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Assessment of *Sidacordata*(burm.f.) Borsswhole plantextracts for Antimicrobial, Anthelmintic activity and Phytochemical analysis.

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ABSTRACT

The present study examined the antimicrobial, anthelminthic activity and phytochemical screening of different extracts of *Sidacordata*(burm.f.) Borss whole plant,extracts were prepared using petroleum ether, chloroform ethylacetate, ethanol and water. The Antimicrobial activity was tested against *Escherichia coli Staphylococcus aureus,Lacto bacillus,Pseudomonas aeroginosa, Candida albicans, Aspergillus niger* by agar well diffusion and MIC by 96 well Resazurin based microtiter dilution method. Significant antibiotic effect was exhibited by aqueous extract followed by ethanol extract. Anthelmintic activity was evaluated against *Pheretimaposthuma* (Indian earthworm), the time of paralysis and time of death were studied and the activity was compared with albendazole as reference standard,all the five extracts exhibited dose dependent activity and maximum activity was seen in aqueous extract. In the qualitative phytochemical analysis importantphyto constituentslike alkaloids, phenolstannins,flavonoids,anthrocyanin, catechin were detected.

Keywords: *Sidacordata, Antimicrobial, MIC, Resazurin, Anthelminthic, Pheretimaposthuma.*

1. INTRODUCTION

The whole plant ***Sidacordata*(burm.f.) Borss** has great medicinal value and is highly reputed plant in Ayurveda system of medicine. It is used as antibacterial, antitumor, antifungal, antiulcer, antitussive, anti-inflammatory, anti-malarial, antioxidant, analgesic, antidepressant, anti-hyperglycemic, hepatoprotective source. The juice of the whole plant is used in urinary infection, dysuria, and spermatorrhoea, it is used in acidity, peptic disorder, constipation, relieves flatulence and heart burn. The whole plant is used in treatment of skin diseases, rheumatism, asthma, cough, bronchitis, and diarrhea. The decoction of the entire plant is given to prevent joint swellings in arthritis. Being a nervine and brain tonic, it is useful in treatment of loss of memory, general debility and muscle wasting. It is also used to convalesce from post- abortion weakness. It has nourishing and rejuvenating properties. It is used in neuromuscular disorders with loss of function and weakness. It is especially used in chronic respiratory disorders where it helps eliminating accumulated mucus, promotes healing and restores the strength. It cures tuberculosis and hoarseness of voice. [1-3].

But it has not been explored properly and remains a silent drug in herbal medicine. Our earlier studies on antimicrobial, anthhelminthic activity and phytochemical analysis of different extracts of ***Sidacordata*(Burm.f.)Borssum** leaf, stem and root [4-5] had proved its efficacy which validates it as a traditional medicine to cure various diseases, hence present study was extended to give a scientific validation to traditional use as a source of medicine.

2. MATERIAL AND METHODS

The Fresh plant *Sidacordata*was collected from its natural habitat, from the forest region of Somawarpetin Madekeri Kodagu district Karnataka. The plant was identified and

authenticated at National Ayurveda Dietetics Research Institute Bangalore, (voucher no: RRCBI-11748). The fresh plants were washed under running tap water, shade dried at room temperature and powdered.

2.1. Extract preparation

The powdered plant samples (100 g/250 ml) were extracted successively with petroleum ether, chloroform, ethyl acetate, ethanol and water using Soxhlet apparatus at 55-85 °C for 8-10 h in order to extract the polar and non-polar compounds. For each solvent extraction, the powdered packed material was air dried and reused. The solvents of the respective extracts were reduced to semi solid and stored at 4 °C for further use.

2.2. Antimicrobial activity

2.2 .1. Test organisms

Human pathogenic organisms (bacteria and fungi) were isolated from clinical samples were used in this study (the samples were collected from Department of Microbiology, Farooqia Dental College & Hospital Mysore, India and were identified and confirmed by Microbiologist.). The organisms used were*Escherichia coli, Staphylococcus aureus, Lacto bacillus,Pseudomonasaeroginosa, Candidaalbicans and Aspergillus niger*.

2.2.2. Antimicrobial activity

Agar well diffusion method andMIC by 96 well Resazurin based Microtiter Dilution:

As described earlier. (Gulnaz.A.R&Savitha.G. 2013)

2.3 Anthelminthic Screening

2.3.1. Worm Collection:

Pheretimaposthuma(Indian earthworm) were collected from Vermiculture tank Maharani's Science College for Women, Mysore, and Karnataka, India The worms were washed with normal saline & water to remove all fecal matter and were identified by the HOD Department of Zoology, Maharani's Science College for Women, Mysore, Karnataka, India.

