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PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL STUDIES OF *DICLIPTERA PANICULATA,* (FORSSK.) I. DARBYSH

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ABSTRACT

Dicliptera paniculata,(Forssk.) I. Darbysh. is a plant belongs to the family Acanthaceae. Considering the less toxicity, notable medicinal property and least public awareness on the usefulness of the plant, the present study focused on the phytochemical, antibacterial, antifungal and antioxidant properties of the aerial part of the plant. Phytochemical screening and antioxidant potential of petroleum ether, acetone, 1-propanol and water extracts were carried out using standard procedures. The antibacterial activity was tested against *Serratia marcescens* (MTCC 97), *Proteus vulgaris* (MTCC 426), *Bacillus cereus* (MTCC 430), *Enterococcus faecalis* (MTCC 439), *Escherichia coli* (MTCC 443) and antifungal activity against *Aspergillus* sps., and *Fusarium* sps., were tested using standard procedures. The results revealed the presence of phytoconstituents such as alkaloids, carbohydrates, glycosides, saponin, phytosterol, tannin, flavanoid etc. The extracts showed a noteworthy antioxidant potential, antibacterial and antifungal activity.

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INTRODUCTION

The plants occurring in our surroundings are of immense medicinal value with therapeutic action against various diseases. The efficacies of most of these plants are not utilised in appropriate situations such as for treating diseases, as antiseptics, as first aids etc. due to the lack of knowledge on the therapeutic potential and medicinal values of them. Due to deficient awareness on the usefulness and lack of scientific studies on the therapeutic potential of these natural sources of medicine, such plants are being displaced as useless or weedy plants by human beings. *Dicliptera paniculata* (Fig. a) is an auxiliary plant species that was first described by Peter Forsskål, and now became known as I.Darbysh. *D. paniculata* is part of the genus *Dicliptera* and the family Akantus plants. The plant has the synonyms *Peristrophe paniculata, Peristrophe bicalyculata* etc. It is one of the traditional herbs recommended in cases of tuberculosis. In Uttar Pradesh, the paste of the plant is used for sprain and bone fracture. The herb is said to possess number of other therapeutic properties also, such as expectorant, analgesic, anti-inflammatory, antipuretic, antibacterial etc (Satyanarayana *et al.,* 1993).

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MATERIALS AND METHODS

Solvent extract preparation: The plant material was collected from different parts of Palakkad district, Kerala and was shade dried, powdered (Banu and Cathrine, 2015) and the extract was prepared using soxhlet apparatus (Azwanida, 2015).

Qualitative phytochemical screening: Various phytochemical tests were carried out using standard procedures in order to determine the presence of phytochemical compounds such as alkaloids, carbohydrates, glycosides etc (Das *et al.* 2015).

Test Organisms: *Serratia marcescens* (MTCC 97), *Proteus vulgaris* (MTCC 426), *Bacillus cereus* (MTCC 430), *Enterococcus faecalis* (MTCC 439), *Escherichia coli* (MTCC 443), *Aspergillus* sps., *Fusarium* sps.

In vitro antibacterial activity test: Antibacterial activity of the plant material was determined by means of standard agar disc diffusion assay (Balouiri, *et al*., 2016). Each disc was impregnated with 200 ug/ml of the extract. Tetracyclin served as positive control whereas the extraction solvents as negative control.

In vitro antifungal activity test: In vitro evaluation of antifungal effect of crude extract as was performed by culturing the fungal pathogens in an extract containing PDA medium. *Aspergillus* sps. and *Fusarium* sps. were isolated from diseased tomato and ginger rhizome respectively (Skidmore and Dickinson, 1976).

In-vitro anti-oxidant activity test: The antioxidant potential of the plant extract was tested using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Saeed *et al.,* 2012)and Modified Ferric Ion Reducing Antioxidant Power (FRAP) Assay (Pulido *et al.,*2000).

RESULTS

Preliminary phytochemical screening: The preliminary phytochemical screening of *D. paniculata* carried out in different solvent extracts of the aerial part showed the presence of various phytoconstituents such as alkaloid, glycosides, flavanoids, carbohydrate, phytosterol, saponin, tannin, protein and amino acid (Table 1).

In vitro antibacterial activity: All the solvent extracts of the plant expressed significant antibacterial activity against different strains of test organisms (Fig. b, c). Highest antibacterial activity was observed in the disc impregnated with acetone and 1-propanol ranging from 12mm to 15mm. (Table 2).

In vitro antifungal activity: When the fungal isolates were allowed to grow in the extract containing PDA medium, considerable inhibition percentage ranging from 33.8% to 56.33% was obtained against *Fusarium* and *Aspergillus* sps. (Table 3) (Fig. d, e).

