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Antioxidant Activity of Various Leaf Extracts of *Mimusops elengi* L.

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ABSTRACT

Mimusops elengi L. is an Indian native plant and is used for a long time in the history of medicine. An investigation was carried out to analyze the antioxidant and free radical scavenging activity of leaves of *Mimusops elengi* L. Healthy and disease free leaves of *Mimusops elengi* L. were collected from Avinashilingam University Campus during the months of August – September 2014. The plant material was identified and authenticated by Department of Botany, Avinashilingam University, Coimbatore-43, Tamilnadu, India. Both enzymatic and non-enzymatic antioxidant assays conducted to evaluate the antioxidant properties of *Mimusops elengi* L. In the case of catalase, peroxidase and polyphenol oxidase the maximum result was shown by chloroform leaf extract. Maximum content of non-enzymatic antioxidants like tannin, α- tocopherol and ascorbic acid oxidase showed by chloroform leaf extract.

INTRODUCTION

Nature has been a source of medicinal agents since time immemorial. The importance of herbs in the management of human ailments cannot be overemphasized. It is clear, that the plant kingdom harbours an inexhaustible source of active ingredients, invaluable in the management of many intractable diseases (Parekh and Chanda, 2006). Potential sources of natural antioxidant have been searched in different types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices and herbs (Sultana *et al*., 2007). Phenolic compounds are commonly found in plants and they have been reported to have multiple biological effects, including antioxidant activity. Many studies had revealed that phenolic content in plants could be correlated to their antioxidant activities. Plants contain phenolic and polyphenol compounds which can act as antioxidants (Fidrianny *et al*., 2013). Hence the present investigation was undertaken with the main objective of screening the plant, *Mimusops elengi* L. (Sapotaceae) for its antioxidant activities.

Mimusops elengi L. is an Indian native plant and is used for a long time in the history of medicine. It is commonly known as bakul in Hindi, magizamaram in Tamil. English common names include Spanish cherry, medlar and bullet wood. Bullet wood is an evergreen tree reaching a height of about 16 m. The tree may reach up to a height of 9–18 m with about 1 m in circumference. Bark is thick and appears dark brownish black or grayish black in colour, with striations and a few cracks on the surface. Leaves are glossy, dark green, oval-shaped, 5–14 cm long and 2.5–6 cm wide. Flowers are creamy, hairy and scented.

MATERIALS AND METHODS

Collection of plant sample

Healthy and disease free leaves of *Mimusops elengi* L. were collected from Avinashilingam University Campus during the months of August – September 2014. The plant material was identified and authenticated by Department of Botany, Avinashilingam University, Coimbatore-43, Tamilnadu, India.

Extraction of plant material

The leaves were cleaned, dried in the shade and pulverized in a mechanical grinder, passed through a 40 mesh sieve and stored in an air tight container. Powdered dried samples (30g) were successively extracted with petroleum ether (300 ml), chloroform (300ml) and distilled water (300ml) by using Soxhlet apparatus until the decolourisation of the solvents.

Estimation of Catalase

The estimation of catalse was done according to the method described by Luck, 1947. Each sample was homogenized in a blender with M / 150 phosphate buffer (assay buffer diluted 10 times) at 4˚C and centrifuged. Stirred the sediment with cold phosphate buffer, allowed to stand in the cold with occasional shaking and then repeated the extraction once. The extraction should not take greater than 24 hours. The combined supernatants were used for the assay. Read against a control cuvette containing the enzyme solution as in the experimental cuvette, but containing H_2O_2 free phosphate buffer (M /150). Pipetted out 3 ml of H_2O_2 phosphate buffer into the experimental cuvette and added 0.01-0.04 of the sample and mixed with a glass or plastic rod flattened at one end. Noted the time (Δt) required for a decrease in absorption from 0.45 to 0.40. The value was used for the calculations. If 't' is more than 60 sec. The measurements have to be repeated with a more concentrated solution of the samples.

Estimation of Peroxidase

The estimation of peroxidase was carried out with the method described by Reddy *et al*., 1995. One part of each sample was macerated with five parts (W / V) of 0.1 M phosphate buffer (pH 6.5) in a homogenate at 500 rpm for 15 min and used the supernatants as the enzyme source. All procedures were carried out at 5˚C. 3 ml of 0.05 M pyrogallol solution and 0.02 ml of enzyme extract were pipetted out in a test tube and adjusted the spectrophotometer to read '0' at 430 nm. Then 0.5 ml of 1% H_2O_2 was added in the cuvette and recorded the change in absorbance for every 30 seconds up to 3 minutes.

Determination of Polyphenol oxidase (Esterbauer et al., 1977)

Take 0.5 ml of the plant sample and made up to 20 ml with the medium containing Tris-HCl, sorbitol and NaCl. Then, the homogenate was centrifuge at 2000 rpm for 10 minutes at 4°C. The supernatant was used for the assay. Phosphate buffer (2.5 ml) and catechol solution (0.3 ml) were

added into a cuvette and the spectrophotometer was set at 495 nm. The enzyme extract (0.2 ml) was added and the change in absorbance was recorded for every 30 seconds up to 5 minutes. One unit of catechol oxidase or laccase is defined as the amount of enzyme that transforms one µmole of dihydrophenol to one µmole of quinine/minute.

