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RESEARCH ARTICLE

PRELIMINARY STUDIES ON PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY OF Delonix elata AND Prospis cineraria

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ABSTRACT

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Key words:

Antimicrobial activity, leaf extracts, phytochemicals, *Delonix elata* and *Prospis cineraria* Delonix elata and Prospis cineraria are used in the treatment of various diseases by local folks. Since, these plants possess many medicinal properties; the present study was designed to evaluate the phytochemicals and the antimicrobial activity of leaf extracts from of Delonix elata and Prospis cineraria. The invitro antimicrobial activity was performed by agar disc diffusion method against bacterial viz. *Staphylococcus aureus, Bacillus subtillus, Klebsiella, E.coli, Proteus sp.* and *Pseudomonas sp.* and fungi viz. *Aspergillus Niger, Pencillium sp., Candida albicans.* The organic extracts especially alcoholic extract, showed maximum against the micro organism. This shows that these plants can be used for medicinal purposes. Both *Delonix elata* and *Prospis cineraria* were deserved to have antimicrobial activity and can be used for medicinal purposes.

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INTRODUCTION

The use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. Medicinal plants are nature's gift to cure a number of diseases. Enormous plants are in use as therapeutic agents for thousands of years in treating different diseases. About 80% of the world's population depends on traditional medicines in numerous treatments and disorders (Farnsworth, 1994). Plants used in traditional medicines contain a wide range of ingredients that can be used to treat chronic as well as infectious The bioactive compounds like alkaloids, diseases. flavonoids, tannins and phenolic compounds are the reason for the medicinal value of plants that produce a definite physiological action on the body (Hemashanmugam et al., 2009).

MATERIALS AND METHODS

Plant materials

The leaves of *Delonix elata* and *Prospis cineraria* were collected from collected *from Kolli hills, Namakkal Distr*ict.

Preparation of extracts for phytochemical analysis

The plant material was allowed to shadow dry and afterwards pulverized by using mortar and pestle. 10 grams pulverized material were dissolved in 100 ml of

solvent (Methanol, ethanol, acetone and water) and kept in a shaker for overnight. The obtained extracts were filtered with Whatmann No.4 filter paper and the filtrate was collected and used for analysis (Kokate, 1994).

Test microorganisms

Fresh cultures of the microorganisms were grown in nutrient broth. The density of microorganisms was adjusted to Mc Farland 0.5 standard. The invitro antimicrobial activity was performed by agar disc diffusion method against bacterial viz. *Staphylococcus aureus, Bacillus subtillus, Klebsiella, E.coli, Proteus sp.* and *Pseudomonas sp.* and fungi viz. *Aspergillus Niger, Pencillium sp., Candida albicans* respectively.

Preliminary Phytochemical analysis Detection of carbohydrates

A minimum amount of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

Molisch's test

The minimum amount of extract was treated with 2 to 3 drops of 1 percent alcoholic alpha-naphthol and 2 ml of concentrated sulphuric acid. This was added along the sides of the test tube. Formation of a violet ring at the junction of two layers will indicate the presence of carbohydrates.

Fehling's test

The minimum amount of extract was treated with 1 ml of Fehling's solution and heated. Formation of a reddish orange precipitate will indicate the presence of reducing sugar.

S.NO	Phytochemical	Test	Acetone extract	Ethanol extract	Methanol extract	Aqueous extract	
1	Carbohydrates &	A. Molish's	+	+	+	+	
	Glycosides	B. Fehling's	+	+	+	+	
	- ,	C. Benedict's	+	+	+	+	
		D. Barford's	+	+	+	+	
2	Alkaloids	A. Mayer's	+	+	+	+	
		B. Wagner's	+	+	+	+	
			+	+	+	+	
3	Phytosterol	Libermann burchard	+	+	+	-	
4	Gums & Mucilages		-	-	-	-	
5	Saponins	Foam test	-	+	+	+	
6	Protein & Amino acids	A. Biuret	+	+	+	+	
		B. Ninhydrin	+	+	+	+	
		C. Xanthoproteic	+	+	+	+	
7	Phenolic compounds	Ferric chloride	-	+	+	-	
8	Flavonoids	A. con H ₂ So ₄	+	+	+	+	