In vitro Antioxidant activity

DPPH scavenging assay: 1-propanol extract possessed highest antioxidant potential than other three extracts with an activity percentage of 58.59%. This is followed by acetone, petroleum ether and extract with highest scavenging activity of 52.00%, 48.80% and 39.84% respectively. Moreover, at a concentration of 20µg, acetone extracts dominates in the activity than rest of the extracts with 23.20%. (Table 4)

Table 1. Summary of preliminary phytochemical analysis for the different solvent extracts of *Dicliptera paniculata*

Qualitative Phytochemical Analysis Of The Plant Extract						
Sl.No	Phytochemical test	Phytoconstituent	Solvent			
			Petroleum ether	Acetone	1-Propanol	Water
1	Hager's test	Alkaloid	+	+	+	-
2	Molisch's test	Carbohydrate	-	+	-	+
3	Legal's test	Glycoside	+	-	-	-
4	Foam test	Saponin	-	-	+	-
5	Salkowski's test	Phytosterol	-	+	-	+
6	Ferric chloride test	Phenol	-	-	-	-
7	Lead acetate test	Tannin	-	-	+	-
8	Alkaline reagent test	Flavanoid	+	+	-	-
9	Xanthoproteic test	Protein	-	-	-	+
10	Ninhydrin test	Aminoacid	-	-	-	+
11		Gums & Mucilage	-	-	-	-

** (+) sign indicates the presence of the compound whereas the (-) sign indicates the absence of the compound.

Table 2. Summary of inhibitory activity of different solvent extracts of *Dicliptera paniculata* against test organisms

Sl. No.	Test bacterium	Zone of inhibition(mm) in different solvent extracts				+ve control
		Petroleum ether	Acetone	1-Propanol	water	Tetracyclin
1	*Serratiamarcescens (MTCC 97)*	12	-	12	-	20
2	*Proteus vulgaris (MTCC 426)*	-	18	13	11	25
3	*Bacillus cereus (MTCC 430)*	-	15	12	12	27
4	*Enterococcus faecalis (MTCC 439)*	-	15	13	-	23
5	*Escherichia coli (MTCC 443)*	-	15	-	-	25

** (-) sign indicates the absence of inhibitory activity against the test organism

Table 3. Summary of antifungal activity of *Dicliptera paniculata* against test organisms

IN VITRO ANTIFUNGAL EFFECT							
Sl.No	Fungus	Concentration					
		5%		10%		15%	
		RG	INH %	RG	INH %	RG	INH %
1	*Aspergillus*sps.	3.9	37.09	3.6	41.93	3.2	48.38
2	Fusariumsps.	4.7	33.8	3.9	45.07	3.1	56.33
	RG - Radius of Growth in cm				INH % - Inhibition percentage(%)		

Table 4. Summary of DPPH radical scavenging activity of *Dicliptera paniculata*

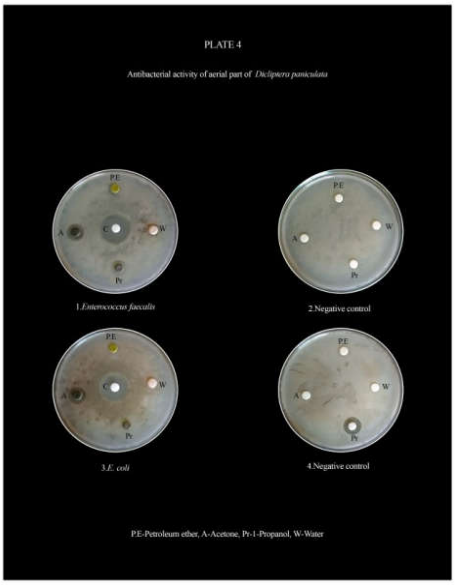
Sl.No.	DPPH Scavenging activity(%)				
	Concentration of extract(µg/ml)	Petroleum ether	Acetone	1-Propanol	Water
1	20	18.40±0.001	23.20±0.002	19.53±0.001	11.71±0.001
2	40	25.60±0.001	30.40±0.003	28.90±0.002	16.40±0.002
3	60	35.20±0.002	37.60±0.001	40.62±0.002	23.43±0.003
4	80	42.40±0.001	44.80±0.001	51.56±0.003	32.81±0.001
5	100	48.80±0.002	52.00±0.001	58.59±0.001	39.84±0.002