2.3.2. Anthelmintic Activity: As described earlier. (Gulnaz.A.R&Savitha.G. 2013).

2.4. Phytochemical tests

Screening of the above six selected medicinal plants for various phytochemical constituents were carried out using standardmethods [6-8]

3. RESULTS

3.1. Percentage of yield

Plant extracts obtained with different solvents are indicative of approximate measures of their chemical constituents extracted with the solvents from a specific amount of air-dried plant material. The percent yields (w/w) of various extracts is shown in table-1. The highest yield was recorded in aqueous medium followed by ethanol. The result indicates water and ethanol serves as a good solvent for the extraction of bioactive compounds from the whole plant ***Sidacordata*(burm.f.) Borss.**

Table -1: Percentage of yield

Solvent	Percentage
A	4.3
B	2.9
C	4.2
D	5.2
E	5.5

3.2. Antibacterial and Antifungal activity

Zone of inhibition and MIC values of different extracts for pathogenic microorganisms are presented in table 2 and 3. Different solvents and aqueous extracts of the whole plant were taken at 50µgms concentration against six important human pathogenic microorganisms. Significant activity was exhibited by aqueous extract followed by ethanol extract against all the clinical isolates in comparison with the standard drugs used Ampicillin for anti-bacterial and Nystatin as antifungal.

Table - 2: Zone of inhibition (millimeter) of different extracts *Sidacordata(burm.f.) Borss* whole plant against clinical isolates.

Source	*E.coli*	*S.aureus*	*P.aeruginosa*	*L.bacillus*	*C.albicans*	*A.niger*
Std(Ampicillin/ Nystatin)	15±0.0^{c}	16±0.23	13±0.0^{c}	16±0.0^{c}	13.5±0.3^{b}	14±0.0^{c}
A	10±0.23	11.68±2.8	10.5±1.32	11.2±0.67	-	-
B	10.84±2.3^{a}	12.3±01^{a}	10.8 ±3. 4	11 ±5.05	-	11.6±7.3^{a}
C	11.8±0.8^{a}	10.5±0.3^{a}	11.2 ±0.6^{c}	13.0±2.6^{a}	11.8±5.5^{b}	11.2±4.3^{c}
D	16.5±5.7^{b}	16.7±5.6^{b}	13. 0±9.5^{a}	16.3±5.7^{b}	12.5±6.3^{a}	13 .0±4.0^{b}
E	17.5±5.0^{c}	18.8 ±4.1^{c}	14.8±4.8^{b}	17.8±5.3^{c}	13.0±2.0^{c}	14.0±9.00^{c}

Table - 3: Minimum inhibitory concentration (MIC, mg/ml) of different extracts of *Sidacordata*(burm.f.) Borss whole plantby Resazurin micro titre-plate assay.

Source	*E.coli*	*S.aureus*	*P.aeruginosa*	*L.bacillus*	*C.albicans*	*A.niger*
Std(Ampicillin/Nystatin	0.195±0.02^{c}	0.097±0.04^{a}	0.390±0.0^{c}	0.195±0.1^{c}	0.390±0.0^{c}	0.390±0.0^{c}
A	6.0±5.0	6.0±2.04	6.0±4.30	6.0±0.19^{b}	-	-
B	6.0±2.06	6.0±0.10^{a}	6.0±0.29	6.0±0.03^{a}	-	6.0±2.0
C	6.0±0.26^{b}	6.0±0.19^{b}	6.0±0.28^{b}	1.5±0.3^{c}	-	6.0±0.39
D	03±0.0^{a}	1.5±0.23^{b}	3.0±0.15^{a}	1.5±0.65	3.0±0.12^{a}	6.0±0.23^{b}
E	1.5±0.33^{b}	0.75±0.11^{a}	3.0±0.21^{c}	0.75±0.1^{b}	3.0±0.5^{c}	3.0±0.46^{b}

Table - 4: Anthelmintic activity of different extracts of the *Sidacordata*(burm.f.) Borss whole plant whole plant.