Table 1. Phytochemical analysis of Leaf extracts of Delonix elata

Table 2. Phytochemical (Qualitative) analysis of Leaf extracts of Prospis cineraria

Sno	Phytochemical	Test	Acetone extract	Ethanol extract	Methanol extract	Aqueous extract
1	Carbohydrates &	A. Molish's	+	+	+	+
	Glycosides	B. Fehling's	+	+	+	+
		C. Benedicts's	+	+	+	+
		D. Barford's	+	+	+	+
2	Alkaloids	A. Mayer's	+	+	+	+
		B. Wagner's	+	+	+	+
		_	+	+	+	+
3	Phytosterol	Libermann burchard	+	+	+	-
4.	Gums & Mucilages		+	-	-	
5	Saponins	Foam test	+	+	+	+
6	Protein & Amino	A. Biuret	+	+	+	+
	acids	B. Ninhydrin	+	+	+	+
		C. Xanthoproteic	+	+	+	+
7	Phenolic compounds	Ferric chloride	-	+	+	-
8	Flavonoids	A. con H_2So_4	+	+	+	+

Benedict's test

The minimum amount of extract was treated with 1 ml of Benedict's solution and heated. Formation of a reddish precipitate will indicate the presence of reducing sugar.

Barford's test

The minimum amount of extract was treated with 1 ml of Barford's solution and heated. Formation of a reddish precipitate will indicate the presence of monosaccharide.

A small quantity of the extract was separately treated with few drops of dilute hydrochloric acid and filtered. The filtrate was used for the following tests. The minimum amount of extract was treated with Mayer's reagent. Cream color precipitates if obtained with the aqueous extracts, will indicate the presence of alkaloids. The minimum amount of extract was treated with dragondroff's reagent. Reddish brown precipitate, if obtained, will indicate the presence of alkaloids.

	Test micro organism	Diameter of Zone Inhibition (mm)									
S.No		Prosopis Cineraria					Prosopis Cineraria				
		Standard	Negative control	Methanolic extract	Ethanolic extract	Acetone extract	Aqueous extract	Methanolic extract	Ethanolic extract	Acetone extract	Aqueous extract
1	Staphylococcus										
	aureus	20	-	18.5	16	11	9	17	10.8	6	8.4
2	Bacillus subtillus	17	-	15	15	6	5.3	18.2	12.5	8.3	7
3	Klebsiella	15	-	18	20	8	6.8	15.4	9.2	14	9
4	E.coli	15	-	21	36	8.4	7.2	15	14.4	10.1	7.3
5	Proteus	15	-	15	30	11	8.2	14.2	13.2	11.1	8
6	Pseudomonas	16	-	16.5	11	26	7	20	13.5	9	7.8

Table 3. Anti-Bacterial activity of Delonix Elata and Prosopis Cineraria leaves in various extracts

Values are mean of three replicates ; Standard: Streptomycin 10 µg/disc; Negative Control: Distilled water

Table 4.Anti-Fungal activity of <i>Prosopis Cineraria</i> and
Delonix Elata leaves in various extracts

S.No	Test micro organism	Diameter of Zone Inhibition (mm)						
5.110		Delonix	: Elata	Prosopis Cineraria				
	0	Methanolic extract	Ethanolic extract	Methanolic extract	Ethanolic extract			
1	Aspergillus Niger	12	10.8	13	12.6			
2	Pencillium sp.,	11.2	10	16	15.4			
3	Candida albicans	14.2	11.2	15.4	9.2			

Detection of phytosterols

A small quantity of the aqueous extract was dissolved in 5 ml of chloroform separately. Then these solutions were subjected to Libermann Buchard test for the detection of phytosterols.

