A STUDY ON PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *CLEOME GYNANDRA*.L AGAINST INFECTIOUS INFANTILE DIARRHOEA.

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Summary

The preliminary phytochemical study and *invitro* antimicrobial activity of *Cleome gynandra* was investigated against some bacterial and fungal pathogens isolated from patients with infectious diarrhoea. The various solvents extract like aqueous, methanol, chloroform, petroleum ether and hexane were screened for antimicrobial activity by disc diffusion method. The preliminary phytochemical analysis of the methanol extract of the plant showed the presence of carbohydrates, alkaloids, flavonoids, amino acids, steroids, sterols and saponins. The results of antimicrobial activity revealed that methanol extract of the plant exhibit good activity compared to chloroform and aqueous extracts against all bacterial pathogens tested. Petroleum ether and hexane extracts did not show any activity. None of extracts exhibited antifungal activity. The antimicrobial activities of extracts were compared with standard antibiotics.

Key words: *Cleome gynandra*, Diarrhoea, Disc diffusion Assay, Medicinal Plants.

Introduction

Diarrhoea is one of the principle causes of death, especially in the infant population [1]. In developing countries, one out of five children dies of diarrhoea before the age of five. Each year more than 5 million people die of diarrhoea, 80% of who are children less than one year of age [2]. *Rotavirus, E.coli, Salmonella, Shigella, Vibrios* sp, *Candida* sp were the major etiological agents encountered in diarrhoea.

Diarrhoeal disease was often treated with antimicrobial drugs, but this treatment is generally ineffective, due to the presence of drug resistance. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistances of these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [3]. Thus, screening of essential compounds for developing new antimicrobial drugs from various sources has been increasing in recent years. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [4]. A wide range of medicinal plants parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twings exudates and modified plant origin. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [5].

Cleome gynandra. L belongs to the family Capparidaceae. It is commonly known as *Spider flower* is a herb indigenous to the tropical regions. It is considered to have many medicinal properties. It has been used for digestive aid and to treat stomach disorders. And used as a medication for oral infection and diarrhoea [6]. The leaves are used as disinfectants. Inhalation of the leaves relieves headache. The seeds have been used as anti-anthelminitic [7]. The present study was aimed to carry out the preliminary phytochemical analysis and to screen *invitro* antimicrobial activity against some major diarrhoeal pathogens.

Materials and Methods

Preparation of extracts

The fresh plant were collected in and around Chidambaram and were identified, confirmed and authenticated by the Department of Botany, Annamalai University, Tamilnadu, India. The voucher specimen was deposited in Herbarium. The whole plant washed, shade dried, powdered and extracted with aqueous, methanol, chloroform, petroleum ether and hexane for 48 hours with occasional shaking in a beaker. The extracts were filtered. The filtrate was dried at 50 to 60 ^{o}. The extracts were weighed and percentage yield was calculated and subjected to preliminary phytochemical analysis.

Evaluation of Antimicrobial Activity

The *in vitro* screening of antimicrobial activity was carried out using Enterotoxigenic *E.coli,* Enteropathogenic *E.coli,, Salmonella typhimurium, Salmonella entertidis, Shigella dysentriae, Shigella flexineri, Candida albicans, Candida tropicalis* and *Candida krusei,* isolated from diarrhoeal patients, attending Rajah Muthiah Medical College and Hospital, Annamalainagar, Tamilnadu, India.

The antimicrobial screening of the extracts were carried out by determining the zone of inhibition using disc diffusion method [8]. The strains were grown to logarithmic phase in nutrient broth and the inoculum was prepared by adjusting the turbidity of bacterial suspension to 0.5 McFarland's tube with nutrient broth [9]. The dried extracts were dissolved in 5% dimethyl sulphoxide (DMSO) to the concentration at 200mg/ml and finally sterilized by filtration. The sterile discs (6mm in diameter) were impregnated with 20 µl of the above extracts to achieve desired concentration of 4mg/ml. The extract discs were placed on Muller-Hinton agar plates (Himedia) for bacteria and Sabouraud's dextrose agar (Himedia) for *Candida*, which were previously inoculated with test strains and incubated at 37^{o}C for 24 hours. Amikacin disc (10µg) and fluconazole disc (10µg) were used as positive control for bacteria and *Candida* respectively. 5% DMSO impregnated discs was used as negative control and the zones of inhibition were recorded. The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay by using two-fold serial dilution method. MIC's were interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity. All the MIC tubes, which did not show any turbidity, were streaked over the Muller–Hinton agar plates The minimum bactericidal concentration was recorded as the lowest concentration (MBC) that did not permit any visible growth on the plates after the period of incubation. Observation and results are given in Table 1 and Table 2.

