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Membrane Stabilizing and Antioxidant Activities of Leaves Extracts of *Salvadora oleoides* (Decne.)

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ABSTRACT

People are using herbal medicines from centuries for safety, efficacy, cultural acceptability and lesser side effects. Plant and plant products have utilized with varying success to cure and prevent diseases throughout history. Due to side effects of synthetic products, herbal products are gaining popularity in the world market. In the present study different extracts of leaves of *Salvadora oleoides* were prepared and evaluated their membrane stabilizing and antioxidant effects. Evaluation of membrane stabilizing and antioxidant activity by hypotonic solution induced hemolysis and DPPH method respectively. All extract were tested for presence of phytoconstituents i.e., alkaloid, carbohydrate, sterols, proteins, amino acids, saponin, and phenolic compounds in different extracts. From the results, we foundout that total methanol extract of leaves was the richest extract for phytoconstituents. It contains maximum tested phytoconstituents viz. Alkaloids, carbohydrates, phenolic compounds, Sterols and Saponin except Protein and amino acids. n-Butanol fraction of leaves showed maximum membrane stabilizing activity (69.77±1.56%) as well as antioxidant activity (79.69±2.04%).

Keywords: *Salvadora oleoides,* Membrane stabilzation, Erythrocyte, DPPH, antioxidant, anti-inflammatory, Ascorbic acid, Aspirin.

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INTRODUCTION

Recenly the use of herbal medicine has been increased because of medicinal plants contains rich source of phytoconstituent and used for the prevention of diseases and ailments all over the world. This universal trend shift synthetic to herbal medicine. There are various ancient civilizations which are still using herbal remedies for diseases. In this context, India has various number of recognized indigenous system of medicine such as Ayurveda, Sidha, Unani, Homeopathy, Yoga and Naturopathy which is unique in all over the world. Herbal drugs gaining popularity and acceptibility in rural and urban community of India because of the herbal medicines are safe, easy availability and cheap [1]. There are many conventional drug which is originated from natural sources in which most of the drug are of plant orgin which includes Quinine (from cinchona bark), morphine (from opium poppy), aspirin (from willow bark) and digoxin (from foxglove) [2]. The herbal medicine has non toxic and no side effects therefore it is being use all over the world as a primary healthcare. Several regulatory models for herbal medicines are currently available including prescription drugs, over-the-counter substances, traditional medicines and dietary supplements [3].

Inflammation is due to irritation, injury or infection characterized by redness, heat, swelling, loss of function and pain which results in increase blood flow, vascular permeability and consequence of activation of primary afferent nerve fibes [4,5]. Many inflammatory mediators are released at the time of tissue injury [6]. Therefore the screening and development of drugs for their anti-inflammatory activity is the needed and there are many efforts for finding the anti-inflammatory drugs from indigenous herbal plants.

The oxidation in the body will effect various enzyme systems and cause damage which may further contribue to conditions such as aging, ischemia, cancer, rheumatoid arthritis and adult respiratory distress [7]. Many chain reactions occurs due to production of free by oxidation reaction which cause damage of cells. Antioxidants removing free radicals and terminate chain reactions. It also inhbits agents which cause oxidation. Low level of antioxidants in body cause oxidative stress and may damage or kill cells. Oxidative stress may contribute many human diseases so the use of antioxidants in pharmacology is intensively studied [8].

Salvadora Linn. a small genus of evergreen trees or shrubs, distributed in tropical Africa and Asia, extending to Egypt, Mascarene Islands and China. Two Species occur in India i.e., Salvadora oleoides decne and *Salvadora persica Linn*. *Salvadora oleoides decne.*, belonging to family Salvadoraceae commonly found in Western region in India. It is known as Jhal, Pilu in Hindi, Pilu in Sanskrit, Khakan in Gujrati, Kalawa, Karkol in Tamil and Diar, Godpilu, Khabbar in Marathi [9].

The leaves of *Salvadora oleoides* are said to possesses anti-inflammatory [10], analgesic [11], antidiabetic activity and antihyperlipidemic activity [12-14], antimicrobial activity [15], antioxidant activity [16]. In present study the selection of plant for evaluation was based on its traditional uses and evaluated for the its membrane stabilizing and antioxidant activity.

MATERIALS ANS METHODS

Collection and Identification of leaves Salvadora oleoides (decne.)

Leaves of *Salvadora oleoides* were collected from locality of Delhi (India). Plant material was authenticated by Dr. H.B. Singh, HOD, Raw materials Herbarium & Museum (RHMD) in National Institute of Science Communication Information Resources (NISCAIR), New Delhi. Authenticated specimen no is-NISCAIR/RHMD/Consult/-2010-11/1675/273.

Preparation of different leaves extracts of Salvadora oleoides

The collected plant material was washed with water to remove other undesirable material and dried under shade.The air-dried leaves (500 gm) of *Salvadora oleoides* were crushed. The crushed leaves extracted with methanol by cold percolation method using percolator. The extract was evaporated till dryness to obtain a residue. From total methanol extract, different fractions were prepared by successive fractionation (separation technique) using increasing polarity of solvents i.e. Petroleum ether, Chloroform, Ethyl acetate & n-Butanol.

Phytochemical analysis of extracts of leaves of Salvadora oleoides

Extracts of leaves and bark of *Salvadora oleoides* were subjected to evaluate the presence of different phytoconstituents such as alkaloids, carbohydrate, steroids, proteins-amino acids, saponin and phenolic compounds.

Invitro membrane stabilizing and antioxidant activities of extracts

Invitro membrane stabilizing activity of leaves extracts [17-20]

Erythrocytic 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 *Salvadora oleoides* extracts and fractions at concentrations of 1000, 1500 and 2000 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. Aspirin was used as a reference standard.

