Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(2): 84-87

Received: 01-01-2016
Accepted: 03-02-2016

Vinay KN
Department of Chemistry, Tumkur University, Tumkur-572101, Karnataka State, India.

V Venkata Lakshmi
AMC Research Centre, Kalkere, Bangalore-560083, Karnataka State, India.

ND Satyanarayan
Department of Pharmaceutical Chemistry, Kuvempu University, Post Graduate Centre, Kadur-577548, Chikmagalur Dt. Karnataka State, India.

Anantacharya R
Department of Pharmaceutical Chemistry, Kuvempu University, Post Graduate Centre, Kadur-577548, Chikmagalur Dt. Karnataka State, India.

Correspondence
V Venkata Lakshmi
AMC Research Centre, Kalkere, Bangalore-560083, Karnataka State, India.

In Vitro antioxidant activity of leaf solvent extracts *Mimusops elengi* Linn

Vinay KN, V Venkata Lakshmi, ND Satyanarayan, Anantacharya R

Abstract
Three different crude extracts viz, n-hexane, dichloromethane and methanol of *Mimusops elengi* Linn. were investigated for *in vitro* total antioxidant activity at three different concentrations (5, 50 and 100 µg). Antioxidant ability is expressed as equivalents of ascorbic acid and was calculated using standard graph. The test results indicated that the leaf dichloromethane extracts exhibited significant antioxidant activity when compared with standard butylated hydroxyl anisole (BHA) and the methanol leaf extract at 50 and 100 µg concentrations was found to be very high. The experimental results revealed that the activity exhibited by the solvent extracts is dose dependent.

Keywords: Antioxidant, *Mimusops elengi*, Sapotaceae, BHA, Ascorbic acid

Introduction
Oxidation reactions are the destructive processes and can produce free radicals in cells which can start chain reactions and can damage cells. Oxidative stress is a result of excessive production of reactive oxygen species (ROS), super oxide, hydrogen peroxide, hydroxyl radicals and these species leads to uncontrolled reactions [1]. Molecular oxygen is an essential component for all living organisms, but suffers from injury if exposed to oxygen concentration of more than 21% [2]. Oxidative free radicals are formed continuously in the human system and have been concerned in several human diseases [3]. When the resistance mechanism is insufficient, oxidative stress can damage proteins, carbohydrates, lipid and nucleic acids leading to the generation of free radicals, other reactive oxygen species or impaired antioxidant defense mechanism and has been concerned in a variety of pathological conditions like rheumatoid arthritis, autoimmune diseases, myocardial infarction, cancer, atherosclerosis and heart diseases[4]. Even if these free radicals can be scavenged by the *in vivo* produced antioxidants, but endogenous antioxidants are insufficient to completely remove them to maintain a balance. As a result, dietary antioxidants are necessary to counteract excess free radicals [5]. Antioxidants are extensively used as constituents in dietary supplements in the hope of maintaining healthiness and preventing diseases such as coronary heart disease, cancer and even altitude sickness. In addition to these uses of natural antioxidants in medication, these compounds have many industrial uses, such as preservatives in cosmetics, food and preventing the dilapidation of rubber and gasoline [4, 6]. Phytochemicals in vegetables, fruits, spices and traditional herbal medicinal plants have been found to play defensive roles against many human chronic diseases. These diseases are associated with oxidative stress caused by surplus free radicals and other reactive oxygen species. Several, steroids, steroidal glycosides, triterpenoids, flavonoids and alkaloids have been reported which shows antioxidant activity [7, 8]. An antioxidant is any substance that when present at smaller concentrations compared to oxidizable substance, considerably delays or prevents oxidation of that substrate. Several aspects of neuroprotection are being examined, focused on different elements leading to loss of nerve cells [9]. These antioxidants may be endogenous or exogenous in origin. Based upon the mode of action, antioxidants may be categorized as chain breaking and preventive antioxidants. In the midst of the most prominent defenses are the enzyme catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) which constitute the main intracellular antioxidant defense systems by removing hydrogen peroxide and superoxide anion. Evidence shows that phytochemicals having antioxidant properties reduce the symptoms of neurodegeneration. Various studies have showed that phenolic substances such as flavonoids are considerably potent antioxidants [10]. Flavonoids have the property of

preventing lipid peroxidation and scavenging free radical [11].

