.\frac{V}{m}$$

Where: C- total content of phenolic compounds, mg/g plant extract, in GAE;

c - the concentration of gallic acid established from the calibration curve, mg/ml;

V - the volume of extract, ml;

m - the weight of pure plant extract

Scavenging of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH• assay)

The free radical-scavenging activity of *Mimusops elengi* methanol extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. In this assay, the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine.[34] The scavenging capacity is generally evaluated in organic media by monitoring the absorbance decrease at 515-528nm until the absorbance remains constant. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.1-20 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Scavenging of nitric oxide radical

Nitric oxide radical (NO•) has a pivotal role in the regulation of diverse physiological and pathophysiological processes. Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide[35] which interacts with oxygen to produce nitric ions that can be estimated by using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate buffer saline (PBS) was mixed with 3.0 ml of different concentrations (50-5000 µg/ml) of *Mimusops elengi* methanol extract and incubated at 25 ºC for 180 min. The samples were added to Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthyl ethylenediamine dihydrochloride). The absorbance of the chromaphore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthyl ethylenediamme was read at 546 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control. The percentage of inhibition was measured by the following formula:

$$\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the Absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Scavenging of ABTS radical

The ABTS assay was employed to measure the antioxidant activity of the bark extract. ABTS was dissolved in

de-ionised water to 7 mM concentration, and Ammonium persulphate added to a concentration of 2.45mM. The reaction mixture was left to stand at room temperature overnight (12 to 16 h) in the dark before usage. 0.5 ml of methanol extract (0.25-300 μg/ml) was diluted with 0.3 ml ABTS solution and made up to the volume with methanol. Absorbance was measured spectrophotometrically at 745 nm. The assay was performed at least in triplicates. Fresh stocks of ABTS solution were prepared every five days due to self-degradation of the radical. The assay was first carried out on ascorbic acid, which served as a standard. The percentage of inhibition was measured by the following formula:

$$\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Scavenging of hydroxyl radical

The hydroxyl radical scavenging capacity was measured using modified method as described previously.[36] Stock solutions of EDTA (1 mM), $FeCl_3$ (0.2 mM), ascorbic acid (1mM), H_2O_2 (10 mM) and deoxyribose (28 mM) were prepared in distilled de-ionized water. The assay was performed by adding 0.1 ml EDTA, 0.1 ml of $FeCl_3$, 0.1 ml of deoxyribose, 0.5 ml of extract (0.1-1000 μg/ml) each dissolved in methanol and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 ºC for 1 h. The above reaction mixture was treated with dodecyl sulphate [8.1%, 0.2 ml], thiobarbituric acid [0.8%, 1.5 ml], and acetic acid [20%, 1.5 ml and pH 3.5] and kept in oil bath maintained at 95 °C for 1 hour and the absorbance was measured at 532 nm. The hydroxyl radical- scavenging activity of the extract was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

$$\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the Absorbance in the presence of the sample of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values. Ascorbic acid was used as a positive control.

RESULTS AND DISCUSSION

Total Phenol content

The content of phenolic compounds (mg/g) in methanol extract was found to be 698.7 ± 0.93 mg/g plant extract and expressed in gallic acid equivalents. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. The same relationship was also observed between phenolic and antioxidant activity in roseship extracts.[37] Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups.[38] The phenolic compounds may contribute directly to antioxidative action. It is well known that phenolic compounds are constituents of many plants, and they have attracted a great deal of public and scientific interest because of their health promoting effects as antioxidants.[39-40] The polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables.[41] The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron-donating agents, and metal ion chelating properties.[42] However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants.[43] Phenolic compounds, at certain concentrations, markedly slowed down the rate of conjugated diene formation. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.[44] Therefore, it would be valuable to determine the total phenolic content of the plant extracts.

