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Membrane stabilizing, antimicrobial and antioxidant effects of *Thevetia peruviana (per.)K.Schum* leaves extracts

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Abstract

Herbal medicine are used throughout the world for the treatment of various diseases. There is huge demand of herbal medicine all over the world. 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. All over the world large population still relies completely on herbs and unrefined extracts as medicine. Herbal drugs are used widely for therapeutic purposes to prevent and treat diseases The objective of present study was to investigate membrane stabilizing, antimicrobial and antioxidant activities of leaves extrats of *Thevetia peruviana*. From the results, methanol extract showed maximum membrane stabilizing (58.50±0.16%), and antioxidant (76.32±0.77%)activities. From antimicrobial studies, methanol extract showed maximum inhibition zone against all the tested bacterial and fungal culture.

Keywords-*Thevetia peruviana*, membrane stabilizing activity, antioxidant, antimicrobial, DPPH

INTRODUCTION

Plants have been playing as the important source of medicine in the health services around the world [1]. Around 75 percent of world population uses the plant and their extrats for treatment of various diseases [2]. People living in rural areas directly depend on herbal remedies for the treatment of different types of diseases. It indicates the importance of the individual plants in the health care system.

Inflammation may be defined as the series of changes that occurs in living tisues following injury. The injury which is responsible for inflammation may be brought about by a variety of conditions such as physical agents like mechanical trauma, ultra-violet or ionizing radiation; chemical agents like organic and inorganic compounds, the toxins of various bacteria; intracellular replication of viruses; hypersensitivity reactions like reaction due to sensitized lymphocytes with antigenic material viz., inhaled organic dusts or invasive bacteria; and necrosis of tissues whereby inflammation is induced in the surrounding tissues[3].

The Infectious diseases all over the world cause the premature death & killing many peoples every day. All over the world faces drug resistance to human pathogenic bacteria are common[4]. The bacterial resistance of antimicrobial compounds over many years are due to abusive and indiscriminate use[5]. The increase in resistance to antibiotics and antimicrobial compounds, the compounds from natural resources could be intresting alternative[6,7]. Antimicrobial properties of plant extracts and phytochemicals can be great importance in the treatment of diseases. Many studies have been demonstrate such efficacy in different countries[8-10].

On the other hand, the major cause of various chronic and degenerative diseases are free radicals. Many diseases like diabetes, neurodegenerative diseases, atherosclerosis, cancer, inflammatory diseases, and aging processes associated with oxidative stress. It is an imblance between free radicals and reactive metabolites called oxidants and their elimination by protective mechanism referred as antioxidative systems. The damgae of tissues, organs and important biomolecules is due to this imbalance which impact on the whole organism. Antioxidants are the oxidizable materials which prevent the oxidation by scavenging the free radicals and reducing the oxidative stress[11,12]. Antioxidants from natural resources have been studied more in last decades to find compounds for the treatment of number of diseases related to oxidative stress and free radicals. Many plants have been studied for such properties and claimed as antioxidant properties[13,14].

From the above aim, this study therefore, reports the membrane stabilizing, antimicrobial and antioxidant activities of different extracts of leaves of *Thevetia peruviana* (Pers.) k.Schum.

MATERIALS AND METHODS

Collection & Identification of plant material

Leaves of *Thevetia peruviana* were collected from Manduwala, Dehradun, Uttarakhand (India). Plant material was authenticated by S. K. Srivastava (Scientist D/HOD), in Botanical Survey of India, Northern regional centre, Dehradun (BSI). Authenticated specimen no. is- Acc. No. 114633.

Extraction of plant material

The collected plant material was washed with water to removed other undesirable material and dried under shade. The air-dried leaves (250gm) of *Thevetia peruviana* were crushed. The crushed leaves extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, acetone and methanol by hot percolation

method using soxhlet apparatus. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure.

Phytochemical Analysis

All extract were tested for presence of phytoconstituents i.e., alkaloid, carbohydrate, sterols, proteins & amino acids, saponin test, and phenolic compounds[15].

Invitro membrane stabilizing activity of extracts

Effect on haemolysis [16-19]

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 leaf extracts at concentration of 500, 1000, 1500, 2000, 2500, 3000 µg/ml while the control sample was *Thevetia peruviana* mixed with drug free solution. The mixtures were incubated for 10 minute at room temperature, and centrifuged at 3000 rpm for 10 minutes. All the experiments were performed in triplicates and the absorbance (O.D.) of the supernatant was measured at 560 nm. Acetyl salicylic acid was used as a reference standard.