Mimusops elengi Linn. (family Sapotaceae) the common name in Hindi is Maulsari, Bakul in Sanskrit, Elengi in Malayalam, Ranja in Kannada and Spanich Cherry and Bullet wood in English. [12] *M. elengi* is a large glabrous evergreen tree with a compact leafy head and short erect trunk, scaly, bark smooth, gray and 12-15 m high. Leaves 6.3-10 by 3.2-5 cm, elliptic shortly acuminate, base acute or rounded, glabrous, petioles 1.3-2.5 cm long [13]. It is cultivated in gardens as a decorative tree for sweets cented flora. It has been used in the traditional Indian system of medication for the treatment of numerous ailments. The different parts of the *M. elengi* plant (flowers, seeds, fruits and bark) have great medicinal value. The flowers, fruits and bark of this plant are used in the treatment of diarrhea, dysentery [14]. Leaves are used as an antidote for snakebite [21]. Seed and fruit of *M. elengi* showed presence of quercitol, ursolic acid, dihydro quercetin, quercetin, β-d glycosides of βsitosterol, α-spinasterol after saponification[15]. Taraxerone, taraxerol, betulinic acid and spinasterol, sodium salt of betulinic acid, fatty acid esters of α-spinasterol and ursolic acid was isolated from the bark. [16] Hentriacontane, lupeol and carotene from the leaves, heartwood and roots were isolated. A new steroidal saponin, 5 α-stigmast-9(11) en-3-obeta- D-glucopyranosyl (1-5)-o-beta dxylofuranoside was isolated from the roots of *M. elengi* [17, 18]. The leaves contain sterols, reducing sugars and tannins [19]. Pulp of the fruit contains a large proportion of sugar and saponin [20]. In view of the above findings in literature we tried to examine the plant *M. elengi* for its total antioxidant property by taking the leaf to compare its antioxidant potential. The results recommended that different solvent crude extracts of *M. elengi* have antioxidant activity, which may oblige in preventing or slowing progress of several oxidative stress related diseases.

Material and methods

Chemicals

Butylated hydroxy anisole (BHA), ammonium molybdate, sodium phosphate and sulfuric acid were purchased from Merck (Mumbai, India). The solvents used for this activity were of analytical grade and purchased from SD fine chemicals, India.

Collection of the plant material

Fresh leaves of *M. elengi* (*Sapotaceae*) was collected in nursery of medicinal plants near Namada Chilume Tumkur and were authenticated at the Department of Botany, Tumkur University, Tumkur, Karnataka State. The leaves were washed thoroughly three to four times with running tap water and once with sterile distilled water. The leaf material was then dehydrated under shade. After complete drying, the sample was cut into small pieces and then slashed to fine powder with the help of mechanical grinder and the powder was stored in a suitable airtight container for further use.

Preparation of the extracts

Extraction is the general process for separation of active constituents by the use of different solvents. Weighed amount (250 gm) of coarsely powdered leaf material was successively extracted with n-hexane, dichloromethane and methanol using soxhlet extraction method nearly for 18 hr. After each extraction, the marcs left over plant material was removed from extractor, dried and reloaded in the extractor for subsequent extraction until the solvent became colorless. The extracts obtained were further concentrated by evaporating solvent using Buchi type evaporator under reduced pressure and controlled temperature (40-50°C). The extracts obtained was dried under vacuum, packed and stored in refrigerator for further use.

Table 1: Details of the extraction and yield of *M. elengi*

Sl. No	Solvent used	Part used	Colour and nature	Yield (gm)
1	n-hexane	Leaf	Green paste	8.58
2	Dichloromethane	Leaf	Green powder	9.74
3	Methanol	Leaf	Brown paste	11.24

Total antioxidant activity

The total antioxidant ability was measured by Spectrophotometric method of Prieto *et al*. [21] various concentrations of solvent extracts of leaf (5, 50 and 100 µg) were taken in a series of test tubes. To this 1.9 ml of reagent solution (28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulfuric acid) was added. The tubes were incubated at 95 °C for 90 min and permitted to cool. The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacities were expressed as equivalents of ascorbic acid and were calculated using standard graph of ascorbic acid and Butylated hydroxy anisole (BHA) was used as reference standard. The values are expressed as ascorbic acid equivalents in µg /mg of extract.

Results

The results from the activity revealed that, total antioxidant capacity was found to be high in methanol extract of *M. elengi* leaves at 50 and 100 µg (**denotes that the total anti-oxidant capacity was so high in terms of absorbance that it was beyond the measurable range of the spectrophotometer) and at 100 µg, dichloromethane leaf extract of *M. elengi* (40 µg), leaf of n-hexane crude extract (24.4 µg) as showed in Table (2)

Table 2: Total Antioxidant capacity of different solvent extracts of *M. elengi*.

concentrations of samples in µg	Leaf extracts of *M. elengi*			Standard
	n-hexane extract	DCM extract	Methanol extract	Butylated hydroxy anisole (BHA)
5	2.15	19.9	60	11
50	11.5	27.4	**	41
100	24.4	40	**	65

** denotes that the total anti-oxidant capacity was so high in terms of absorbance that it was beyond the measurable range of the spectrophotometer.