Table 5. Summary of Ferric ion reducing power of *Dicliptera paniculata*

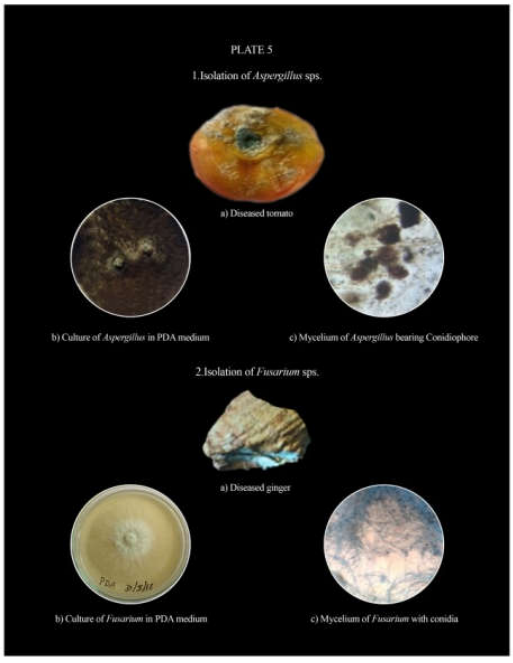
Sl.No	Concentration of extract(µg/ml)	% inhibition				
		Ascorbic acid	Petroleum Ether	Acetone	1-Propanol	Water
1	20	48.72%	19.60%	28.47%	42.94%	18.10%
2	40	52.10%	21.33%	32.80%	46.16%	19.23%
3	60	56.31%	26.35%	39.65%	50.79%	20.37%
4	80	63.42%	28.93%	46.10%	58.03%	21.59%

a

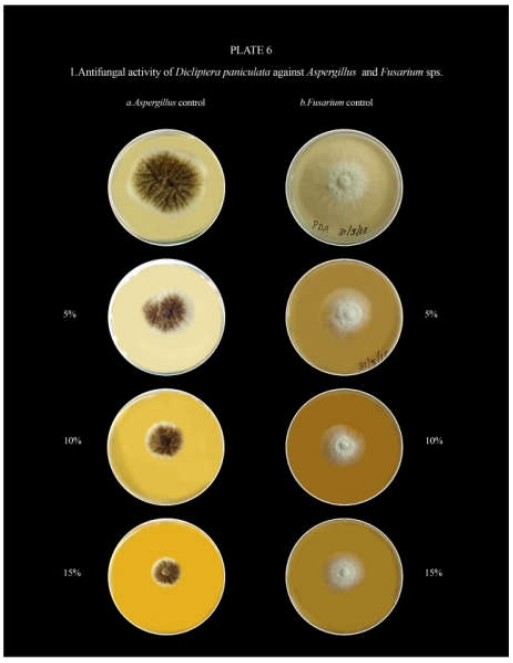
b



c



d



e

a – Habit
b – Antibacterial activity of *Dicliptera panilculata*
c - Antibacterial activity of *Dicliptera panilculata*
d – Isolation of fungal pathogen
e – Antifungal activity of *Dicliptera paniculata*

FRAP assay: The FRAP assay reveals that, 1- propanol extracts shows highest reducing power compared to other extracts with a maximum activity of 58.03% at a concentration of 80µg/ml. This is followed by acetone with its highest activity of 46.10% at the same concentration. The highest activity of 28.93% and 21.59% was performed by petroleum ether and water respectively at this concentration. Meanwhile, the positive control ascorbic acid showed an activity percentage of 63.42 at 80µg/ml (Table 5).

DISCUSSION

Results of qualitative phytochemical screening of different solvent extract correspond to the outcomes of the work carried out in *Dicliptera paniculata* – a review (Rashmi *et al.,* 2010) and screening of antimicrobial ethanolic extract of *D. paniculata* (Giwa *et al.,*2010). As there are no previous records of the analysis of phytoconstituents in 1-propanol extract of *D. paniculata*, this finding will be a new contribution to the knowledge base for further study and evaluation of pharmaceutical property of the plant. Antibacterial studies carried out in *D. paniculata,* the acetone extracts of *D. paniculata* performed maximum zone of inhibition against *S. aureus* (14 mm) followed by *B.cereus* (11 mm) and *S. typhi* (10 mm). The acetone extracts were failed to demonstrate the inhibition against *E. aerogenes* and *E. coli* (Janakiraman *et al*., 2012). No other records of antibacterial studies are found in petroleum ether, 1-propanol and water extract of *D. paniculata.* Antimicrobial potency of crude ethanolic extract of *Dicliptera paniculata* against *Aspergillus niger, Aspergillus clavatus, Rhizopus stolonife r*recorded as 1.8cm, 1.5cm and 2.2cm (zone of inhibition) respectively (Giwa *et al.,* 2010). All of the test samples showed significant levels of antioxidant properties and that varied according to the solvent taken. 1-Propanol extract of *D. paniculata* performed strongest DPPH radical scavenging activity when compared with the other three extracts. The activity range of different extract against DPPH and FRAP is as follows: 1-Propanol > acetone > petroleum ether > water. The works on ferric ion reducing power of extracts of *D. paniculata* has not been found to be enumerated much. Thus the present reports of the ferric reducing power of different extracts of the plant could be significant and can be considered for further studies and analysis. This significant activity may be due to environmental impact on phytochemical production.

Conclusion

The aerial part extracts of *Dicliptera paniculata* can be considered for effectively healing the bacterial borne diseases as well as against against Aspergillosis and other fungal borne diseases. By considering the therapeutic potential of the plant, a detailed analysis of individual plant parts can be contribute a leap towards the drug discovery for various diseases.

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