Indian Journal of Pharmaceutical Education and Research | Association of Pharmaceutical Teachers of India

Invitro Antioxidant Activity and Total Phenolic Content of *Mimusops elengi* Bark

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ABSTRACT

The aim of this study was to assess the in vitro potential of chloroform extract of *Mimusops elengi* bark. The IC_{50} values of chloroform extract in DPPH radical, nitric oxide, ABTS radical and hydroxyl radical were obtained to be 2.43 μg/ml, 152.76 μg/ml, 14.12 μg/ml and 6.31 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 chloroform extract of M. elengi was achieved using Folin–Ciocalteau reagent containing 375.9 mg/g of phenolic content, which was found signicantly higher when compared to reference standard gallic acid. The results obtained in this study clearly indicate that *M. elengi* has a significant potential to use as a natural anti-oxidant agent.

Key words: Mimusops elengi; anti-oxidant activity; total phenolic content; chloroform bark extract.

Submitted: 5-10-2010 *Revised: 8-12-2010* *Accepted: 13-3-2011*

INTRODUCTION

The human body produces reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical and hydrogen peroxide by many enzymatic systems through oxygen consumption. In small amounts, these ROS can be beneficial as signal transducers[1] and growth regulators[2]. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to overproduction of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins[3] occur which increases risk of more than 30 different disease processes[4]. The most notorious among them being neurodegenerative conditions like Alzheimer's disease[5-6], mild cognitive impairment (MCI)[7] and Parkinson's disease. To circumvent the damage caused ROS, multiple defense systems collectively called antioxidants are present, with protective efficiency depending on the balance between ROS and availability of antioxidants in the microenvironment of a cell[8]. Antioxidants are the vital substances which possess the ability to protect the body from damage caused by the free radical induced oxidative stress[9]. 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[10] and a powerful scavenger activity against free radicals. Human body has multiple mechanisms especially enzymatic and non-enzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species induced damage. However, the innate defense may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in human body. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and are used for industrial processing but they possess potential health risk and toxic properties to human health and should be replaced with natural antioxidants[11]. Hence, compounds especially from natural sources capable of protecting against ROS mediated damage may have potential application in prevention and/or curing of diseases.

It is reported that some medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants. Many investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases[12]. *Mimusops elengi* Linn commonly known as Bakul (in India) belongs to the family *Sapotaceae* and is a small to large evergreen tree found all over the different parts of Bangladesh, Pakistan and India. 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*. 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

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gargle. 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[13]. Several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this species[14]. Phytochemical review shows the presence of taraxerol, taraxerone, ursolic acid[15,] betulinic acid, V-spinosterol, W-sitosterol, lupeol, alkaloid isoretronecyl tiglate, pentacyclic triterpenes, such as mimusopgenone and mimugenone in the seeds triterpenoid saponins, such as mimusopsides A and B, mimusopin, mimusopsin, mimusin, Mi-saponin A from the seeds. *In vitro* free radical scavenging activity of methanol extract of the leaves of *M. elengi* was reported in the literature[16]. Antioxidant capacity and phenolic content of *Mimusops elengi* fruit extract was also been reported[17]. 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 chloroform extract of *Mimusops elengi* bark through various *in vitro* assay models were assessed. The possible relationship between phenolic content and antioxidant activity was also seen.

MATERIALS AND METHODS

Chemicals:

Folin ciocalteu Reagent (Merck, Mumbai), Gallic acid (Hi media Mumbai), Sodium carbonate (Merck, Mumbai), DPPH (Hi Media, Mumbai), ABTS (Hi Media, Mumbai), Ammonium peroxysulphate (Merck Limited, Mumbai), Ascorbic acid (vitamin C)(Merck Limited, Mumbai), Sodium Nitroprusside (Merck Limited, Mumbai), Sodium dihydrogen phosphate (Merck Limited, Mumbai), Sulfanilamide (Loba cheme Pvt. Limited, Mumbai), Naphthylethylenediamine dihydrochloride (Loba cheme Pvt. Limited, Mumbai), Orthophosphoric acid (Merck Limited, Mumbai), Methanol L.R. grade (Merck Limited, Mumbai), Ferric chloride (Merck Limited, Mumbai), Thiobarbituric acid (Merck Limited, Mumbai), Acetic acid (Merck Limited, Mumbai), 2 deoxy ribose (Hi media Mumbai), EDTA (Merck 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 was deposited in the herbarium of Roland institute of pharmaceutical sciences, Berhampur under the number (RIPS/H/0109).