Inhibition of DPPH radical

The DPPH radical is a stable organic free radical with an absorption maximum band around 515-528 nm and thus, it is a useful reagent for evaluation of antioxidant activity of compounds.[45] In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidants.[46] It has been documented that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g., hydroquinone, pyrogallol, gallic acid), reduce and decolorize 1,1-diphenyl-2-picrylhydrazine by their hydrogen donating capabilities.

The methanol extract of *Mimusops elengi* demonstrated a concentration dependent scavenging activity by

quenching DPPH radicals. The hydrogen donating activity, measured using DPPH test, showed that *Mimusops elengi* extract contained 2.2 µg ascorbic acid equivalents/g extract of activity. The concentration of *Mimusops elengi* needed for 50% inhibition (IC_{50}) was found to be 2.2 µg/ ml, whereas 0.66 µg/ml (Table 1) was needed for ascorbic acid. The different concentrations of methanolic bark extract of *Mimusops elengi* (MBEM) (0.1-20 µg/ml) showed antioxidant activities in a dose dependent manner (5.61% ± 0.21-90.61% ± 0.16) on the DPPH radical scavenging assay (Figure 1). A higher DPPH radical scavenging activity is associated with a lower IC_{50} value. The results were also found to be statistically significant and calculated the *P* value using ANOVA by Bartlett's test for equal Variance (Table 3).

Inhibition of Nitric oxide radical

Nitric oxide plays an important role in various types of inflammatory processes in the animal body. Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal

Table 1. Effect of methanolic bark extract of *Mimusops elengi,* on DPPH and Nitric oxide radical scavenging activities

DPPH radical scavenging activity			Nitric oxide radical scavenging activity		
Conc. (mcg/ml)	Mean (%) inhibition ± SD[a]		Conc. (mcg/ml)	Mean (%) inhibition ± SD[a]	
	M. elengi	Ascorbic acid		M. elengi	Ascorbic acid
0.1	5.61 ± 0.21	18.05 ± 0.60	50	16.67 ± 0.43	21.73 ± 0.32
0.5	6.56 ± 0.18	25.03 ± 0.82	100	32.02 ± 0.37	35.89 ± 0.06
0.75	11.25 ± 0.26	39.52 ± 0.79	200	41.77 ± 0.46	46.17 ± 0.50
1	14.0 ± 0.32	74.3 ± 1.19	300	48.84 ± 0.81	57.84 ± 0.38
2.5	46.03 ± 0.28	78.77 ± 1.02	500	58.31 ± 0.96	67.4 ± 0.19
5	73.79 ± 0.27	80.63 ± 0.68	750	68.06 ± 0.24	73.45 ± 0.03
10	79.78 ± 0.38	84.05 ± 0.61	1000	72.57 ± 0.31	76.66 ± 0.48
20	90.61 ± 0.16	93.15 ± 0.82	2500	76.24 ± 0.44	86.87 ± 0.35
			5000	78.26 ± 0.28	92.92 ± 0.29
IC50 values	2.2	0.66		158.49	200

[a]Values are given as mean of three replicates.
M. elengi: Mimusops elengi bark methanolic extract.
SD: Standard deviation

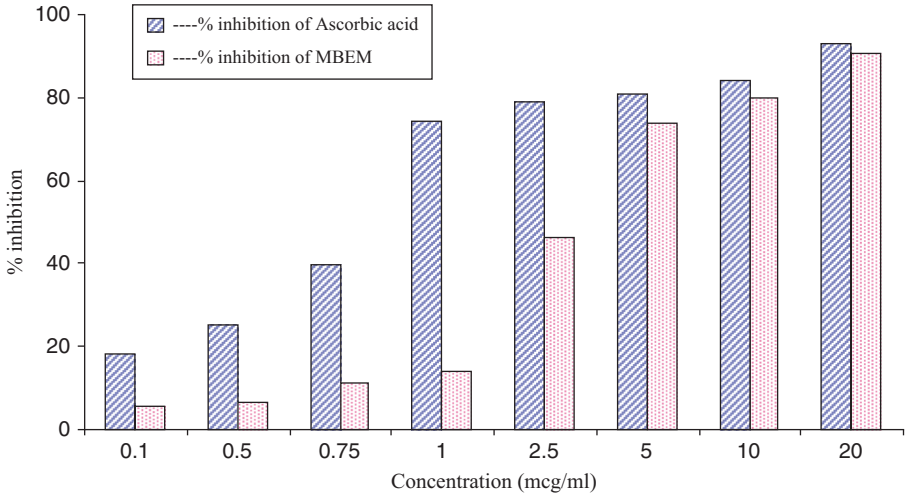

Figure 1. DPPH radical scavenging activity of the methanolic bark extract of *Mimusops elengi.*

signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.[47] In the present study the crude methanol extract of the *Mimusops elengi* was checked for its inhibitory effect on nitric oxide production. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by *Mimusops elengi*. *Mimusops elengi* extract at varied concentrations showed remarkable inhibitory effect of nitric oxide radical- scavenging activity. Results showed the percentage of inhibition in a dose dependent manner. The various concentrations of MBEM (50-5000 µg/ml) showed the percentage of inhibition in a dose dependent manner (16.67% ± 0.43-78.26% ± 0.28) (Figure 2). The concentration of *Mimusops elengi* needed for 50% inhibition (IC_{50}) was found to be 158.49 µg / ml, whereas 200 µg /ml (Table 1) was needed for ascorbic acid. The results were found to be statistically significant and calculated the P value using ANOVA by Bartlett's test for equal Variance (Table 3).

Inhibition of ABTS radical

The reduction capability of ABTS radical was determined by the decrease in its absorbance at 745 nm which is induced by antioxidants. The methanol extract of *Mimusops elengi* bark at quantities of (0.25 - 300 µg/ml) scavenged the ABTS radicals in a dose dependent manner. Ascorbic acid at a concentration of (0.25-5 µg/ml) also found to produce dose dependent inhibition of ABTS radicals. The various concentrations of MBEM on ABTS radical scavenging activity (0.25-300 µg/ml) showed (6.68% ± 0.24-93.95% ± 0.14) inhibition respectively. Results showed the percentage of inhibition in a dose dependent manner (Figure 3). The quantity of *Mimusops elengi* extract required to produce 50% inhibition of ABTS

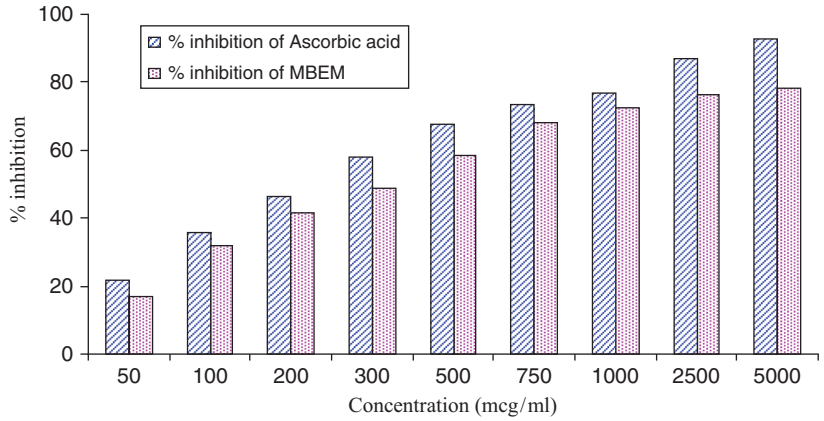

Figure 2. Nitric oxide radical scavenging activity of the methanolic bark extract of *Mimusops elengi*.

