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Phytochemical and Antimicrobial Activity of *Digera Muricata* (L.) Mart.

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Abstract: The successive Soxhlet extract of *Digera muricata* (L.) Mart. (Amaranthaceae) were extracted using petroleum ether, chloroform, ethanol and distilled water in ascending order of the polarity. These extracts were subjected to screening of preliminary phytochemicals tests. The results indicate the presence of alkaloids, flavonoids, phenols, tannins, terpenes and saponins. These extracts further subjected to the antimicrobial activity. Among the bacteria used, the petroleum ether extract gave highest zone of inhibition at 400 µg/well against *V. cholerae*. Similarly, in fungi the ethanol extract exhibited highest zone of inhibition at 400 µg/well against *Candida albicans.*

Keywords: Digera muricata, Phytochemicals, Antimicrobial activities.

Introduction

The *Digera muricata* (L.) Mart (Amaranthaceae) wild edible plant commonly known as 'Cancali soppu'. It is commonly distributed throughout the India. In Ayurveda the herb is considered as a cooling, astringent to the bowels and also used as laxative. The flowers and seeds are used to treat urinary discharges[1]. Boiled root infusion given to mother after child birth for lactation purpose[2].

The bacterial and fungal diseases are a major threat to human population and domestic animals, the death caused by infectious diseases ranked 5th, has become the third leading cause of death[3]. Plant derived antimicrobial agents have been largely overlooked. Hence, the *D. muricata* (L.) Mart. is used in both folk and traditional system of medicine. The present investigation is carried out to rationalize the therapeutic claims as antimicrobial activity using few pathogenic bacteria and fungi.

Experimental

The plant material of *Digera muricata* was collected from the agricultural field of Humnabad Taluk in Bidar district, Karnataka, India in January 2008 and identified with the help of

Flora of Gulbarga District[4] and authenticated. The voucher specimen was deposited (HGUG - 811) in herbarium at the Department of Botany, Gulbarga University, Gulbarga.

Extraction of plant material

The plant, *Digera muricata* was washed with acetone and shade dried, coarsely powdered and extracted with petroleum ether, chloroform, ethanol (Hi-media, Mumbai) and distilled water sequentially in Soxhlet for 24 h each. The extracts were evaporated to dryness *in vacuo,* percentage of extracts colour and odour was calculated.

Preliminary phytochemical tests

The extracts of *Digera muricata* were dissolved in their respective organic solvents and were subjected for preliminary phytochemicals test[5] to screen the presence of various secondary metabolites like alkaloids, flavonoids, phenols, glycosides, sterols, saponins, terpenoids, lignin and tannins.

Antimicrobial activity assay

Antimicrobial activity of organic solvent extracts was determined by cup diffusion method on nutrient agar medium[6], for bacterial and potato dextrose agar (PDA) medium for fungi. Cups were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 10^{-6} CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µL each of all solvent extracts were placed and the cups medium inoculated plates. The treatments also included 50 µL of DMF separately which served as control. Streptomycin sulphate and Nistatin 1 µg/mL at their respective recommended dosage were treated for comparative efficacy. The plates were incubated for 24 and 38 h for bacterial and fungi and temperature maintained 37 ^{0}C and 27 ^{0}C respectively. The zone of inhibition around the wells was measured in mm (millimeter). For each treatment five replicates were maintained. The data was subjected to statistical analysis using SPSS for windows software.

Results and Discussion

The preliminary phytochemical tests result indicate the presence of phenol, flavonoids, alkaloids, terpenes, sterols, tannins, glycosides and lignins test indicate (Table 1). The quantitative estimation of primary and secondary metabolite results shows higher amount of terpenoids and tannins secondary and carbohydrates from primary metabolites (Table 2).

Antibacterial study

The organic successive Soxhlet extracts of *Digera muricata* (L.) Mart. *i.e.,* petroleum ether, chloroform, ethanol and distilled water, have shown significant zone of inhibition of bacterial growth at the concentrations of 200 and 400 µg/well against these bacterial strains (Table 3).