Non – enzymatic antioxidants

Estimation of Tannin (Folin and Ciocalteau Method, 1927)

The tannins were determined by Folin and Ciocalteau method. 0.1 ml of the sample extract was added with 7.5 ml of distilled water, 0.5 ml of folin- ciocalteau reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes. An absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of tannic acid is treated in the same manner as described earlier and read against a blank.

Estimation of α-Tocopherol (Emmeric Method as described by Rosenburg, 1992)

2.5 g of the homogenized sample was weighed accurately into a conical flask. Added 50 ml of 0.1 N sulphuric acid slowly without shaking. Stoppered and allowed to stand overnight. The next day contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper discarding the initial 10-15 ml of filtrate. Aliquots of the filtrate were used for the estimation. In to three stoppered centrifuged tubes (three leaf samples, standard and blank) pippetted out 1.5 ml of each leaf extract, 1.5 ml of standard and 1.5 ml of distilled water respectively. To the three leaf samples and blank 1.5 ml of ethanol, 1.5 ml of water and 1.5 ml of xylene were added, mixed well and centrifuged. Transferred 1.0 ml of xylene layer into another stoppered tube, taking care not to include any ethanol or protein. Pipetted out 1.5 ml of the mixture into the spectrophotometer cuvette and read extinction co-efficient of the test and standard against the blank at 460 nm. This in turn, beginning with the blank, added 0.33 ml of ferric chloride solution, mixed well and after exactly 15 minutes, test and standard against the blank at 520 nm were read.

Assay of Ascorbic acid (Roe and Keuther, 1943)

For the estimation of ascorbic acid, 1 g of the different parts of sample were homogenized by 4% of TCA, (Trichloro Acetic Acid) after centrifugation a pinch of activated charcoal was added, mixed vigorously using cyclo mixer and kept for 5 minutes. The tubes were centrifuged again to pellet the charcoal particles. To 0.5 ml of charcoal treated supernatant, 2.0 ml of 4% TCA (Trichloro Acetic Acid) and 0.5 ml of Di Nitro Phenyl Hydrazine were added followed by 2 drops of thiourea solution and mixed well. The tubes were incubated for 3 hours, removed, placed in ice cold water and added 2.5 ml of 85% H_2SO_4 drop by drop and the absorbance were recorded at 540 nm.

RESULTS AND DISCUSSION

The different solvent extracts of *Mimusops elengi* L. leaf (chloroform, methanol and aqueous) were taken for the analysis of enzymatic and non enzymatic antioxidants which are depicted in Table 1.

A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic potential. Natural antioxidants may function as reducing agents, free radical scavengers, complexes of pro-oxidant metals and quenchers of singlet oxygen. They can be used in the food industry and there is evidence that they may exert antioxidant effects within the human body (El-Agbar, 2008).

Enzymatic antioxidants of leaf extracts of *Mimusops elengi* L.

Table1.Estimation of enzymatic antioxidants of leaf extracts of *Mimusops elengi* L.

Solvents	**Catalase** (@**Units** - g tissue)	**Peroxidase** (#**Units** - g tissue)	**Polyphenol Oxidase** (□**Units**-g tissue)
Petroleum ether	1.019 ± 0.002	1.21 ± 0.07	0.164 ± 0.005
Chloroform	1.023 ± 0.004	1.32 ± 0.05	0.167 ± 0.006
Aqueous	1.020 ± 0.001	1.18 ± 0.02	0.160 ± 0.004
SEd CD (p<0.05)	0.0018 0.0043	0.0357 0.0621	0.0043 0.0105

@Unit = Amount of enzyme required to decrease the optical density 0.5 units at 230 and 250 nm.

[#]Unit = Change of Absorbance/minute at 430 nm

[□]Unit = Change in OD of 0.01/minute

In the case of peroxidase the result was higher in chloroform extract (1.32 ± 0.005 $^{\#}unit^{-g\ tissue}$) but lower in aqueous extract (1.18 ± 0.02 $^{\#}unit^{-g\ tissue}$) Table 5 and Figure 4. The activity of polyphenol oxidase in various extracts of *Mimusops elengi* L. is represented in Table 1, which showed significant activity in chloroform extract (0.167 ± 0.006 $^{\square}Unit^{-g\ tissue}$).

Studies on antioxidant enzymes revealed that *Hyptis suaveolens* extracts treated animals showed significant increase in the levels of superoxide dismutase and catalase, the two powerful antioxidant enzymes of the body that are known to quench superoxide radicals (Shiruaikar *et al*., 2003). Premkumar *et al*. (2010) have reported the antioxidant potential of *Andrographis* and *Boerhaavia* sp and confirmed that the ethanol extract of both the plants showed significant enzymatic and non enzymatic antioxidants.

