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In vitro anti oxidant activity of ethanol extract of *myxopyrum serratulum a.w.hill*

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ABSTRACT

Objectives: Free radical induced oxidative stress is involved in the pathogenesis of various diseases and disorders. Antioxidants play an important role against this oxidative stress to protect our body. The present study was carried out to evaluate the in vitro antioxidant properties ofethanolic extract of *myxopyrum serratulum* (EEMS).

Methods:EEMS was assayed ondifferent in vitro free radical models like DPPH, suproxide radical scavenging assay, hydroxyl scavenging assay.

Result: The extract showed good antioxidant activity in in-vitro free radical scavenging models. Various concentrations ranging from 200 µg/ml and 400 µg/ml of ethanol extract of the leaves of *M. serratulum* were tested for their antioxidant activity in various in-vitro models. Free radicals were scavenged by the test compounds in a concentration dependent manner within the given range of concentration (IC50) in the DPPH (36.30 ± 1.47%), superoxide scavenging activity (55.30 ± 1.29%), and hydroxide scavenging activity (43.70 ± 2.43%).

Conclusion: All the results of the in vitro antioxidant assays revealed potent antioxidant and free radical scavenging activity of the ethanol extract of *myxopyrum serratulum a.w.hill*, equivalent to that of standard quercetin and this antioxidant property may be attributed to its high phenolic and flavonoid contents.

Keyword: Antioxidant, *myxopyrum serratulum*, superoxide scavenging activity, ascorbic acid

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. The freeradicals start attacking the cell proteins, lipids and carbohydratesand this leads to a number of physiological disorders. Free radicals are involved in the development of degenerative diseases. They have also been implicated in the pathogenesis of diabetes, liver damage, inflammation, cancer, cardiovascular disorders, neurological disorder, and in the process of aging. [1]Phytochemicals in medicinal plants have received a great deal of attention mainly on their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen and various radicals as a damaging side-effect of aerobic metabolism. Antioxidant is a molecule which terminates the chain reaction by removing free radical intermediates. Plants and animals maintain complex system of multiple type of antioxidant. The natural plant based antioxidants have played an important role in the maintainance of human health for the past three decades.[2]

Myxopyrum serratulum A.W.Hill is one of the medicinally valuable plants belonging to the family Oleaceae. Traditionally leaves are used as astringent, acrid, cough, asthma, rheumatism, cephalalgia, nostalgia, consumption, fever, otopathy, neuropathy and cuts and wounds. Iridoid glycosides were found on the leaves of *Myxopyrum smilacifolium*.[3]

The present study has been directed to investigate the antioxidant activity of the ethanolic extract of the leaves of *Myxopyrum serratulum A.W.Hill.* in different *in vitro* and *in vivo* models.[4]

MATERIALS AND METHODS

Collection of plant material and authentication

The plant was collected in the month of September from Trivandrum, Kerala, India and was identified by Dr. V.Chelladurai, Research Officer (Botany). Central Council of Research in Ayurveda and Siddha, Government Siddha Medical College, Palayamkottai, Tamilnadu, India. A voucher specimen (MSU/PHAR/HER-139) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli - 627 012. [5]

Extraction of plant material

The leaves of *Myxopyrum serratulum* were dried under shade and powdered. The dried powder (500 g) was successively extracted using petroleum ether (40º-60°C) ethanol by using a Soxhlet apparatus. The last trace of the solvent was removed under reduced pressure by rotary evaporator. The dried crude ethanol extract has been used for the study.

Animals

Wistar albino rats of either sex weighing between 180 g and 200 g were selected for the acute toxicity and antioxidant activity studies. The study was approved by the Institutional Ethics Committee for animal experimentation (KMCRET/PH.D/17/2012-13), KMCH college of Pharmacy, Coimbatore. The animals were stabilized for 1 week. They were maintained in standard conditions at room temperature, 60 ± 5% relative humidity and 12 hours light dark cycle. They were given standard pellet diet supplied by Hindustan Lever Co., Mumbai and water *ad libitum* throughout the course of the study.

Drugs and chemicals

Silymarin (Silybin 140) was purchased from Microlabs Limited, Goa and Thioacetamide from Lova Laboratories Pvt. Ltd., Mumbai. All others chemicals used in this study were of analytical grade.