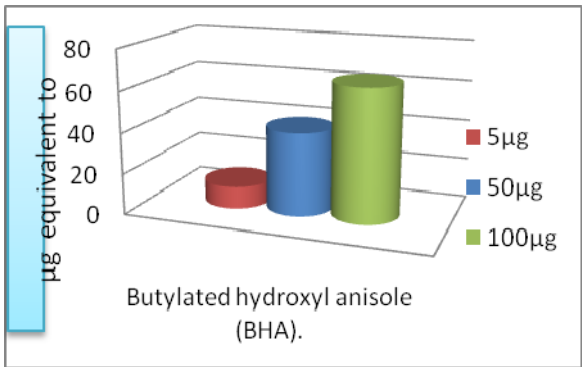

Fig 1a: Butylated hydroxyl anisole (BHA)

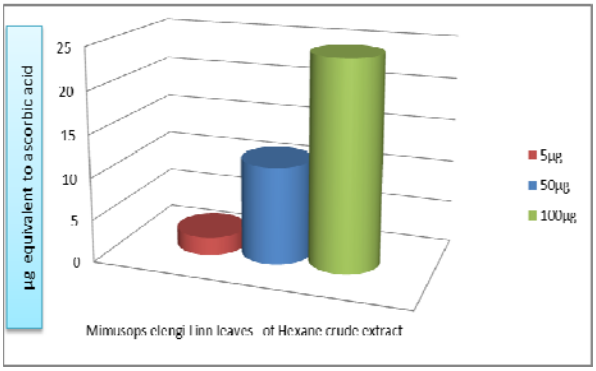

Fig 1b: Mimusops elengi leaves n-hexane crude extract

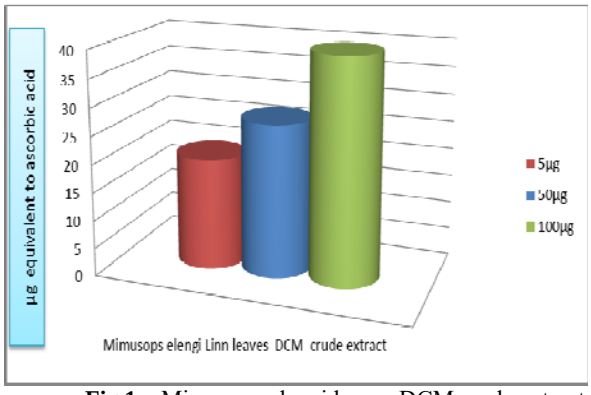

Fig 1c: Mimusops elengi leaves DCM crude extract

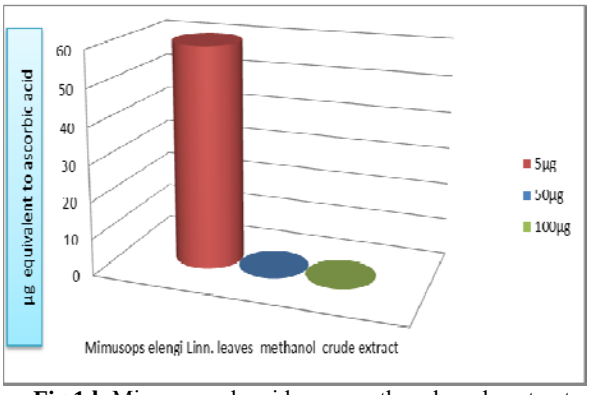

Fig 1d: Mimusops elengi leaves methanol crude extract

Fig 1: Total antioxidant activity of samples and butylated hydroxyl anisole (BHA) equivalent to ascorbic acid

Discussion

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyzed and the consequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative because the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. [21] In the present work, we investigated the antioxidant activity of different solvent extracts of the leaf of *M. elengi*. The total antioxidant capacity of the extract was calculated based on the formation of phosphomolybdenum complex which was analyzed spectrometric ally at 695 nm. The antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated by standard graph of ascorbic acid. Butylated hydroxy anisole (BHA) was used as reference standard. The different solvent extracts of the leaf of *M. elengi* showed very good total antioxidant capacity. The ascorbic acid equivalents and their optical density results are presented in (Fig-1). The total antioxidants capacity was found to be high in *M. elengi* leaf of methanol crude extract (**denotes that the total antioxidant capacity was so high in terms of absorbance that it was beyond the measurable range of the spectrophotometer.) at 100µg, dichloromethane leaf extract of *M.elengi* (40 µg) followed by leaf of n-hexane crude extract (24.4 µg of ascorbic acid/mg of extract) (Table-2).

The present study show that all extracts exhibited increased antioxidant activity or decreased pro-oxidant activity with escalating concentration. However, their activities differed according to the type of extract added to the system. The results suggested that various solvent crude extracts of *M. elengi* have antioxidant activity, which may be supportive in preventing or slowing development of different oxidative stress related diseases.