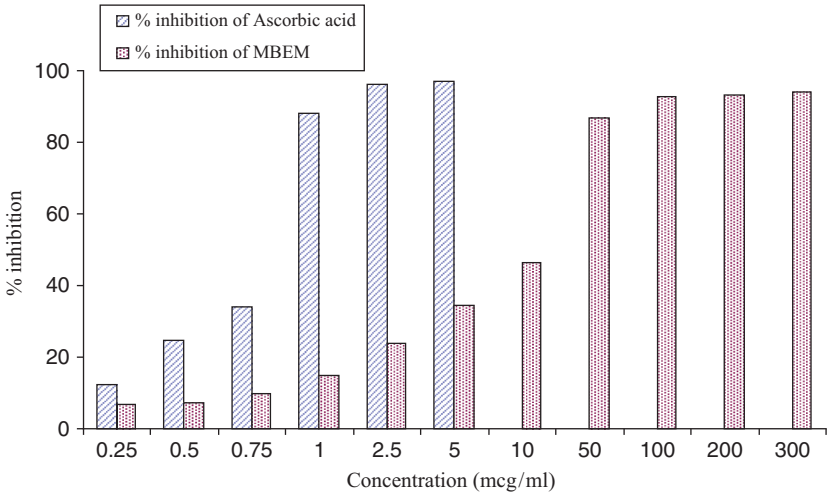

Figure 3. ABTS radical scavenging activity of the methanolic bark extract of *Mimusops elengi*.

radical was 7.69 μg/ml. similar effects was produced by ascorbic acid nearly at concentration of 0.7 μg/ml (Table 2). The results were also found to be statistically significant and calculated the P value using ANOVA by Bartlett's test for equal Variance (Table 3).

Inhibition of Hydroxy radical

Several *in vitro* methodologies for determination of HO• scavenging capacity are available mostly based on Fe^{3+} EDTA + H_2O_2 + ascorbic acid system to generate a constant flux of HO• radicals. Those radicals attack the sugar 2-deoxy-d-ribose (used as target), degrading it into a series of fragments, some or all of which react upon heating with thiobarbituric acid at low pH to give a pink chromogen. If a HO• scavenger is added to the reaction mixture, it will compete with deoxyribose for HO• radicals, inhibiting the degradation of the target species. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly

Table 2. Effect of methanolic bark extract of *Mimusops elengi,* on ABTS and Hydroxyl radical scavenging activities

ABTS radical scavenging activity			Hydroxyl radical scavenging activity		
Conc. (mcg/ml)	Mean (%) inhibition ± SD[a]		Conc. (mcg/ml)	Mean (%) inhibition ± SD[a]	
	M. elengi	Ascorbic acid		M. elengi	Ascorbic acid
0.25	6.68 ± 0.24	12.24 ± 0.45	0.1	10.81 ± 0.24	17.85 ± 0.23
0.5	7.1 ± 0.10	24.48 ± 1.19	0.5	15.65 ± 0.25	33.4 ± 0.32
0.75	9.71 ± 0.49	34.12 ± 0.46	1	19.51 ± 0.53	50.32 ± 0.37
1	14.71 ± 0.13	88.02 ± 0.45	5	46.39 ± 0.44	58.6 ± 0.24
2.5	23.79 ± 0.49	96.1 ± 1.36	10	59.02 ± 0.44	66.53 ± 0.21
5	34.53 ± 0.36	97.14 ± 0.44	20	72.77 ± 0.12	74.17 ± 0.53
10	46.38 ± 0.14		30	74.39 ± 0.24	74.8 ± 0.13
50	86.79 ± 0.60		50	75.23 ± 0.12	75.16 ± 0.0
100	92.68 ± 0.28		70	75.31 ± 0.13	75.79 ± 0.0
200	93.32 ± 0.24		100	75.72 ± 0.12	76.77 ± 0.12
300	93.95 ± 0.14		200	76.42 ± 0.21	78.88 ± 0.12
			300	77.05 ± 0.21	82.46 ± 0.44
			500	77.82 ± 0.12	85.05 ± 0.0
			700	78.95 ± 0.21	89.51 ± 0.15
			1000	82.88 ± 0.48	93.96 ± 0.53
IC50 values	7.69	0.7		3	1

[a]Values are given as mean of three replicates.
M. elengi: Mimusops elengi bark methanolic extract.
SD: Standard deviation

Table 3. Effect of methanolic bark extract of *Mimusops elengi* (*P* values), on different radical scavenging activities

