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Membrane Stabilizing and antioxidant activities of extracts from leaves of *Elaeocarpus sphaericus*

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Abstract : Medicinal plants have played an essential role in the development of human culture. Plant and plant products have utilized with varying success to cure and prevent diseases throughout history. In the present study different extracts of leaves of *Elaeocarpus sphaericus* 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 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. Total methanol extract of leaves showed maximum membrane stabilizing activity (66.65±3.22%) and ethyl acetate fraction of leaves showed maximum antioxidant activity (90.28±1.03%).

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

Introduction

Herbal medicine is used as a primary healthcare of about 80% of the world population. Herbal medicine is used because of their acceptibility, compatibility with human body and lesser side efffects. There is increase in use of herbal medicine in last few years throughout the world[1]. The important part of indigenous medical system is medicinal plants in all over the world by providing rich resources of phytoconstituents for natural drug research and development[2]. The substantial historical use of herbal medicine is true for many product which are available in traditional herbal medicines[3]. In developing countries, peoples utilizes medicinal plants on regular basis as traditional medicine[4]. The ingredients present in medicinal plants can be used in drug development and synthesis[5]. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs[6].

Reaction due to living tissue towards injury is cause of inflammation which involves systemic and local responses[7]. It is a complex biological process of vascular tissues to harmful stimuli[8]. Inflammation is protective attempt of organism by initiaing the healing process of damage cell and removing injurious stimuli[9].

Oxidation is a chemical reaction in which electron is transfer from one substance to another. Free radicals can also produced by oxidation reactions. These free radicals initiates many chain reactions that damage cells. An antioxidant is capable of inhibiting these chain reaction by removing free radicals and inhibit other oxidation agents[10]. Low level of antioxidants in the body cause oxidative stress and may damage or kill cells[11].

Elaeocarpus sphaericus is belonging to family Elaeocarpaceae. *Elaeocarpus sphaericus* (syn. *Elaeocarpus ganitrus*) commonly known as rudraksha in sanskrit and rudraki in hindi is grown in Assam and himalayan region of India for its attractive fruit stones and medicinal properties[12]. Nearly 360 species of Elaeocarpus trees are found in different parts of the world. It is widely distributed from Madagascar in the west through India, Southeast Asia, Malaysia, Southern China and Japan, through Australia to New Zealand, Fiji, and Hawaii in the east with its approximately 350 species. Various phytochemical investigations shows that the common phytochemical present in Elaeocarpus species are Elaeocarpine, Isoelaeocarpine, Epiisoelaeocarpiline, Myricitrin, Mearnsetin 3-*O*-*β*-D-glucopyranoside cucurbitacins and Ellagic acid derivatives[12-14]. Literature reports are available on various pharmacological activities which include analgesic and anti-inflammatory, CNS activities, typical behavioral actions, sedative, tranquillizing, hypnosis potentiating, antidepressant, antiasthmatic, anti-diabetic, cardio stimulation, antihypertensive, anticonvulsant, etc[15-16]. the selection of plant for evaluation was based on its traditional uses and evaluated for the its membrane stabilizing and antioxidant activity.

Materials and Methods

Collection and Identification of leaves *Elaeocarpus sphaericus*:

Leaves of *Elaeocarpus sphaericus* were collected from locality of Dehradun. Plant material was authenticated by Kumar Ambrish (Scientist C), in Botanical Survey of India, Northern regional centre, Dehradun (BSI). Authenticated specimen no is- Acc. No. 114849.

Preparation of different extracts of leaves of *Elaeocarpus sphaericus*:

The collected plant material was washed with water to remove other undesirable material and dried under shade.The air-dried leaves (500 gm) of *Elaeocarpus sphaericus* 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 *Elaeocarpus sphaericus*:

Extracts of leaves and bark of *Elaeocarpus sphaericus* 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 *Elaeocarpus sphaericus* extracts 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 3000g 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 extracts of leaves of *Elaeocarpus* sphaericus[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 *Elaeocarpus sphaericus* extracts:

Ascorbic acid is an strong antioxidizing agent. It is taken as standard. Standard solution of ascorbic acid as well as extracts was prepared. viz. 50, 100, 200, 300, 400, and 500 µg/ml.