Preparation of the extract:

The sundried bark of *Mimusops elengi* (350 g) was powdered and then extracted with 1.5L of petroleum ether in a soxhlet apparatus for 24 h, 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 marc obtained after the extraction with petroleum ether was further extracted with 1.8L of chloroform by using soxhlet apparatus for 24 h. The crude chloroform extract was filtered and evaporated under reduced pressure using rotary evaporator which was a light brown coloured mass with a percentage yield of 2.37 % (w/w). The chloroform extract thus obtained was dissolved in methanol and used for the assessment of antioxidant activity.

Determination of total phenol content:

Total phenolic content was determined by Folin-ciocalteau reagent, using gallic acid as a standard phenolic compound[18]. 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 chloroform 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[19]. All the determinations were performed in triplicate. Total content of phenolic compounds were expressed as Gallic acid equivalents (GAE) calculated by the following formula:

$$C = c. V/m$$

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 activity against the DPPH (1-1-diphenyl 2-picryl hydrazyl) radical:

The free radical-scavenging activity of the *Mimusops elengi* chloroform extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH[20]. 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:

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

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 activity against the Nitric oxide radical:

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[21] 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 the *Mimusops elengi* chloroform extract and incubated at 25°C 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 napthylethylenediamme 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 similar to that of DPPH assay.

Scavenging activity against the ABTS radical:

The ABTS assay was employed to measure the antioxidant activity of the bark extract[22]. ABTS was dissolved in de-ionised water to 7 mM concentration, and Ammonium persulphate added to a concentration of 2.45 mM. 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 (0.1 – 200 µg/ml) was diluted with 0.3 ml ABTS solution and made up to the volume with methanol. Absorbance was measured spectrophotometrically at 745nm. 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 similar to that of DPPH assay.

Scavenging activity against the hydroxyl radical:

The hydroxyl radical scavenging capacity was measured[23] using modified method as described previously. Stock solutions of EDTA (1 mM), $FeCl_3$ (0.2 mM), ascorbic acid (1 mM), 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°C 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 h 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 formula using ascorbic acid as a positive control.

Statistical analysis:

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Bartlett's test for equal Variance test ($P < 0.05$) using the Graph pad prism 4 software. Difference was considered significant when P-value was < 0.05.

RESULTS AND DISCUSSION

Total Phenol content:

The content of phenolic compounds (mg/g) in chloroform extract was found to be 375.9 ± 0.88 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. Phenolic compounds (flavanoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, aminoacids and amines), carotenoids, lignans and terpenes were reported to possess antioxidative activity in suppressing the initiation or propogation of the chain reactions. The same relationship was also observed between phenolics and antioxidant activity in roseship extracts[24]. Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups[25]. 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[26]. The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen- or electron-donating agents, and metal ion chelating properties[27].

However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants[28]. 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. The phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators. Therefore, it would be valuable to determine the total phenolic content of the plant extracts.

Inhibition of DPPH radical:

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[29], with an absorption maximum band around 515–528 nm and thus, it is a useful reagent for evaluation of antioxidant activity of compounds[30]. 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. The chloroform extract of *Mimusops elengi* demonstrated a concentration dependent scavenging activity by quenching DPPH radicals. The hydrogen donating activity, measured using DPPH test, showed that the *Mimusops elengi* extract contained 2.43 µg ascorbic acid equivalents/g extract of activity. The concentration of *Mimusops elengi* needed for 50% inhibition (IC_{50}) was found to be 2.43 µg/ ml, whereas 0.66 µg/ml was needed for ascorbic acid. The results were found to be statistically significant and reported in the table 1. The different concentrations of chloroform bark extract of *Mimusops elengi* (CBEM) (0.1, 0.5, 0.75, 1, 2.5, 5, 10 and 20 µg/ml) showed antioxidant activities in a dose dependent manner (3.05 ± 0.21%, 8.32 ± 0.28%, 11.12 ± 0.32%, 15.82± 0.26 %, 35.37 ± 0.38%, 54.24± 0.37%, 77.4 ± 0.37%, 83.47 ± 0.18% inhibition respectively) on the DPPH radical scavenging assay (Fig. 1). A higher DPPH radical scavenging activity is associated with a lower IC_{50} value.

