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Membrane Stabilizing and Antioxidant Activity of *Myrica esculenta* Leaves Extracts

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Abstract: In the present study, different extracts of leaves of *Myrica esculenta* were prepared and evaluated their membrane stabilizing and antioxidant effects. All extract were tested for presence of phytoconstituents i.e., alkaloid, carbohydrate, sterols, proteins, amino acids, saponin, and phenolic compounds. Membrane stabilizing effect was studied by hypotonic solution induced haemolysis of erythrocyte and Antioxidant activity was studied by DPPH method at a different concentration. From the study, Phytochemical analysis showed that methanol extract was the richest extract for the tested phytoconstituents. Maximum membrane stabilizing activity of leaves of *Myrica esculenta* showed in Methanol extract (61.11±1.10) at a concentration of 1000 µg/ml in comparison to standard drug aspirin. From antioxidant studies, methanol extract showed maximum antioxidant activity (91.36±1.27) at a concentration of 1000 µg/ml than other extract and comparison to standard drug ascorbic acid. From above studies it could be concluded that methanol extract showed maximum membrane stabilizing and antioxidant activities.

Keywords: *Myrica esculenta*; erythrocyte membrane stabilization; anti-inflammatory; antioxidant; DPPH; aspirin; ascorbic acid.

INTRODUCTION

Traditional herbal medicine and their preparations have been widely used in developing and developed countries from many years because of their natural origin, lesser side effects and non-toxic in nature [1]. Many herbal drugs from plants origin have been widely used medicinally in different countries [2].

The presence of secondary metabolites in plants is found to be active principles of many drugs [3]. The plant phytoconstituents are the best source of variety of medicinal agents. There is increase in demand of phytoconstituents from all over of world for the treatment of diseases [4].

Inflammatory diseases are increasing all over the world. These are activated in disease condition and it involves complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defence [5].

Oxidation reaction involved in many processes and producing harmful product such as free radicals or reactive oxygen species These free radicals in human being initiates many chain reaction and cause damage to the cell results in problems like asthma, cancer, cardiovascular diseases, liver diseases, muscular degeneration and other inflammatory processes. An anti-oxidant inhibits these chain reactions of free radicals by removing free radicals and inhibits other oxidation agents [6, 7]. Antioxidants are the agent that protects cell damage caused by free radicals by termination of chain reactions of free radicals [8]. There is an increasing interest all over the world for searching medicinal plants for anti-inflammatory and antioxidant activities. Hence, the present study was aimed for exploring the membrane stabilizing and antioxidant activities of *Myrica esculenta* leaves.

MATERIALS AND METHODS

Collection & Identification of leaves of *Myrica esculenta*

Leaves of *Myrica esculenta* were collected from Raxaul, Bihar, India. Plant materials were authenticated by Dr. Vidit Tyagi, Botanist (Dept. of Botany, Dolphin PG Institute of Biomedical & Natural

Sciences, Dehradun). DPPH, aspirin and ascorbic acid were purchased from HIMIDEA, Mumbai, India.

Extraction of Leaves of *Myrica esculenta* in different Solvents

The collected plant material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (100 gm) of *Myrica esculenta.* were crushed. The crushed leaves extracted with different solvent of increasing polarity viz. Petroleum ether, chloroform, acetone and methanol. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure [9].

Phytochemical analysis

All extracts were tested for presence of phytoconstituents i.e., alkaloid, carbohydrate, sterols, proteins, amino acids, saponin test, and phenolic compounds [9].

Membrane Stabilizing activity of leaves extracts
Effect on haemolysis
Erythrocyte suspension

Whole blood was collected from goat from slaughter house and NIH-ACD (National Institute of Health-Acid Citrate Dextrose) solution was added to it to prevent clotting. The blood was centrifuged three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 100 ml of distilled water: $NaH_2PO_4.2H_2O$, 0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g (10 mm sodium phosphate buffer). The isotonic buffer solution was composed of 154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4).

Hypotonic solution-induced haemolysis

Stock erythrocyte suspension (30 µl) was mixed with 5 ml of the hypotonic solution containing the *Myrica esculenta* leaves extracts at concentrations of 500 and 1000 µg/ml, while the control sample was mixed with drug free solution. The mixtures were incubated for 10 min at room temperature, and centrifuged at 3000 g for 10 min. All the experiments were performed in triplicates and the absorbance (O.D.) of the supernatant was measured at 560 nm. Acetyl Salicylic acid (Aspirin) was used as a reference standard of concentration 200 µg/ml [10-11].

Calculation and statistical analysis

The percentage inhibition or acceleration of haemolysis in tests (b) and (c) was calculated according to the equation:

$$\%\ \text{inhibition of haemolysis} = \frac{OD_1 - OD_2}{OD_1} \times 100$$

Where,
OD_1 = Optical density of hypotonic saline solution + blood (control)
OD_2= Optical density of test sample in hypotonic saline solution + blood
Results are expressed as percentage mean values ± standard error (n = 3)

Antioxidant activity of leaves extracts
DPPH method

Weigh accurately 20 mg DPPH and dissolved in 100 ml methanol. Standard solution of ascorbic acid is prepared as 100 µg/ml. Different concentration of the test samples of *Myrica esculenta* leaves extracts which is to be examined for anti-oxidant activity is prepared in their respective solvent viz. 250, 500, 750 and 1000 µg/ml. For the analysis of test samples, 3 ml of different concentration of test sample of *Myrica esculenta* extracts were mixed with 1 ml of DPPH solution in dark. For analysis of Standard drug Ascorbic acid, 3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test samples was incubated for 30 minutes. When Procedure is done; absorbance is taken with the help of U.V. Spectrophotometer at 517 nm.