Results and Discussion

Results of antimicrobial assay showed that aqueous, methanol, chloroform extracts exhibited antimicrobial activity against *E.coli*, *Salmonella* spp and *Shigella* spp. The antimicrobial activity in terms of zone of inhibition is shown in Table 1. The methanol extract exhibited relatively wide zone of inhibition on the three enteric bacterial pathogens as compared with chloroform and aqueous extracts. Petroleum ether and hexane extract did not exhibit any activity. None of the extract exhibit antifungal activity. Minimum inhibitory concentration of active extracts is shown in Table. 2. The MIC values observed for methanol extract was 2.5 mg/ml for *E.coli* strains and 5 mg/ml for *Salmonella* sp. The MBC values observed for *E.coli* was 5 mg/ml and for *Salmonella* sp was 10 mg/ml. Aqueous and chloroform extracts exhibited MIC and MBC values for *E.coli* and *Salmonella* sp. were 5 mg/ml and 10 mg/ml respectively. The total yield for methanol extract was 8.5%, aqueous extract 5.8%, chloroform extract was 5.5%, petroleum ether extract 5% and hexane extract 4.2%. Preliminary phytochemical analysis of methanol extract showed the presence of carbohydrates, flavonoids, Alkaloids, amino acids, steroids and saponins.

Table.1 Antibacterial activity of *Cleome gynandra* . L.

Solvent extracts	Conc. of disc	Zone of inhibition of *Cleome gynandra* (mm)							
		ETEC	EPEC	S.t	S.e	Sh.d	Sh.f	C.a	C.t
Aqueous	4mg/ml	12	12	12	14	16	14	-	-
Methanol	4mg/ml	18	16	18	18	16	14	-	-
Chloroform	4mg/ml	14	14	14	14	14	14	-	-
Petroleum ether	4mg/ml	-	-	-	-	-	-	-	-
Hexane	4mg/ml	-	-	-	-	-	-	-	-
Amikacin	10 µg	24	22	20	20	24	24	Nt	Nt
Fluconazole	10 µg	Nt	Nt	Nt	Nt	Nt	Nt	-	-
DMSO	5%	-	-	-	-	-	-	-	-

Above values are the means of three assays. -: no activity, Nt – not tested
ETEC- Enterotoxigenic *E.coli*, EPEC- Enteropathogenic *E.coli*, S.t- *Salmonella typhimurium*, S.e- *Salmonella enteritidis*, Sh.d- *Shigella dysenteriae*, Sh.f- *Shigella flexineri*, C.a- *Candida albicans*, C.t- *Candida tropicalis* and C.k- *Candida krusei*.

Table. 2 Antimicrobial activity of *Cleome gynandra*. L extracts by two-fold serial dilution method

Extracts		MIC and MBC values (mg/ml)			
		ETEC	EPEC	S.t	S.e
Aqueous	MIC	5	5	5	5
	MBC	10	10	10	10
Methanol	MIC	2.5	2.5	5	5
	MBC	5	5	10	10
Chloroform	MIC	5	5	5	5
	MBC	10	10	10	10

MIC- Minimum inhibitory concentration, MBC- Minimum bactericidal concentration, ETEC- Enterotoxigenic *E.coli*, EPEC-Enteropathogenic *E.coli*, S.t–*Salmonella typhimurium*, and S.e - *Salmonella enteritidis*.

The results reveal that the methanol extract of *Cleome gynandra* was effective against *E.coli* and *Salmonella* strains associated with infectious diarrhoea. Studies by Ajaieoba, shows methanol extract of leaves and stem *Cleome gynandra* exhibited activity against gram positive and gram negative bacterial pathogens and also possess antifungal properties [10].

From this study it is evident that the methanol extract has the *invitro* inhibition potential against various pathogens that cause infectious diseases. However, further identification and isolation of the single compound and *invivo* activity was required for development of novel bioactive antimicrobial compounds that are responsible for the antibacterial effects.

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References

1. Zavala MA, Perez S, Perez C, et *al*. Antidiarrhoeal activity of *Walthria Americana*, *Commelins cuelestries* and *Altermanthra repens*. J Ethnopharmacol 1998; 61: 41-47.
2. Bern C, Martines J, DeZoysa I, Glass OL. The magnitude of the global problem of diarrhoeal diseases: A ten-year update. Bull World Health Organ 1992; 70: 705-714.
3. Cohen ML. Epidemiology of drug resistance: implication for a post antimicrobial era. Science 1992; 257: 1050-1055.
4. Newman DJ, Cragg GM, Snader KM. Influence of natural products upon drug discovery. Nat Prod Res 2000; 17: 215-234.
5. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: sources of industrial and medicinal materials. Science 1985; 228: 1154-1160.
6. Kirikar KR, Basu BD. Indian Medicinal Plants, Vol II, International Book Publication, Distribution. Dehradun. 1981; 956-960.
7. Ajaiyeoba OE, Onocha PA, Olarenwaju OT. Invitro anthelminitic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra*. J Pharmaceut Biol 2000; 43: 334-338.
8. Sahoo S, Kar D, Mohapatra S, Rout SP, Dash SK. Antibacterial activity of *Hybanthus enneaspermus* against selected UTI pathogens. Indian J Pharm Sci 2006; 68: 653-655.
9. Mc Farland J. Standardization of bacterial culture for disc diffusion assay. J Am Med Ass 1987; 49: 1176-1178.
10. Ajaiyeoba OE. Phytochemical and antimicrobial studies on *Gynandropsis Gynandra and Buchholzia coriaceae* extracts. Afr J Biomed Res 2000; 3: 161-165.