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 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[31]. In the present study the crude chloroform extract of *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 CBEM (50 – 5000 µg/ml) showed 15.82 ± 0.76%, 32.02 ± 0.49%, 43.32 ± 0.32%, 47.88 ± 0.39%, 59.25 ± 0.51%, 65.23 ± 0.37%, 70.43 ± 0.32%, 74.61± 0.39%, 76.69 ± 0.55% inhibition respectively. Results showed the percentage of inhibition in a dose dependent manner (Fig. 2). The concentration of *Mimusops elengi* needed for 50% inhibition (IC_{50}) was found to be 152.76 µg/ ml, whereas 200 µg/ml (Table 1) was needed for ascorbic acid.

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 chloroform extract of *Mimusops elengi* bark at quantities of (0.1- 200 µg/ml) scavenged the ABTS radicals in a dose dependent manner. Ascorbic acid at a concentration of (0.1-5µg/ml) also found to produce dose dependent inhibition of ABTS radicals. The various concentrations of CBEM on ABTS radical scavenging activity showed 3.91 ± 0.1%, 4.16 ± 0.1%, 4.44 ± 0.1%, 4.69

Fig.1: DPPH radical scavenging activity of the chloroform bark extract of *Mimusops elengi*

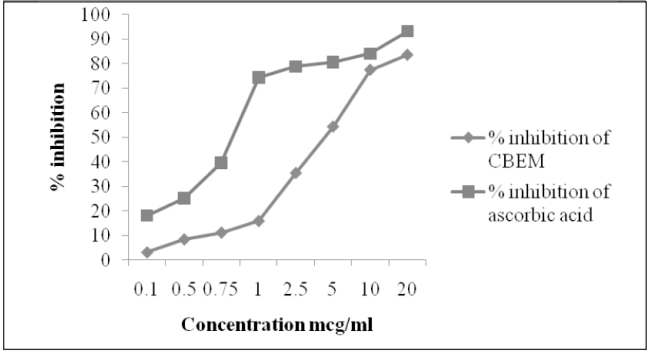


Fig.2: Nitric Acid radical scavenging activity of the chloroform bark extract of *Mimusops elengi*

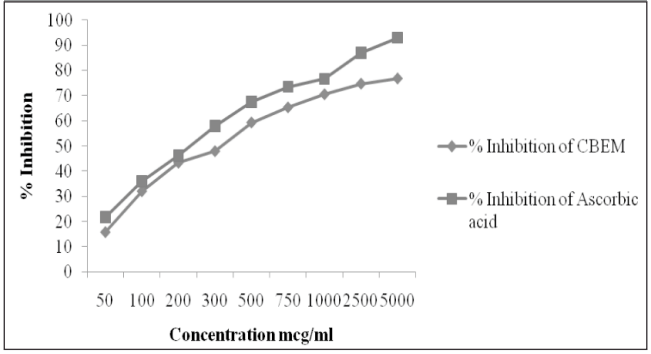


Fig.3: ABTS radical scavenging activity of the chloroform bark extract of *Mimusops elengi*