The growth of *K. pneumonia*, *E. coli*, *S. aureus, S. typhi* and *V. cholerae* was inhibited considerably particularly at the higher dose (400 µg/well). The highest growth of inhibition were recorded in *V. cholerae* in petroleum ether extract (19.13±0.38) followed by *K. pneumonia* (18.16±0.94), *S. typhi* (16.12±0.98), *E. coli* (15.18±0.17) and *S. aureus* (15.16±0.12). Similarly, in chloroform extracts, the maximum zone of inhibition were observed in *E. coli* (18.62±0.17) followed by *S. aureus* (18.09±1.32), *K. pneumonia* (17.93±0.65), *S. typhi* (15.18±1.03) and *V. cholera* (13.17±0.99). In ethanol extracts maximum zone of inhibition was observed in *V. cholerae* (18.16±0.88) followed by *S. aureus* (17.16±0.19, *E. coli* (16.93±017), *K. pneumonia* (16.14±1.07) and *S. typhi* (16.19±0.33). However, in aqueous extracts the moderate activity is recorded in *V. cholerae* (13.16±1.20), followed by *E. coli* (12.12±0.16, *S. typhi* (10.93±1.80), *S. aureus* (10.16±0.13) and *K. pneumonia* (09.16±0.96) respectively.

Table 1. Preliminary phytochemicals screening tests of secondary metabolites in *Digera muricata.*

Tests	Pet. ether	$CHCl_3$	Et-OH	Aqu
Test for Alkaloids				
a) Meyers test	+	+	+	+
b) Dragendroff's	+	+	+	+
c) Wagner's	+	-	+	-
Flavonoids				
a) Shinoda test (Mg/HCl)	+	+	-	+
b) Lead acetate	+	+	+	-
c) Pew's test	-			
d) NaOH test	+	-	+	+
Phenols Test				
a) Ellagic acid test	+	+	+	+
b) Phenols test	-	+	-	+
c) Hot water	-	-	-	-
Glycosides tests				
a) Keller-Kiliani	+	+	+	+
b) Conc. H_2SO_4	+	-	+	+
c) Molisch's test	+	-	-	-
Sterols tests				
a) Liebermann -Burchard	+	+	+	+
b) Salkowski test	+	+	+	+
Saponins				
a) Foam test	+	+	-	-
Terpenoids				
a) Salkowski test	+	+	+	+
b) Liebermann -Burchard	+	+	+	+
Lignin Test				
a) Labat	+	+	+	-
b) Lignin Test	-	-	+	-
Tannins				
a) Gelatin	-	-	+	-

Pet. ether- Petroleum ether; $CHCl_3$ - Chloroform; Et. OH- Ethanol; Aqu- Aqueous
+ - Indicates presence; - indicates absence

Table 2. Estimation of primary metabolites in *Digera muricata* (L.) Mart**.**

Metabolites	Whole plant powder, mg/100 g
Primary Metabolites	
Proteins	08.305 ± 1.13
Carbohydrates	20.900 ± 3.33
Chlorophylls	08.040 ± 2.17
Amino acids	02.382 ± 0.19
Reducing sugar	04.040 ± 1.37
Lipids	02.800 ± 0.48
Prolines	01.090 ± 0.57
Secondary Metabolites	
Phenols	0.414 ± 0.19
Flavonoids	0.214 ± 0.13
Alkaloids	0.013 ± 0.15
Terpenoids	1.270 ± 1.03
Saponins	0.857 ± 0.89
Tannins	1.412 ± 0.73

Table 3. Antibacterial activity of *Digera muricata* (L.) Mart. extracts on bacteria

Name of the bacteria	Streptomycin	Extracts (Diameter of the zone of inhibition in mm)							
		Pet. ether		Chloroform		Ethanol		Aqueous	
		I	II	I	II	I	II	I	II
Klebsiella pneumonia	16.03±1.34	16.07±1.03	18.16±0.94	16.19±0.31	17.93±0.65	15.31±1.03	16.14±1.07	-	09.16±0.96
Escherichia coli	16.17±1.16	14.06±0.83	15.18±0.17	17.16±0.93	18.62±0.17	14.16±0.91	16.93±0.17	10.16±0.12	12.12±0.16
Staphylococcus aureus	18.15±0.93	13.03±0.89	15.16±0.12	18.01±0.31	18.09±1.32	15.18±0.87	17.16±0.19	08.12±0.93	10.16±0.13
Salmonella typhi	17.05±1.94	15.18±0.39	16.12±0.98	10.13±0.21	15.18±1.03	10.93±1.93	15.19±0.33	10.93±1.60	10.93±1.60
Vibrio cholerae	17.17±1.63	17.89±1.62	19.13±0.38	07.12±1.23	13.17±0.99	16.16±1.32	18.16±0.88	11.03±0.92	13.16±1.20