PROCEDURES

Antioxidant activity studies

In-Vivo Biochemical Parameter Models

Rats were randomly divided into four groups of six animals each and each group was kept in a separate cage. All the groups were treated orally for 7 days.

Group I served as control and was treated with vehicle (0.5% carboxyl methyl cellulose). Group II served as standard and was treated with silymarin (25 mg/kg bw). Group III was treated with ethanol extract of the leaves of *M. serratulum* (200 mg/kg bw). Group IV was treated with ethanol extract of the leaves of *M. serratulum* (400 mg/kg bw).

On the 8th day, the rats were sacrificed for the determination of antioxidant enzymes. Liver was dissected out, washed in the ice-cold saline, and the liver homogenate was prepared in 0.1 M Tris-HCl buffer (pH

7.4). The homogenate was centrifuged and supernatant was used for the assay of antioxidant enzymes, namely superoxide dismutase, catalase (CAT), and lipid peroxidation (LPO).

In-vitro free radical scavenging models

Determination of DPPH radical scavenging activity

The capacity of the samples in scavenging 1,1 diphenyl-2-picryl hydrazyl radical (DPPH) was measured according to the method of Yamaguchi *et al*. The stock solutions (10 mg/ml) were diluted with 70% ethanol into different concentrations, 200 µg/ml, 400 µg/ml. The wells in a 96 well plate were pipetted with 25 µl of sample solutions and 171 µl of 0.3 mM DPPH solution, and then left to stand at room temperature for 30 min in the dark before the absorbance was measured at 517 nm. The inhibition percentage (%) of radical scavenging activity was calculated according to the following equation:-

$$\textbf{Inhibition (\%)} = [(A_o - A_s) / A_o] \times 100$$

Where A_o is the absorbance of the control and A_S is the absorbance of the sample at 517 nm.IC_{50} value was the attentive concentration at which 50% of DPPH radicals were scavenged and obtained by interpolation from linear regression analysis. A lower IC_{50} value indicated a greater antioxidant activity.[5]

Determination of superoxide radical scavenging activity

Superoxide scavenging was determined by the nitro blue tetrazolium reduction method. The reaction mixture consisted of ethylene diamine tetra aceticacid (EDTA, 6 µm), sodium cyanide (µg), riboflavin (2 µm), nitro blue tetrazolium (50 µm), various concentrations of ethanolic extract of leaves of *M. serratulum* (200 µg/ml, 400 µg/ml) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent visible light for 15 minutes, and the optical density was measured at 530 nm before and after the illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and experimental tubes.[6]

Determination of hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to a modified method of Yamaguchi *et al*Stock solutions of EDTA (1mM), $FeCl_3$ (10mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxy ribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, 0.36 ml of deoxy ribose, 1.0 ml of *Myxopyrum serratulum* ethanolic extract (200 µg/ml, 400 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37ºC for1 hour. A 1.0 ml portion of the incubated mixture was mixed with1.0 ml of 10 g/100g TCA and 1.0 ml of 0.5g/100g TBA (in 0.025M NaOH containing 0.025 g/100g TBA) to develop the pink chromogen and measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as percentage inhibition of deoxyribose degradation.[7]