Calculation

The percentage inhibition or acceleration of hemolysis in test (b) and (c) was calculated according to the equation-

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

Where, OD1=Optical density of hypotonic saline solution + blood (control) and OD2=Optical density of test sample in hypotonic saline solution + blood

Antimicrobial activity of extracts [20]

The antimicrobial activity of the leaves of *Thevetia peruviana* was carried out using well diffusion method. The leaves extract were screened for antibacterial and antifungal activities.

Antibacterial activity of extracts

The antibacterial activity was studied against the micro-organism and the bacterial cultures used in the study were Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa & Staphylococcus aureus. These bacteria were provided by Department of Microbiology, Dolphin (PG) institute of Biomedical and Natural Sciences, Dehradun checked for purity by convention biochemical method. To test antibacterial activity, the well diffusion method used. The concentration of different extracts were 200 mg/ml.

Antifungal activity of extracts

In this study, the antifungal activity was against the microorganism and the fungi culture used for this study were Aspergillus niger, Alternaria solani, Fusarium oxysporum, Penicillium chrysogenum. These cultures were obtained from the standard cultures maintained in the Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun . To test antifungal activity, the well diffusion method was used. The concentration of different extracts were 200 mg/ml.

Antioxidant activity of extracts [21, 22]

Preparation of standard solution & different concentration of *Thevetia peruviana* leaves extract

Ascorbic acid is an strong antioxidizing agent. It is taken as standard. Standard solution of ascorbic acid is prepared as 100 µg/ml. Different concentration of the test sample *Thevetia peruviana* extract which is to be examined for antioxidant activity is prepared. viz. 500, 1000, 1500 µg/ml.

Preparation of test sample & standard sample

DPPH is a highly oxidisable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in ethanol. 3 ml of different concentration of test sample *Thevetia peruviana* leaves extracts and standard (ascorbic acid) were mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test sample was incubated for 30 minutes. When procedure was done then 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-

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

Abs. = Absorbance

RESULTS AND DISCUSSION

The yield (in grams) of leaves extracts in different solvents system are petroleum ether (9.88gm), chloroform (9.37gm), acetone (4.75gm) and methanol (24.9gm). The extract of leaves of *Thevetia peruviana* undergone various qualitative phytochemical tests. We found out that methanol extract was the highly active extract for phytoconstituents. It contained some tested phytoconstituents viz. alkaloids, carbohydrates, and phenolic compounds, saponins, proteins and amino acid, except triterpinoids of sterols, fats and fixed oil which are absent in methanol. Acetone extract contained carbohydrates and phenolic compounds and petroleum ether contains sterols only.

Membrane stabilizing activity

The invitro membrane stabilizing activity of the different extracts was compared with standard drug aspirin. From the study it was observed that the concentration of 3000 µg/ml of methanol extract showed maximum activity 58.5±0.16 % at 560nm, as compared to acetone (52.33±1.26%), chloroform (35.84±0.57%), and petroleum ether (28.82±1.12%).

The release of lysosomal constituents causes inflammation and damage of cells. Stabilization of lysosomal membrane inhibits the release of lysosomal constituents from it[23]. Lysosomal membrane and erythrocyte membrane are resemblance with each other & by stabilize the erythrocyte membrane by extract may also stabilize lysosomal membrane[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.

Antimicrobial activity

The antibacterial & antifungal activity of different extracts of *Thevetia peruviana* and standard drugs were tested for different strains of bacteria & fungi and zone of Inhibition was recorded in mm.

Methanol and acetone extracts showed antibacterial activity against all bacterial culture at a concentration of 200 mg/ml. Methanol extract showed inhibition zone 24.4±0.5 mm against E. coli, 10.7±0.9 mm against Bacillus cereus, 11±0.2 mm against S. aureus, 12.3±0.6 mm against Pseudomonas aeruginosa. Acetone extract showed inhibition zone 23.4±0.9 mm against E. coli, 11.1±0.4 mm against Bacillus cereus, 10.4±0.6 mm against S. aureus, 11.4±0.5 mm against Pseudomonas aeruginosa. Standard drug Chloramphenicol showed antibacterial activity against E. coli, Bacillus cereus, Pseudomonas aeruginosa and S. aureus, and the inhibition zone of Chloramphenicol, 20.2±0.1 mm against E. coli, 17.2±0.15 mm against Bacillus cereus, 27.3±0.2 mm against Pseudomonas aeruginosa, and 15.4±0.2 mm against S. aureus.

All extracts showed antifungal activity against all fungal culture at a concentration of 200 mg/ml. Methanol and acetone extracts showed maximum antifungal activity in comparision to other extracts.

Acetone extract showed inhibition zone 14.4±0.5 mm against Aspergillus niger, 23.4±0.5 mm against Alternaria solani, 22.1±0.2 mm against Penicillum chrysogenum and 19.1±0.4 mm against Fusarium oxysporum. Methanol extract showed inhibition zone 16.4±0.5 mm against Aspergillus niger , 25.3±0.7 mm against Alternaria solani, 26.9±0.9 mm against Penicillum chrysogenum and 15±0.2 mm against Fusarium oxysporum.