Calculate the % activity of individual concentration of individual extract from the following formula:- [12-13].

$$\%\ \text{Antioxidant Activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Individual Concentration}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION

The crushed leaves are extracted by different solvents i.e. petroleum ether, chloroform, acetone and methanol by hot percolations method and the yield of leaves extracts of *Myrica esculenta* in different solvent systems are Petroleum ether (2.17 gm), Chloroform (4.20 gm), Acetone (2.39 gm) and Methanol extract (4.66 gm).

The extracts of leaves of *Myrica esculenta* undergo various qualitative phytochemical tests. They showed their presence and absence in the different solvent systems. From the results, we found out that methanol extract was the richest extract for phytoconstituents. It contains all tested phytoconstituents viz. Alkaloids, carbohydrates, phenolic compounds, saponins and proteins and amino acids except sterols. Acetone extract showed presence of alkaloids, carbohydrates, phenolic compounds, proteins and amino acids only. Petroleum ether extract showed only presence of steroid and phenolic compounds. Chloroform extract showed the presence of phenolic compounds only.

Membrane Stabilizing activity

The membrane stability activity of the different extracts of leaves of *Myrica esculenta* was compared with activity of standard drug aspirin at 560 nm. It was observed that the concentration of 1000 µg/ml of methanol extract showed maximum activity of 61.11 % in comparison with other extract and standard drug aspirin (Table-1).

Table-1: Effect of different extract and standard drug on membrane stabilizing activity

Concentration of extracts (µg/ml)	% Membrane Stabilizing activity of extracts & Standard drug					
	Myrica esculenta leaves extracts				Standard Drug	
	Petroleum ether	Chloroform	Acetone	Methanol	Acetyl Salicylic acid	Concentration of Acetyl Salicylic acid (µg/ml)
500	11.55±1.32	10.20±0.65	20.16±1.06	33.90±1.04	58.31±0.62	**200**
1000	14.44±0.44	13.88±1.04	41.28±1.36	61.11±1.10		

Results are expressed as mean values ± standard error (n = 3)

The clinical features of inflammation have been recognised since ancient times as swelling, redness, pain and heat. A normal inflammatory response is essential to fight infections and is part of the repair mechanism and removal of debris following tissue damage. Inflammation can also cause disease, due to damage of healthy tissue. Inflammation response occurs in vascularised tissues in response to injury; it is part of the innate (nonspecific) immune response. Inflammatory responses require activation of leukocytes: neutrophils, eosinophils, basophils, mast cells, monocytes and lymphocytes, although not all cell type need be involved in an inflammatory episode. The cells migrate to the area of tissue damage from the circulation and become activated [14].

The release of lysosomal constituents by inflammation results in damage of cell. Inhibition of lysosomal constituents by the stabilization of lysosomal membrane results in reduction of inflammation. The erythrocyte membrane resembles to lysosomal membrane and as such the effect drug on the stabilization of erythrocyte membrane could be stabilize the lysosomal membrane [15]. Stabilization of RBC membrane by hypotonic solution induced RBC membrane lysis can be taken as an in vitro measure of anti-inflammatory activity of the drugs or plant extracts.

Antioxidant activity

Methanol extract of leaves of *Myrica esculenta* showed maximum antioxidant activity in comparison to all extracts and standard drug ascorbic acid. The concentration of 1000 µg/ml of methanol extract showed 91.36 % antioxidant activity (Table 2).

Table-2: Effect of different extract and standard drug on antioxidant activity

Concentration of extracts (µg/ml)	% Antioxidant Activity of extracts & Standard drug					
	Myrica esculenta leaves extracts				Standard Drug	
	Petroleum ether	Chloroform	Acetone	Methanol	Ascorbic Acid	Concentration of Ascorbic Acid (µg/ml)
250	19.38±0.96	20.81±0.93	23.20±1.11	62.72±1.2	96.50±0.19	**100**
500	37±1.16	47.54±1.62	44.45±1.31	85.68±1.32		
750	43.18±0.84	53.33±1.05	57.66±1.77	89.70±1.37		
1000	59.15±2.05	64.54±1.30	69.01±0.98	91.36±1.27		

Results are expressed as mean values ± standard error (n = 3)

The free radical scavenging activity by DPPH is based on the addition of radical species and antioxidants which effect the change in colour of DPPH solution. The change in colour of the DPPH solution depends upon the concentration and potency of antioxidants. The decrease in intensity of colour of DPPH solution indicates antioxidant activity [16].

CONCLUSION

From the above study it is concluded that methanol extract of *Myrica esculenta* leaves showed remarkable membrane stabilizing activity and antioxidant activities. Further study needed for the isolation of active principle.

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