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In vitro antioxidant activity of *Meyna laxiflora* seeds

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ABSTRACT

Antioxidants in normal diet and practice of using it in daily life can reduce the chances of various aliments like diabetes, cancer, cardiovascular diseases, aging, gastrointestinal diseases, arthritis etc. Various studies have been done to identify antioxidants from plant sources and efforts have been taken to incorporate in conventional therapy. In our present study, methanol extract of the seeds of the plant *Meyna laxiflora* has been evaluated for *in vitro* antioxidant activity by using three different methods. The methanol extract is found to possess free radical scavenging property in concentration dependent manner. The IC_{50} values were determined and are found to be 84.2±2.1, 91.0±3.0, and 104.5±3.4 µg/ml for DPPH, H_2O_2, and NO radical scavenging method respectively. This observation indicates the presence of free radical scavenging potential by the methanol extract of *Meyna laxiflora* seeds.

Keywords: *Meyna laxiflora*, antioxidant activity, methanol, seeds.

1. INTRODUCTION

Meyna laxiflora Robyns. family Rubiaceae commonly known as manakkarai (Tamil), muyna, muduna (Hindi, Bengali) and gobergally (Kannada) is a spinescent or unarmed shrub or a small tree found in Western UP, West Bengal, North-east India and Deccan peninsula. Leaves are ovate to elliptic; flowers in lax cymes, greenish white; drupes are subglobose, green to brown. Seeds are albuminous with a membranous testa. Different parts of the plant were used in the treatment of boils, dysentery, diphtheria etc.[1-2] Antioxidant activity of the *Meyna laxiflora* fruit pulp has already been reported.[3]

Free radicals are generated as part of the body's normal metabolic process and play a dual role in our body as both deleterious and beneficial species. Excess production of reactive oxygen species (ROS) and/or a decrease in antioxidant levels, this may leads tissue damage and causes different diseases.[4] Antioxidant plays a major role to protect our body from disease by reducing the oxidative damage to cellular component caused by ROS.[5] Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis and aging process. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, relatively plant based medicine confer less side effect than the synthetic drug.[6] Several investigations showed that seeds of various fruits are an important source of natural antioxidant.[7]

In view of the above observation our interest was to find out natural antioxidant

from seeds of *Meyna laxiflora* using *in vitro* antioxidant activity.

2. MATERIALS AND METHODS

2.1. Plant material

The seeds of *Meyna laxiflora* were collected from the dried ripe fruits from Jampui Hill located in North Tripura district, Tripura and the plant was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, West Tambaram, Chennai. The voucher specimen was preserved in Department of Pharmacognosy, CES College of Pharmacy for future reference.

2.2. Chemicals

The standards (curcumin, α-tocopherol and ascorbic acid) were procured from Natural Remedies, Bangalore. Chemicals like 1,1-diphenyl-2-picryl hydrazyl (DPPH), hydrogen peroxide (H_2O_2), sodium nitroprusside, sulfanilic acid, naphthylethylenediamine dihydrochloride, glacial acetic acid were obtained from Hi-Media Laboratory Ltd, Mumbai. All other solvents and chemicals used were of analytical grade.

2.3. Preparation of the extract

Dried seeds of *Meyna laxiflora* were crushed into a coarse powder using mechanical grinder. Weighed quantity of about 250 g powder was charged in Soxhlet apparatus and extracted using methanol for 18 h. Extract obtained was concentrated in vacuum under reduced pressure using rotary flash evaporator. Methanol extract of *Meyna laxiflora* seeds (MEML) was further concentrated and dried in the desiccator for further studies.

2.5. Preliminary phytochemical screening

Phytochemical screening of methanol extract of *Meyna laxiflora* seeds were performed for the presence of carbohydrates, glycosides, alkaloids, steroids, tannins, saponins, terpenoids, gums and mucilage.[8]

2.6. Antioxidant study

2.6.1. DPPH radical scavenging activity

DPPH radical scavenging activity was determined as per following method.[9] Briefly, 1.0 ml of 0.3 mM DPPH ethanol solution was added to 2.5 ml of sample solution of various concentrations of methanol extract of *Meyna laxiflora* and allowed to react at room temperature for 30 min. Absorbance were measured at 517 nm. Ethanol (1.0 ml) with 2.5 ml of MEML solution was used as blank and DPPH solution (0.3 mM, 1.0 ml) with 2.5 ml of ethanol served as negative control. Ascorbic acid was used as standard.