**Correlation is significant at the 0.05 level ; **correlation is significant at the 0.001 level; 200 µg/well ; 400 µg/well*

Table 4. Antifungal activity of *Digera muricata* (L.) Mart. extracts on fungi

Name of the fungi	Nystatin	Extracts (Diameter of the zone of inhibition in mm)							
		Pet. ether		Chloroform		Ethanol		Aqueous	
		I	II	I	II	I	II	I	II
Aspergillus flavus	16.17±0.98	13.01±0.78	16.19±0.33	14.12±0.32	17.06±1.62	12.12±1.32	15.12±1.26	10.06±0.63	12.01±0.16
Aspergillus niger	20.16±1.76	15.16±1.62	19.18±1.32	16.12±0.16	19.03±1.23	18.16±1.23	20.12±1.63	11.07±0.16	13.01±0.73
Aspergillus terreus	16.19±1.32	14.17±0.37	18.33±0.58	15.13±0.76	17.17±0.58	14.32±0.92	16.16±1.09	10.01±0.92	11.07±0.12
Candida albicans	18.18±1.06	16.16±0.97	19.16±0.92	17.08±0.37	20.32±0.82	18.39±0.73	21.12±1.06	14.02±0.77	16.03±0.83

**Correlation is significant at the 0.05 level ; **correlation is significant at the 0.001 level; 200 µg/well ; 400 µg/well*

The above results clearly indicate that, the organic extracts showed maximum activity against all the tested pathogens. However, the poor activity recorded in aqueous extracts. Among the tested organisms *V. cholerae* shows higher zone of inhibition in petroleum ether extract at 400 µg/ml (19.13 ± 0.38) compared to all the tested extracts and Streptomycin sulphate a standard drug. Similar, activities were reported by Alam *et al.*[7], in amaranthaceae and in *achyranthes aspera* by Sunil *et al.*[8], in *alternanthera sessile* by Lina and Manuel[9], in *Amaranthus hypochondriacus*. While, least zone of inhibitions were recorded in aqueous extract. These results indicate the presences of high amount of phytoconstituents in the organic plant extracts are the responsible for the antimicrobial activity.

Antifungal studies

The organic extracts *i.e.,* petroleum ether, chloroform, ethanol and aqueous extracts have exhibited moderate to significant activity at the concentrations of 200 and 400 µg/well against *Aspergillus flavus, A. niger, A. terreus* and *Candida albicans* (Table 4).

Among which a good antifungal activity is exhibited by petroleum ether against *C. albicans* (19.6 ± 0.58) whereas, chloroform extract against the *C. albicans* (20.32 ± 0.87) and *A. flavus (*17.06 ± 1.63). However, the aqueous extract has displayed feeble activity against all the fungi.

The results clearly indicate that both the (200 and 400 µg/well) tested concentrations the maximum zone of inhibition were observed in the higher doses (400µg/well) and all the tested fungi and all the extracts. The maximum inhibition was observed against the *C. albicans* compared to standard (Nystatin). Nystatin is the most commonly used standard antifungal agent (positive control) which has strong affinity towards sterols particularly ergosterols. Similar, reports reported in Amaranthaceae by Quetin - Leclereq *et al.*[10] The antifungal activity against C*andida species* reported by Sunil *et al.*[8], in *Alternathera sessilis*.

Conclusion

D. muricata is commonly found as a weed on road side and in agricultural fields throughout India. The wild plant is edible and used in traditional medicine for curing various ailments. The presence of wide range of phytochemical constituents indicates that plant could serve as lead for the development of novel agents for various pathological disorders. However, less information is available regarding chemical constituents and there is a lack of phytochemical studies of *Digera muricata* plant. So, further in detailed phytochemical study is needed.

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