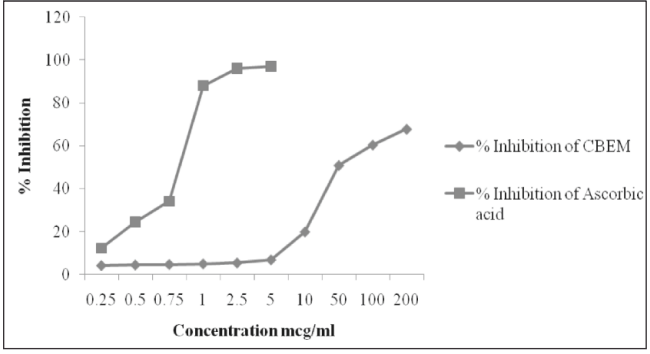
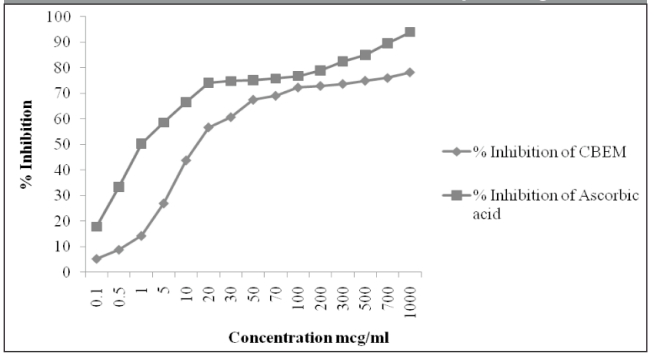


Fig.4: Hydroxyl Acid radical scavenging activity of the chloroform bark extract of *Mimusops elengi*



± 0.11%, 4.95 ± 0.21%, 5.38 ± 0.30%, 6.84 ± 0.10%, 19.88 ± 0.15%, 50.87 ± 0.15%, 60.41 ± 0.26%, 67.79 ± 0.84% inhibition respectively. Results showed the percentage of inhibition in a dose dependent manner (Fig. 3). The quantity of *Mimusops elengi* extract required to produce 50% inhibition of ABTS radical was 14.12 µg/ml. Similar effect was produced by ascorbic acid nearly at concentration of 0.7 µg/ml (Table 1).

Inhibition of hydroxyl radical:

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells[32]. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. Among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to the adjacent biomolecules[33]. 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 thiobarbituric acid form a pink chromogen. When chloroform 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 Fig.4. *Mimusops elengi* was also capable of reducing DNA damage at all concentrations used. The IC_{50} value of chloroform extract of *Mimusops elengi* on hydroxyl radical were found to be 6.31 µg/ml and 1 µg/ml for ascorbic acid, respectively (Table 1). The various concentrations of CBEM on hydroxyl radical scavenging activity (0.1 – 1000 µg/ml) showed 5.33 ± 0.32%, 8.91 ± 0.32%, 14.32 ± 0.43%, 27.02 ± 0.32%, 43.86 ± 0.32%, 56.76 ± 0.32%, 60.77 ± 0.24%, 67.58 ± 0.21%, 69.12 ± 0.32%, 72.35 ± 0.24%, 72.98 ± 0.12%, 73.75 ± 0.12%, 75.02 ± 0.12%, 76.07 ± 0.32%, 78.31 ± 0.42% inhibition respectively. Results showed the percentage of inhibition in a dose dependent manner (Fig. 4). 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

We have demonstrated the chloroform 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

Table 1: Effect of chloroform bark extract of *Mimusops elengi,* on different radical scavenging activities

Parameters	DPPH radical scavenging activity		ABTS radical scavenging activity		Hydroxyl radical scavenging activity		Nitric oxide radical scavenging activity	
	M. elengi	Ascorbic acid	*M. elengi*	Ascorbic acid	*M. elengi*	Ascorbic acid	*M. elengi*	Ascorbic acid
IC 50 value	2.2	0.66	7.69	0.7	3	1	158.49	200
P value of CBEM	< 0.05		< 0.05		< 0.05		< 0.05	
BTEV	0.0026		0.0261		< 0.0001		< 0.0001	

CBEM: Chloroform bark extract of M. elengi , BTEV: Bartlett ' s test for equal Variance

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 chloroform 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 chloroform extract of *Mimusops elengi* bark can be used as an accessible source of natural antioxidants with consequent health benefits.

ACKNOWLEDGEMENTS

Authors are grateful to Roland Institute of Pharmaceutical Sciences, Berhampur for providing laboratory facilities and Dr. Malaya Kumar Mishra, Professor, Berhampur University, Berhampur, Orissa., for identifying the plant.

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