2.6.2. Hydrogen peroxide scavenging activity

Different concentrations of MEML solution mixed with 0.6 ml of hydrogen peroxide solution. Hydrogen peroxide solution (2.0 mmol/l) was prepared with standard phosphate buffer (pH 7.4). Absorbance of mixture was determined spectrophotometrically at 230 nm after 10 min using a blank solution containing phosphate buffer without H_2O_2, α-tocopherol were used as standard.[9]

2.6.3. Nitric oxide scavenging activity

Various concentrations of the extract (0.5 ml) was mixed with 2.0 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) and the mixture was incubated at 25°C for 150 min. Incubated mixture (0.5 ml) was taken out and mixed with 1.0 ml of sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml of naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. Curcumin used as positive control or standard. Absorbance was measured at 540 nm using spectrophotometer and nitric oxide (NO) radical scavenging activity was calculated.[6]

2.7. Determination of IC_{50} values

Concentration of methanol extract required to scavenge 50% of the radical was calculated by using the percentage scavenging activities at different

concentrations. Percentage inhibition was calculated using the formula,

Percentage inhibition = (Ac-As) × 100/Ac

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

3. RESULTS AND DISCUSSION

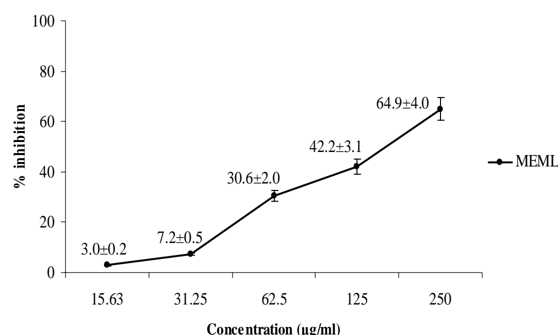
Preliminary phytochemical studies revealed the presence of alkaloids, saponins, glycosides, steroids and carbohydrates in methanol extract.

MEML produced *in vitro* antioxidant activity observed by DPPH radical, H_2O_2 and nitric oxide scavenging activity methods. DPPH radical scavenging activity of MEML is presented in Figure1. The DPPH antioxidant assay is based on the reduction of DPPH, a stable free radical contains a free odd electron. Any molecule or antioxidant compound that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption, which can be quantitatively measured from the changes in absorbance at 517 nm.[10,11] IC_{50} value of MEML in DPPH radical scavenging activity method was found to be 84.2±2.1 µg/ml.

Activity of MEML on hydroxyl radical has been shown in Fig. 2. Hydrogen peroxide itself not very reactive but can cause cytotoxic effect by giving rise to hydroxyl radical in cells. Hydroxyl radical is highly reactive oxygen centered radical attacks proteins, DNA, polyunsaturated fatty acid in membranes results death of cell.[6,12] MEML exhibited concentration dependent scavenging activity against hydroxyl radical and IC_{50} value was found to be 91.0±3.0 µg/ml.

Nitric oxide is a potent pleiotropic mediator of different physiological process and plays a vital role in smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical, important as an effectors molecule in different biological systems including neuronal messenger, vasodilatation and antimicrobial and anti-tumor activities. But over production of the radical is responsible for pathogenesis of different inflammatory diseases. MEML produced concentration dependent inhibition of NO radical (Fig. 3). IC_{50} value of MEML was found to be 104.5±3.4 µg/ml.

Fig. 1: Effect of MEML on DPPH radical scavenging activity



It is suggested that ROS may contribute to various pathophysiological conditions and endogenous defense mechanisms. The plants may be considered as potential sources of natural antioxidants for medicinal uses against different diseases related to radical mechanisms. Therefore antioxidant potential of the MEML may due to presence of different phytochemicals in the methanol extract. Moreover a detailed work needs to carry out to isolate active constituents responsible for antioxidant activity.