P values of MBEM			
DPPH radical scavenging activity	Hydroxy radical scavenging activity	Nitric oxide radical scavenging activity	ABTS radical scavenging activity
BTEV	BTEV	BTEV	BTEV
0.0013	<0.0001	<0.0001	0.0055
$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

MBEM: Methanolic bark extract of *M. elengi ;* BTEV: Bartlett's test for equal Variance

damaging species in free radical pathology, capable of damaging almost every molecule found in living cells.[48] Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity.[49] Among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to the adjacent biomolecules.[50] The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins Ferric EDTA incubated with H_2O_2 and ascorbic acid during which hydroxy radicals were formed in the free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA form a pink chromogen. When methanolic extract of *Mimusops elengi* and the reference compound, ascorbic acid, added to the reaction mixture they removed hydroxyl radicals from the sugar and prevented degradation. The results are shown in Figure 4. *Mimusops elengi* was also capable of reducing DNA damage at all concentrations used. The IC_{50} value of methanolic extract of *Mimusops elengi* on hydroxyl radical were found to be 3 μg/ml and 1 μg/ml for ascorbic acid, respectively (Table 2). The various concentrations of MBEM on hydroxyl radical scavenging activity (0.1-1000 μg/ml) showed (10.81% ± 0.24-82.88% ± 0.48) inhibition respectively. Results showed the percentage of inhibition in a dose dependent manner (Figure 4) also found to be statistically significant and calculated the P value using ANOVA by Bartlett's test for equal Variance (Table 3). The ability of the above mentioned extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of the chain reaction.

CONCLUSION

Free radicals particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in the pathogenesis of several chronical and degenerative diseases such as inflammation, cardiovascular diseases, neurodegenerative diseases, cancer and aging related disorders. We have demonstrated the methanol extract of *Mimusops elengi* bark contained high level of total phenolic compounds and were capable of inhibiting, quenching free radicals to terminate the radical chain reaction, and acting as reducing agents. Furthermore, phenolic compounds present in the plant kingdom are mainly responsible for the antioxidant potential of plants. Accordingly in this study, a significant and linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to antioxidant activity. The methanol extract of *Mimusops elengi* bark showed strong antioxidant activity by inhibiting DPPH, hydroxyl radical, nitric oxide and ABTS radical scavenging activities when compared with standard ascorbic acid. In addition, the *Mimusops elengi* found to contain a noticeable amount of total phenols which plays a major role in controlling antioxidants. Although the antioxidant activities found *in vitro* experiment were only indicative of the potential health benefit, these results remain important as the first step in screening antioxidant activity of *Mimusops elengi* bark. Thus, it can be concluded that methanol extract of *Mimusops elengi* bark can be used as an accessible source of natural antioxidants with consequent health benefits.

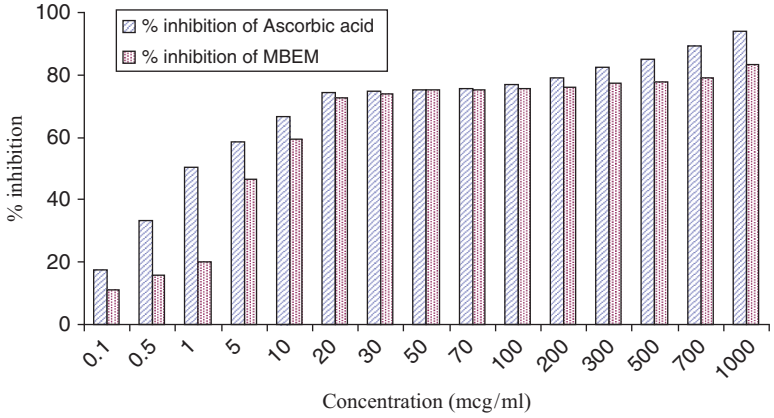

Figure 4. Hydroxy radical scavenging activity of the methanolic bark extract of *Mimusops elengi*.

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