Libermann burchard's test

The chloroform solution was treated with few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. Purple color change was observed. It showed the presence of phytosterols.

Detection of gums and mucilages

Add about 10 ml of aqueous extract slowly to 25 ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

Detection of saponins

Dilute 1 ml of alcoholic and aqueous extracts separately with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponins. The saponins content was classified as follows. No froth = Negative; Froth less than 1 cm = weakly positive; Froth 1.2 cm high = Positive and froth greater than 2 cm high = strongly positive (Segelman and Farnsworth, 1969).

Detection of proteins and free amino acids

Dissolve small quantities of extracts in a few ml water and subject the solution to Biuret, Ninhydrin and Xanthoproteic tests.

Detection of phenolic compounds and tannins

Small quantity of the aqueous extract was dissolved in water and tested for the presence of phenolic compounds

and tannins with dilute ferric chloride solution (5%), 1 percent solution of gelatin, containing 10 percent sodium chloride, 10 percent lead acetate and aqueous bromine solution. Formation of a white precipitate will show the presence of phenolic compounds and tannins.

Detection of flavonoids

5 ml of dilute ammonia solution were added to the extract of each sample followed by addition of concentrated sulphuric acid. A yellow coloration was observed and it indicates the presence of flavonoids.

Antibacterial assay

The antibacterial activity of the extracts was determined by the disc diffusion method. Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth (O.D. 600=0.08) to obtain a bacterial suspension of 10 8 CFU/ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. The test samples were applied on sterile paper discs (6 mm diameter) and placed on the inoculated agar surface. A 20 µl of the extracts (100 mg/ml) were loaded on to the filter paper discs and were allowed to dry completely. Standard antibiotic Streptomycin 10 µg/disc was placed as standard. Plates were incubated at 37° C for 24 h. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicate.

Antifungal assay

For the evaluation of antifungal effects, PDA medium was incubated with fungal cells. The plates were incubated for 3 days at 25° C. Further processes were repeated as above mentioned.

RESULTS

Phytochemical analysis revealed that Methanol, ethanol, acetone and aqueous extracts of *Delonix elata* and *Prospis cineraria* leaves contains, alkaloids, flavonoids, phytosterol, sapponins, tannins and phenolic compounds (Table 1 and 2). Table 3 shows the invitro antibacterial activity of various extracts of *Delonix elata* and *Prospis cineraria* leaves. Alcoholic extracts of leaves showed maximum inhibition against all the bacterial species.

Table 4 expressed antifungal activity of all the extracts of *Delonix elata* and *Prospis cineraria*.

DISCUSSION

Screening of the two plant extracts and plant products for antimicrobial activity has shown that higher plants represent potential sources of new-anti-infective agents. The organic extraction of plants (especially alcoholic extracts) greater activity than aqueous extracts. Hence the study suggests that the organic solvent especially alcoholic solvent is suitable to screen for the antibacterial activity.

The result of present study reveals that the employed extracts of plants exhibited potential antibacterial activity against the tested pathogens. The study also supports the view that several medicinal plants might be useful as antimicrobial agents. In the present study the notable activity was observed against all tested micro organisms. This shows that these two plants can be used for medicinal purposes. Since earlier studies on phytochemicals reported the antibacterial activity of terponids, saponins, tannin, alkaloids and flavonoids isolated from plant materials (Mahmoud et al., 1999; Tsuchiya et al., 1996). The presence of phytochemicals in this study might be a factor for the antibacterial activity of Delonix elata and Prospis cineraria leaves. In the present study the maximum activity was observed against all the species using Delonix elata and Prospis cineraria leaves. Thus, these plants can be useful, seems to be a potential source for arresting the growth and metabolic activities of various general bacteria and fungi. The exact dosage concentration and the synergistic antimicrobial activity of Delonix elata and Prospis cineraria leaves will be studied further.

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