Conclusion

The data presented here shows that the marked antioxidant activity of *M. elengi*, seems to be due to presence of triterpenoid saponins, [22] which may act in a similar fashion as reductions by donating the electrons and reacting with free radicals to convert them to more stable product and discontinue the free radical chain reaction. The plant may be useful for the treatment of various diseases by free radicals. Herbal drugs containing free radical scavengers are gaining importance in treating various diseases.

In terms of antioxidant activity, more attention has been paid to oxidative stress and therapeutic plant products. Herbal products have opened up a completely new field for exploration and, in the next to expectation and nutritional modulation of diseases may come forward as an unconventional mode of treatment. The present study has attempted to understand total antioxidant activity to advocate the use of *M. elengi* extracts as potential antioxidants thus helping in the treatment of many diseases mediated by reactive oxygen species. Hence, it can be used for herbal pharmaceutical formulation.

Acknowledgement

The author Vinay K.N expresses thanks to AMC research centre and Tumkur University, for providing necessary facilities to carry out the present work.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Vinay KN, Lakshmi VV, Satyanarayan ND, Anantacharya GR. Antioxidant activity of leaf and fruit extracts of Rauwolfia tetraphylla Linn. International Journal of Pharmaceutical Sciences and Research 2016; 7(4):1000-

05.
2. Bhattacaharya SK, Sen P, Ray A. Pharmacology. Edn 2, Elisver publication, 2003.
3. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health, Pharmacognosy Reviews. 2010; 4(8):118-126.
4. Rang HP, Dale MM, Ritter JM, Moore PK. Rang and Dale's. Pharmacology Edn 5, Elisver published by India private limited, New Delhi, 2005.
5. Li Peiyuan, Lini H, Wei S, Rumei L, Chaocheng D. Free radical-scavenging capacity, antioxidant activity and phenolic content of Pouzolzia zeylanica. Journal of the Serbian Chemical Society. 2011; 76(5):709-717.
6. Trease GE, Evans MC. Textbook of Pharmacognosy. Edn 12, Balliere Tindal, London, 1983.
7. Jahan N, Ahmed W, Malik A. New steroidal glycosides from Mimusops elengi. Journal of Natural Products. 1995; 8(8):1244-1247.
8. Sahu NP, Koike K, Jia Z, Nikaido T. Triterpenoid saponins from Mimusops elengi. Phytochemistry 1997; 44(6):1145-9.
9. Halliwell B. Free Radicals, Antioxidants, and Human Disease: Curiosity, Cause, or Consequence? Lancet 1994; 344:721-724.
10. Cao G, Sofic E, Prior L. Antioxidant and pro-oxidant behavior of Flavonoids, structure activity relationship. Free Radical Biology and Medicine 1997; 91:84-1.
11. Brissot P, Cillard P, Cillard J. Antioxidant and iron chelating activities of flavonoids catechin, quercetin, and diosmetin on iron loaded rat hepatocyte cultures. Biochemical Pharmacology. 1993; 45:13-19.
12. Bailey LH, Bailey EZ. The staff of the Liberty Hyde Bailey Hortorium. 1976. Hortus third: A concise dictionary of plants cultivated in the United States and Canada. Macmillan, New York. Brock. J Top End Native Plants, 1988.
13. Kirtikar KR, Basu BD. Indian Medicinal Plants, 2nd ed. Oriental Enterprises, Uttranchal, India. 2001; IX:3068-3069.
14. Jahan N, Ahmed W, Malik A. New steroidal glycosides from Mimusops elengi. Journal of Natural Product's. 1995; 8(8):1244-1247.
15. Mishra G, Mitra CR. Constituents of fruit and seed of Mimusops elengi. Phytochem 1967; 6:453.
16. Mishra G, Mitra CR. Constituents of bark of Mimusops elengi linn. Phytochem 1967; 6:1909.
17. Saxena VK, Shrivastava K. New steroidal saponins from the roots of Mimusops elengi. Fitoterapia 1988; 59(5):418.
18. Mishra G, Mitra CR. Constituents of leaves, heartwood and root of Mimusops elengi linn. Phytochem 1968; 7:501502.
19. Anonymous. The Wealth of India, Raw materials. J-Q. NISCIR, New Delhi, 2003, IV:134-135.
20. Nadkarni KM, Nadkarni AK. Indian Materia Medica. 3rd revised and enlarged edition. Popular Prakashan Private Limited, Mumbai, 2009; I:800-801.
21. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E1. Analytical Biochemistry. 1999; 269:337.
22. Sahu NP, Koike K, Jia Z, Nikaido T. Novel triterpenoid saponins from Mimusops elengi. Tetrahedron 1995; 51(48):13435-13446.