RESULTS

DPPH radical scavenging activity

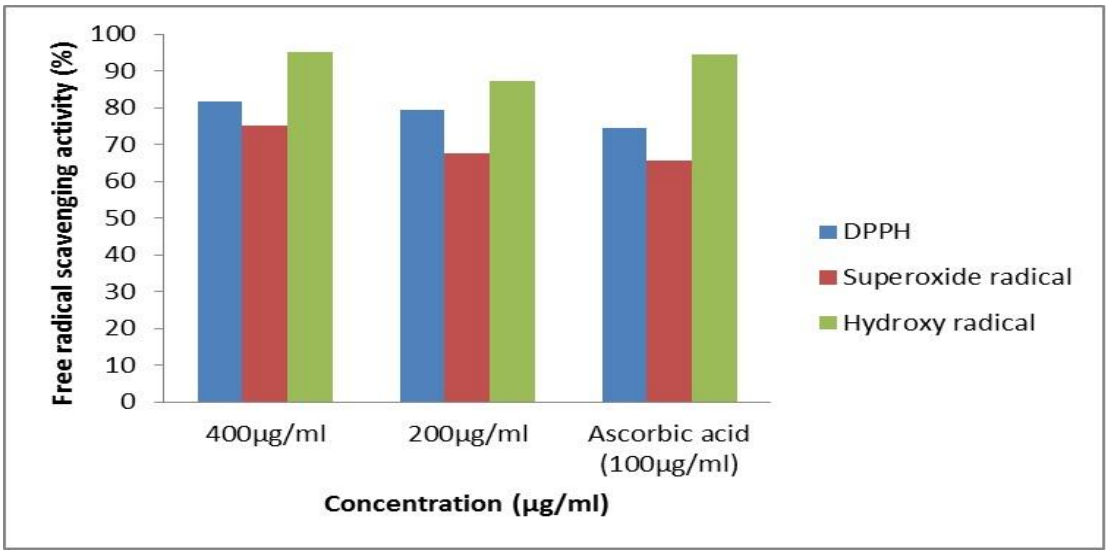

Fig 1: Free radical scavenging activity of different concentration of theethanolic extract of the leaves of *M. serratulum.* and the reference antioxidant, ascorbic acid.

Table 1:Free radical scavenging activities of ethanolic extract of leaves of *Myxopyrum serratulum A.W.Hill.*

Extract Concentration (µg/ml)	Inhibition (%)		
	DPPH	Superoxide	Hydroxyl radical
400 µg/ml	81.67 ± 2.34	79.41 ± 3.65	74.41 ± 4.43
200 µg/ml	75.22 ± 3.74	67.51 ± 2.78	65.52 ± 2.76
Ascorbic acid (100 µg/ml)	95.11 ± 4.22	87.32 ± 0.87	94.44 ± 4.71
IC50	36.6 ± 1.47	55.3 ± 1.29	43.7 ± 2.43

Table 2: Enzymatic biochemical parameter activities of ethanolic extract of leaves of *Myxopyrum serratulum A.W.Hill.*

Groups	SOD	CAT
Control	9.06 ± 0.06	55.93 ± 1.57
Silymarin (25 µg/ml)	8.65 ± 0.12***	55.31 ± 1.64
200 µg/ml	6.99 ± 0.05***	36.58 ± 1.19***
400 µg/ml	7.26 ± 0.12***	43.10 ± 0.03***

DISCUSSION

The extract showed good antioxidant activity in *in-vitro* free radical scavenging models. Various concentrations ranging from 200 µg/ml and 400 µg/ml of ethanol extract of the leaves of *M. serratulum* were tested for their antioxidant activity in various *in-vitro* models (Table-1). Free radicals were scavenged by the test compounds in a concentration dependent manner within the given range of concentration (IC_{50}) in the DPPH (36.30 ± 1.47%), superoxide scavenging activity (55.30

± 1.29%), and hydroxide scavenging activity (43.70 ± 2.43%). [8], [9]

The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation.[10]

Antioxidant can act by converting the unpaired electrons to paired ones. The dose dependent inhibition of DPPH (Fig. 1) indicates that the ethanolic extract of the leaves of *M. serratulum* causes reduction of DPPH radical. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of *M. serratulum* extract towards generation of superoxide in the *in-vitro* reaction mixture. [11]

Enzymatic antioxidants like, SOD, CAT activities in liver were found significantly reduced ($p<0.001$) when compared to that of the control and the standard. There is a significant increase in activity from lower to higher dose. The dose of 400 µg/ml treated group was found to be effective similar to silymarin treated group(Table-2). The SOD and CAT activities of 400 µg/ml dose were found to be almost the same as that of silymarin.[12]

A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom. The reducing property of *Myxopyrum serratulum* implies that it is capable of donating hydrogen atom in a dose dependent manner. [13]

CONCLUSION

The results obtained thus indicate that the ethanolic extract of the leaves of *Myxopyrum serratulum* has potent antioxidant activity, achieved by scavenging abilities observed against DPPH, superoxide radical and hydroxyl radical. Also the wide use of the plant in the Indian System of Medicine for wound healing may be in part due to the antioxidant potential of the ethanolic extract of the leaves.

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