Calculation

The percentage inhibition or acceleration of hemolysis in test was calculated according to the equation:

$$\textbf{\% acceleration or inhibition of hemolysis} = \textbf{100 x } [\frac{OD1-OD2}{OD1}]$$

Where, OD^1 = Optical density of hypotonic saline solution + blood (control) and OD^2= Optical density of test sample in hypotonic saline solution + blood

Antioxidant activity of leaves of Salvadora oleoides [21, 22]

Preparation of DPPH

DPPH is a highly oxidisable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in 100 ml methanol.

Preparation of standard Ascorbic acid solution & different concentration of Salvadora oleoides extracts

Ascorbic acid is an strong antioxidizing agent. It is taken as standard. Standard solution of ascorbic acid as well as extracts is prepared. viz. 500 µg/ml, 1000 µg/ml and 1500 µg/ml.

Preparation of test sample & standard sample

3 ml of different concentration of test sample of *Salvadora oleoides.* Extracts and standard (ascorbic acid) were mixed separately with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test sample was incubated for 1/2 half an hour. When procedure is done than absorbance is taken with the help of U.V. Spectrophotometer at 517 nm.

We calculate the % activity of individual concentration of individual extract from the following formula:-

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$$\% \text{ Activity} = \frac{\text{Abs. of control} - \text{Abs. of individual concentration}}{\text{Abs. of control}} \times 100$$

Abs. = Absorbance

RESULTS AND DISCUSSION

The collected leaves of *Salvadora oleoides* were dried under shade. The air-dried leaves (500 gm) of *Salvadora oleoides* were crushed. The crushed leaves extracted with methanol by cold percolation method using percolator. The extract was evaporated till dryness to obtain a residue of 140 gm. From total methanol extract, different fractions were prepared by successive fractionation (separation technique) using increasing polarity of solvents & yield were, Petroleum ether (15.92 gm), Chloroform (14.07 gm), Ethyl acetate (5.40 gm) & n-Butanol (32.16 gm).

The methanol extract & its fractions of leaves of *Salvadora oleoides* undergo various qualitative phytochemical tests. we found out that total methanol extract of leaves was the richest extract for phytoconstituents. It contains maximum tested phytoconstituents viz. Alkaloids, carbohydrates, phenolic compounds, Sterols and Saponin except Protein and amino acids. Petroleum ether fraction showed the presence of sterols and saponin only. Chloroform and Ethyl acetate fractions showed the presence of Carbohydrate and Phenolic compounds only. Butanol extract showed presence of alkaloids, carbohydrates, phenolic compounds, except sterols saponin, proteins and amino acids.

Membrane Stabilizing activity

Membrane stabilizing activity of leaves extract & its fractions of *Salvadora oleoides* were compared with activity of standard drug Aspirin. It was observed that the concentration of 2000 µg/ml of n-Butanol fraction of leaves showed maximum membrane stabilization activity 69.77±1.56 percent. The Ethyl acetate fraction showed the 65.19±1.91percent activity (Table 1).

Concentration of extracts (µg/ml)	% membrane stabilizing activity of extracts & standard drug						
	Salvadora oleoides leaves extracts					Standard Drug	
	Petroleum ether	Chloroform	Ethyl acetate	Butanol	Total Methanol extract	Acetyl Salicylic acid	Concentration of Acetyl Salicylic acid (µg/ml)
1000	10.66±0.78	26.30±0.92	29.83±2.15	60.27±2.28	27.33±1.24	49.14±0.77	**100**
1500	11.73±0.67	27.89±1.06	40.55±1.43	65.90±1.99	31.33±1.31	55.66±0.75	**150**
2000	15.34±1.37	40.87±1.58	65.19±1.91	69.77±1.56	37.85±0.91	58.13±0.71	**200**

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

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

Inflammation mediated release of lysosomal constituents are main cause of damage of cells. Lysosomal membrane resemblance with erythrocyte. By stabilization of the lysosomal membrane inhibits the release of lysosomal constituents. So stabilization of erythrocyte membrane with extract may also stabilize the lysosomal membrane [23]. Stabilization of erythrocyte cell membrane by hypotonic solution induced erythrocyte membrane lysis can be taken as an invitro measure of anti-inflammatory activity of the drugs or plant extracts.

Antioxidant activity

n-Butanol fraction of leaves of *Salvadora oleoides* showed maximum antioxidant activity in comparision to all extracts. The concentration of 1500 µg/ml of n-Butanol fraction of leaves showed 79.69±2.04 percent antioxidant activity (Table 2).

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

Concentration of extracts (µg/ml)	% antioxidant activity of extracts & standard drug						
	Salvadora oleoides leaves extracts					Standard Drug	
	Petroleum ether	Chloroform	Ethyl acetate	Butanol	Total Methanol extract	Ascorbic Acid	Concentration of Acetyl Salicylic acid (µg/ml)
500	26.45±1.58	13.18±0.79	45.57±1.36	68.66±2.1	26.13±1.36	96.50±0.19	**100**
1000	38.32±2.25	22.45±2.1	46.21±0.78	78.52±1.16	26.50±1.39	96.45±0.11	
1500	39.29±2.11	32.03±2.11	53.80±2.31	79.69±2.04	27.37±1.76	96.67±0.17	

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

DPPH molecule scavenges radical species and antioxidant molecule by change the colour of DPPH solution and the intensity of colour of DPPH solution depends on the concentration and potency of antioxidants. Low absorbance of the reaction mixture indicates significantly the increase in antioxidant activity [24].

CONCLUSION

From the above studies it could be concluded that n-Butanol fraction of leaves showed maximum membrane stabilizing activity as well as antioxidant activity. So further study is needed for the isolation of active principle.

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