Standard drug Nystatin showed antifungal activity against Aspergillus niger, Alternaria solani, Fusarium oxysporum, and Penicillium chrysogenum, and the inhibition zone of Nystatin, 13.5±0.3 mm against Aspergillus niger, 18.5±0.2 mm against Alternaria solani, 22.2±0.1 mm against Fusarium oxysporum, and 22.5±0.3 mm against Penicillium chrysogenum.

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

S. No.	Test organism	Inhibition zone (in mm)				
		Petroleum ether	Chloroform	Acetone	Methanol	Chloramphenicol
1.	E. coli	-	-	23.4±0.9	24.4±0.5	20.2±0.1
2.	Bacillus cereus	-	-	11.1±0.4	10.7±0.9	17.2±0.15
3.	Pseudomonas aeruginosa	-	-	10.4±0.6	11.0±0.2	27.3±0.2
4.	Staphylococcus aureus	-	-	11.4±0.5	12.3±0.6	15.4±0.2

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

Table 2 Antibacterial activity of different extracts and standard drug (Chloramphenicol)

Concentration of extracts (µg/ml)	% membrane stabilizing activity of extracts & standard drug					
	Thevetia peruviana leaves extracts				Standard Drug	
	Petroleum ether	Chloroform	Acetone	Methanol	Acetyl Salicylic acid	Concentration of Acetyl Salicylic acid (µg/ml)
500	3.68±0.62	6.63±0.52	9.93±0.18	11.47±0.31	49.79±1.0	**100**
1000	7.56±0.96	11.31±0.90	14.80±0.76	18.12±0.44		
1500	12.83±0.88	17.28±0.62	22.50±0.63	26.84±0.53	55.85±0.74	**150**
2000	17.41±1.59	23.40±1.9	33.73±0.58	40.18±1.24		
2500	21.32±1.61	29.24±0.35	46.44±0.77	51.16±0.46	59.0±0.32	**200**
3000	28.82±1.12	35.84±0.57	52.33±1.26	58.50±0.16		

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

Table 3 Antifungal activity of different extract and standard drug (Nystatin)

S. No.	Test organism	Inhibition zone (in mm)				
		Petroleum ether	Chloroform	Acetone	Methanol	Nystatin
1.	Aspergillus niger	-	-	14.4±0.5	16.4±0.5	13.5±0.3
2.	Alternaria solani	-	-	23.4±0.5	25.3±0.7	18.5±0.2
3.	Fusarium oxysporum	-	-	19.1±0.4	15±0.2	22.2±0.1
4.	Penicillium chrysogenum	-	-	22.1±0.2	26.9±0.9	22.5±0.3

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

Table 4 Effect of different extract and standard drug on antioxidant activity

Concentration of extracts (µg/ml)	% antioxidant activity of extracts & standard drug					
	Thevetia peruviana leaves extracts				Standard Drug	
	Petroleum ether	Chloroform	Acetone	Methanol	Ascorbic acid	Concentration of Acetyl Salicylic acid (µg/ml)
500	15.58±1.56	37.37±0.52	24.09±0.25	35.75±0.74	96.50±0.19	**100**
1000	20.99±0.40	38.53±1.3	33.26±0.69	55.17±0.57		
1500	38.11±0.88	43.46±0.41	38.84±0.48	76.32±0.77		

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

Antioxidant activity

Methanol extract showed maximum antioxidant activity in comparision to all extracts. The concentration of 1500 µg/ml of methanol extract showed maximum antioxidant activity 76.32±0.77% in comparision to all extracts and standard drug.

DDPH scavenging method is based on the addition of radical species and antioxidants which scavenges by DPPH which inturn change in colour of DPPH solution. The change iin colour of DPPH solution depends on the concentration and potency of antioxidants. Decrease in absorbance of reaction mixture indicates significantly activity of drugs or plant extracts [25].

CONCLUSION

Membrane stabilizing activity was done by use of hypotonic solution induced haemolysis method at a different concentration & from the above study is could be concluded that methanol extract showed maximum membrane stabilizing effect in comparison with other extract. Antimicrobial activity was done by well diffusion method and results showed that methanol extract showed maximum antimicrobial activity in comparison to other extract. Antioxidant activity was done by used of DPPH method from which methanol extract showed maximum antioxidant activity is comparison with other extract. So in present study we concluded that methanol extract showed maximum membrane stabilizing, antimicrobial and antioxidant activities. Phytochemical analysis showed that methanol extract was richest extract for phytoconstituent so further study needed for the isolation of active principle for the same.

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