Non enzymatic antioxidants of leaf extracts of *Mimusops elengi* L.

Non enzymatic antioxidant activity of leaf is presented in Table 2. In the case of tannin the result was higher in chloroform extract (0.555 ± 0.003 mg^{-gTAE}) but lower in aqueous extract (0.446 ± 0.001 mg^{-gTAE}). The maximum α-tocopherol content was present in aqueous extract (0.626 ± 0.064 $mg^{-g\ tissue}$) but lower in chloroform extract (0.620 ± 0.069 $mg^{-g\ tissue}$). Tannin is an important antioxidant that is found to detoxify toxic substance by conjugation (Peklak *et al*., 2005).

Table 2. Estimation of non enzymatic antioxidants of leaf extracts of *Mimusops elengi* L.

Solvents	**Tannin** mg^{-gTAE}	**α-tocopherol** **mg/g tissue**	**Ascorbic acid Oxidase** **mg/g tissue**
Petroleum ether	0.548 ± 0.01	0.622 ± 0.070	1.793 ± 0.042
Chloroform	0.555 ± 0.03	0.620 ± 0.069	1.829 ± 0.043
Aqueous	0.446 ± 0.01	0.626 ± 0.064	1.724 ± 0.041
SEd CD ($p<0.05$)	0.0018 0.0043	0.0573 0.1402	0.0310 0.0758

TAE - Tannic Acid Equivalent per gm

It is evident that the ascorbic acid was maximum in chloroform extract (1.829 ± 0.043 $mg^{-g\ tissue}$) but minimum in aqueous extract (1.724 ± 0.041 $mg^{-g\ tissue}$).

Zongo *et al*. (2010) evaluated the antioxidant activities of aqueous and acetone extracts from the rhizome of *Ampelocissus grantii*, where the acetone extracts exhibited the higher level of total polyphenol content when compared to aqueous extract. Krishnaiah *et al.* (2009) supported the present study and validates the importance of non-enzymatic antioxidant like reduced glutathione, ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E), to be good source of antioxidants.

REFERENCES

1. El-Agbar, Z.A., Ashok K. Shakya, Nooman A. Khalaf and Makbula Al-Haroon, (2008). Comparative antioxidant activity of some edible plants, Turk J Biol., 32: 193-196.
2. Esterbauer, H., E. Schwarzyl and M. Hayn, (1977). A rapid assay for catechol and laccase using 2-nitro-5-thio-benzoic acid, Anal. Biochem., 77 (1 & 2): 486-497.
3. Folin, C. and V. Ciocalteu, (1927). Tyrosine and tryptophan determination in protein J. Biol. Chem., 73: 627-650.
4. Fidrianny, I., Ira Rahmiyani, Komar Ruslan Wirasutisna, (2013). Antioxidant capacities from various leaves extracts of four varieties mangoes using DPPH, ABTS assays and correlation with total phenolic, flavonoid, carotenoid, International Journal of Pharmacy and Pharmaceutical Sciences, 5 (4): 189-194.
5. Krishnaiah, D., T. Devi, A. Bono and R. Sarbatly, (2009). Antioxidant properties and stability of ethanolic extracts of holy basil, J. Med. Plants Res., 3 (2): 67-72.
6. Premkumar, P., J. Priya and M. Suriyavathan, (2010). Evalution of antioxidant potential of *Andrographis echioides* and *Boerhaavia diffusa*, International Journal of Current Research, 3: 59-62.
7. Parekh, J. and S.V. Chanda, (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants, Turk J Biol., 31: 53-58.
8. Peklak, S.C., A.J. Townsena and C.S. Morrow, (2005). Dynamics of glutathione conjugation and conjugate efflux in detoxification of the carcinogen, 4-nitroquinoline l-oxide, contributions of glutathione, glutathione transferase and MRPI, Biochem., 44: 4426-4433.
9. Roe, J.H. and C.E. Keuther,(1953). The determination of ascorbic acid in whole blood and wine through 2,4-dinitrophenyl hydrazine derivative of dehydro ascorbic acid, J. Biol. Chem., 147 (5): 399-405.
10. Rosenberg, H.R.,(1992). Chemistry and physiology of the vitamins, Interscience, Publishers Inc., 5th Edition New York, PP. 452-543.
11. Sultana, B., Farooq Anwar and Roman Przybylski, (2007). Antioxidant activity of phenolic components present in barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica* and *Eugenia jambolana* Lam. Trees, Food Chemistry 104: 1106-1114.
12. Shiruaikar, A., S. Ghosh and P.G.M. Rao, (2003). Effect of *Gmelina arborea* Roxb leaves wound healing in rats, Journal of Natural Remedies, 15 (2): 43-48.
13. Zongo, C., A. Savadogo, L. Quathara, I.H.N. Bassole, C.A.T. Qualtara, A.S. Qualtara, N. Barro, J. Koudou and A.S. Traore,(2010). Polyphenol content, antioxidant activity of *Ampelocissus granti* Baker, Int. J. Pharmacol., 5 (6): 880 - 887.