Preparation of test sample & standard sample:

3 ml of different concentration of test sample of *Elaeocarpus sphaericus*. Test 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 30 minutes. When procedure is done then absorbance is taken with the help of U.V. Spectrophotometer at 517 nm.

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

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

Abs. = Absorbance

Results and Discussion

The collected leaves of *Elaeocarpus sphaericus* were dried under shade. The air-dried leaves (500 gm) of *Elaeocarpus sphaericus* 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 134.5 gm. From total methanol extract, different fractions were prepared by successive fractionation (separation technique) using increasing polarity of solvents & yield were, Petroleum ether (6.1 gm), Chloroform (16.03 gm), Ethyl acetate (9.42 gm) & n-Butanol (7.19 gm) yield.

The methanol extract & its fractions of leaves of *Elaeocarpus sphaericus* 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 *Elaeocarpus sphaericus* were compared with activity of standard drug aspirin. It was observed that the concentration of 2000 µg/ml of total methanol extract of leaves showed maximum membrane stabilization activity 66.65±3.22 percent (Table 1). Infection, injury and contaminants exposure are severe perturbation of homeostasis which initiates the phenomenon of Inflammation[23]. The release of lysosomal constituents during inflammation are main cause of damage of cells. Stabilisation of lysosomal membrane inhibits the release of lysosomal constituents. Lysosomal membrane resemblance with erythrocyte and stabilisation of erythrocyte membrane may also stabilize the lysosomal membrane which inhibit the cell damage and initiation of inflammation[24]. 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.

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						
	Elaeocarpus sphaericus leaves extracts					Standard Drug	
	Petroleum ether	Chloroform	Ethyl acetate	n-Butanol	Total Methanol extract	Acetyl Salicylic acid	Concentration of Acetyl Salicylic acid (µg/ml)
1000	3.29±0.49	11.56±0.71	12.45±0.52	15.35±0.59	19.58±0.50	48.99±0.51	**100**
1500	6.07±0.74	13.47±0.38	13.73±0.95	28.92±0.82	54.46±2.23	55.86±1.13	**150**
2000	8.92±0.39	15.56±1.07	24.83±0.47	33.76±1.03	66.65±3.22	58.20±1.23	**200**

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

Antioxidant activity

Ethyl acetate fraction of leaves of *Elaeocarpus sphaericus* showed maximum antioxidant activity in comparision to all extracts. The concentration of 500 µg/ml of ethyl acetate fraction of leaves showed 90.28±1.03 percent antioxidant activity where as total methanol extract of leaves showed 89.25±0.88 percent 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					
	Elaeocarpus sphaericus leaves extracts					Standard Drug
	Petroleum ether	Chloroform	Ethyl acetate	n-Butanol	Total Methanol extract	Ascorbic Acid
50	13.41±0.41	13.06±0.76	81.86±1.27	41.26±0.53	15.50±1.15	96.50±0.19
100	21.82±0.74	17.93±0.88	83.50±2.09	44.67±1.54	20.04±0.86	96.45±0.10
200	32.65±1.22	30.62±1.67	85.56±2.91	46.50±1.48	25.97±1.01	96.67±0.16
300	38.07±0.83	37.20±0.97	86.31±1.55	48.37±1.26	38.62±1.32	96.59±0.17
400	41.25±1.28	45.42±1.3	87.10±2.61	50.84±0.78	45.68±1.37	96.25±0.17
500	49.58±0.97	59.39±1.22	90.28±1.03	55.21±1.16	89.25±0.88	96.49±0.15

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

Free radicals are the chemical moiety which cause damage cells. The human body uses oxygen as energy by various chain reactions in the body. Formation of free radicals from various chain reactions in body can harm surrounding tissues which initiates lipid peroxidation resulting in membrane destruction. An antioxidants prevents oxidation of other molecule and terminate all chain reactions and inhibits oxidation reactions being oxidized themselves[25,26]. DPPH molecule scavenges the free radical species by change the

colour of DPPH solution. An antioxidant molecule prevents the formation of free radicals, and the intensity of colour of DPPH solution depends on the concentration and potency of antioxidants. Low intensity of DPPH colour and low absorbance of the reaction mixture indicates significantly the increase in antioxidant activity[27].

Conclusion

From the above studies it could be concluded that total methanol fraction of leaves showed maximum membrane stabilizing activity and ethyl acetate fraction showed maximum antioxidant activity. So further study is needed for the isolation of active principle.

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