Group	Extract	Dose (mg/ml)	Response	
			Time taken for paralysis (min)	Time taken for death (min)
I	Normal Control		NR	NR
	Albendazole	5	68.2±0.50	72.02±0.30
II		25	46.6±4.32	60.06±0.23
		50	34.5±1.00	30.24±0.20
		100	30.0±0.32	34.0 0±0.46
III a		5	72.00±0.33	122.3 ±0.80
III b		25	53.80±0.44	94.00±0.11^{a}
III c	A	50	44.5 ±0.22^{b}	51.23±0.30^{c}
III d		100	39.0 ±0.5	40.6 ±0.10^{a}
IV a		5	88.88±0.2^{c}	143.0±0.33
IV b		25	76.33 ±0.0^{a}	104.33±0.54
IV c	B	50	56.34±0.1^{a}	74.88±0.13^{b}
IV d		100	42.55 ±0.0^{a}	65.00 ±0.11^{a}
V a		5	86.50±0.2^{c}	135.45±0.78
V b		25	63.88±0.52	98.87±0.34
V c	C	50	41.54 ±0.3^{b}	48.55±0.13^{a}
V d		100	39.00±0.0^{a}	40.34±0.22^{b}
VI a		5	48.5 ±0.02^{c}	70.00±0.81
VI b	D	25	31.66±0.3^{a}	58.22±0.62^{b}
VI c		50	27 .54±0.1^{b}	45 .45±0.11^{a}
VI d		100	28.00±0.0^{a}	40.54 ±0.19^{b}
VII a		5	40.50±0.5^{c}	60.33 ±0.20^{b}
VII b		25	29.0±0.11^{b}	49.50±0.11^{b}
VII c	E	50	25.54±0.11^{a}	35.0±0.00^{a}
VII d		100	25.0±0.00^{a}	31.0±0.00^{a}

(A-Petroleum ether, B-Chloroform, C-Ethyl acetate, D-Ethanol, E-Water, - : No activity.) The values are means of triplicates ± standard deviation, the values followed by different superscript differ significantly ^{a} p<0.001, ^{b} p <0.01, ^{c}p<0.05

Table - 4: Phyto chemical analysis of different extract of *Sidacordata*(burm.f.) Borss.whole plant

		Solvents				
Phyto Compounds	**Test**	**A**	**B**	**C**	**D**	**E**
Alkaloids	a. Mayer's test	+	+	+	+	+
	b. Wager'test	+	+	+	+	+
	c. Dragondoff' test	+	+	+	+	+
	d. Hager's test	+	+	+	+	+
Phytosterols /triterpenoids	a. Liebermann test	+	+	+	+	-
	b. Salkowski test	+	+	+	+	-
Saponins	a. Froth test	-	-	+	+	+
	b. Foam test	-	-	+	+	+
Tannins	Gelatin test	+	-	+	+	+
Flavonoids	a.Alk.reagent test	+	+	+	+	+
	b. Lead acetate test	+	+	+	+	+
Glycosides	Borntrager's test	+	+	+	+	+
Fixed oil/fat	Spot test	-	-	-	-	-
Phenol	Ferric chloride test	-	+	+	+	+
Gum		-	-	-	-	-
Volatile oil		-	-	-	-	-
Coumarin		+	+	+	+	+
Emodin		+	-	+	+	+
Anthrocyanin		+	-		+	+
Anthraquinones	Sulphuric acid Test	+	+	+	+	+
Catechins	Erhlish test	+	+	-	+	+

(A-Petroleum ether, B-Chloroform, C-Ethyl acetate, D-Ethanol, E-Water,+: Present, - : Absent

3.3. Anthelmintic activity

Anthelmintic activity of different extracts and the standard are shown in table:4.The aqueous extract of the whole plant showed significantly better effect as anthelmintic source in comparison to the standard drug Albendazole.

3.4. Phytochemical analysis

The qualitative phytochemical analysis of crude extract revealed the presence of most of the phytochemicals which are tabulated in table-5. Phyto constituent extracted varied with the solvent used. Coumarins were detected in all the extracts, but steroids and anthrocyanins were detected in pet.ether, ethanol and water. Alkaloids were present in all the extracts. Phenols and emodine were present in all the extract except phenol in petroleum ether and emodine in chloroform. Tannin were found in petroleum ether, ethyl acetate, ethanol and water. Flavonoids and glycosides were found in all the extracts. Anthrocyanin and Catechin were found in all extract except chloroform and ethyl acetate.

4. DISCUSSION

The aqueous extract was found to be more potent source for both antimicrobial and anthelmintic activity.The phyto constituents responsible for were not investigated however in the qualitative phytochemical analysis of the extracts important phyto constituents like steroids, alkaloids, terpenoids, saponins, tannins, flavonoidsetc, were detected.Antimicrobial activity could be due to the presence of flavonoids, tri terpenoids and other natural poly phenolic compounds or free hydroxyl group. Presence of flavonoids, saponins, alkaloids and tannins are responsible for anthelmintic activity.

5. CONCLUSION

From the results of the present study it can be concluded aqueous extract of *Sidacordata Burma (brossa*) whole plant can be used as an easily available and cost effective natural antimicrobial, anthelminthic source.

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