Fig. 2: Effect of MEML on hydrogen peroxide scavenging activity

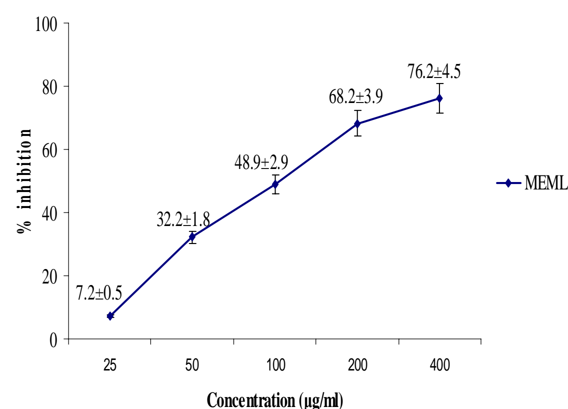
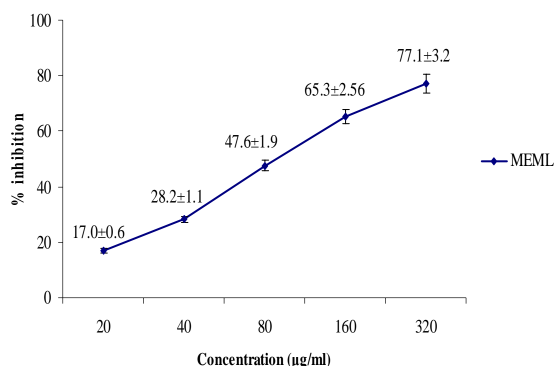


Fig. 3: Effect of MEML on nitrous oxide scavenging activity



4. CONCLUSION

In summary, the plant may be considered as good source of natural antioxidants for medicinal uses related to radical mechanisms. Further investigation on the isolation and identification of antioxidant constituent(s) in the plant probably lead to chemical moiety with potential for therapeutic use. The isolation of bioactive constituents in the extract could certainly help to find out the individual potency of the compound and possibly the exact mechanism of action.

5. REFERENCES

1. Anonymous. **The Wealth of India, Raw materials**, Vol VI (L-M). Council of Scientific and Industrial Research, New Delhi, 1992; 353-354.
2. Kashyapa K and Chand R. **The Useful Plants of India**. National Institute of Science Communication and Information Resources, New Delhi, 2006; 369.
3. Haripyaree A, Guneshwor K and Damayanti M. Evaluation of antioxidant properties of some wild edible fruit extracts by cell free assays. **Electronic Journal of Environmental, Agriculture and Food Chemistry**. 2010; 9: 345-350.
4. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M and Telser J. Free radicals and antioxidants in normal physiological functions and human disease. **The International Journal of Biochemistry & Cell Biology**. 2007; 39: 44–84.
5. Huda AWN, Munira MAS, Fitrya SD and Salmah M. Antioxidant activity of *Aquilaria malaccensis* (Thymelaeaceae) leaves. **Pharmacognosy Research**. 2009; 1: 270-273.
6. Nagulendran KR, Velavan S, Mahesh R and Begum VH. *In vitro* antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. **E-Journal of Chemistry**. 2007; 4: 440-449.
7. Moure A, Cruz, JM, Franco D, Dominguez JM, Sineiro J, Dominguez H, Nunez MJ and Parajo JC. Natural antioxidants from residual sources. **Food Chemistry**. 2001; 72: 145-171.
8. Trease GE and Evans MC. **Pharmacognosy**, 12th ed. Balliere-Tindal, London, 2002; 174-393.
9. Asokkumar K, Umamaheswari M, Bahrudeen A, Sivashanmugam AT, Subhadhradevi V and Ravi TK. Antioxidant and hepatoprotective activities of the fraction of *Ficus microcarpa* using *in vivo* and *ex vivo* models. **Functional Plant Science and Biotechnology**. 2010; 4: 17-27.
10. Zahin, M. Aqil F and Ahmad I. The *in vitro* antioxidant activity and total phenolic content of four Indian medicinal plants. **International Journal of Pharmacy and Pharmaceutical Sciences.** 2009; 1: 88-95.
11. Ara N and Nur H. *In vitro* antioxidant activity of methanolic leaves and flowers extracts of *Lippia alba*. **Research Journal of Medicine and Medical Sciences**. 2009; 4: 107-110.
12. Bhujbal SS, Kewatkar SMK, More LS and Patil MJ. Bhujbal SS. Antioxidant effects of roots of *Clerodendrum serratum* Linn. **Pharmacognosy Research**. 2009; 11: 294-298.