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Research Article

BIO-PROSPECTING OF *CLEISTANTHUS COLLINUS* AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: This present study was planned to resolve the phytochemical profile of the plant leaf extracts prepared by two different (hot and cold) extraction methods and to determine the bactericidal efficiency of *Cleistanthus collinus.*

Methods: Preliminary phytochemical analysis of *C. collinus* leaf crude extracts (methanol, ethanol, ethyl acetate and aqueous) were performed according to the Odebiyi method. The bactericidal efficiency of *C. collinus* leaf crude extracts were tested by standard microbiological methods against *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus, Listeria monocytogenes* (Gram-positive).

Results: The presence of secondary metabolites like alkaloids, flavonoids, steroids, terpenoids, tannins, phlobatannins, glycosides and saponins were observed in preliminary phytochemical analysis of *C. collinus* leaf crude extracts. Analysis of bactericidal efficiency for the various solvent based extracts revealed that methanol extract of *C. collinus* was found to be intensive antibacterial activity [> 18 mm inhibition zone; MIC (0.27 - 0.42 mg/ml); MBC (0.35 – 0.50 mg/ml)] which is followed by ethanol extract and other extracts.

Conclusion: From this study, it is obvious that the *C. collinus* leaf crude extract could be exploited for the preparation of new and efficient green medicine to treat various infectious diseases for both human being and veterinary animals.

Keywords: *Cleistanthus collinus,* Phytochemistry, Bioprospecting, Antibacterial activity, MIC, MBC.

INTRODUCTION

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First it is very likely that these phytochemical will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested in humans. Scientists realize that the effective life span of any antibiotic is limited, so new sources especially plant sources are also being investigated. Second the public is becoming increasingly aware of the problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over the counter from herbal suppliers and national food stores and the self medication with these substances is a common practice to certain extent [1].

Cleistanthus collinus (Euphorbiaceae) is a naturally distributed in the dry forests of the southern and central parts of India [2-3]. Many parts of the plants were reported as toxic and the aqueous extract of the crushed leaves is used as a cattle and fish poison, abortifacient, suicidal and homicidal agent in Malaysia and Africa. *C. collinus* leaves contain saponin, tannin, oduvin and the poisonous effect attributed to oduvin usually occurs after drinking the decoction of the leaves of *C. collinus* leading to death within 1-3 days [4-5]. *In-vitro* studies carried out by Pradheep Kumar *et. al.,* [6] revealed that Cleistanthin B, isolated from the poisonous plant *C. collinus* (Rox B) showed potent anticancer properties. The alcoholic extract of the whole plant was tested for anticancer activity in human epidermoid carcinoma of nasopharynx in culture, Walker carcinoma sarcoma 256 in rats and L-1210 lymphoid leukaemia in mice. The extract showed significant anticancer activity against human epidermoid carcinoma of the nasopharynx [7].

C. collinus is used as washing agent for clearing septic wound, cure fungal diseases [8]. With this backdrop information we had carried out preliminary phytochemical screening and antibacterial bioassay test for methanol, ethanol, ethyl acetate and aqueous solvents based leaf extract of *C. collinus* against *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa* (Gram-negative), and *Staphylococcus aureus, Listeria monocytogenes* (Gram-positive).

MATERIALS AND METHODS

Collection of Plant sample

The plant material *C. collinus* used for this research work was collected in August, 2011 from plains of Virallimalai, Tamilnadu, India.

Plant extraction

Cold extract: The powdered leaf material was soaked (1:4w/v) with selected solvents like aqueous, ethanol, ethyl acetate and methanol at 24 h in room temperature. The crude material was filtered through muslin cloth and the filtrate was concentrated naturally.

Hot extract: 350g of plant sample was used for the hot crude extract preparation. Distilled aqueous, ethanol, ethyl acetate and methanol were used as solvents, crude extract were concentrated and used for further analysis.

Phytochemical screening

Preliminary phytochemical screening was carried out in powder and extracts (wherever required) using standard procedure described by Odebiyi method [9] with few modification to identify the constituents such as Tannins, Phlobatannins, Glycosides, Saponins, Flavonoids, Steroids, Terpenoids and Alkaloids.

Collection of test pathogens

Bacterial strains examined in this study were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacterial strains used in this work were as follows *Escherichia coli (MTCC-433), Salmonella typhi (MTCC-733), Klebsiella pneumoniae (MTCC-432), Pseudomonas aeruginosa (MTCC-741), Staphylococcus aureus (MTCC-1430)* and *Listeria monocytogenes (MTCC-1143)*.

Antimicrobial activity assay

The antibacterial potentialities of plant extracts were evaluated by agar well diffusion method using Mueller Hinton agar medium [10]. The pathogens were activated by inoculating a loopful of the strain in the nutrient broth (20 ml) in a 100 ml Erlenmeyer flask and incubated at 37°C on a rotary shaker for 24 h. Then 0.1 ml of fresh inoculum (containing around 1-2 × 10^6 cfu / ml as per McFarland

standards) was spread onto the surface of sterile Mueller Hinton agar using a sterilized glass spreader. Wells were made on the seeded plates with the help of a sterilized cork borer (8mm Hi-Media). The collected different extracts were further dissolved in DMSO (dimethyl sulfoxide, 4%, v/v). The diluted extracts (100µl of 10mg/ml concentration) were dispended into the well and the plates were incubated aerobically at 37°C for bacteria. In the same way a positive control wells were made with only streptomycin (20µg) respectively. The entire microbial assay was carried out under strict aseptic conditions. The zones of inhibition (mm) of the different extracts were examined after 24 h.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC of the plant extracts were determined by diluting the raw extracts in various concentrations (0.10mg-1mg/ml) in nutrient broth were mixed in a test tube and 0.1 ml of active inoculum was added to each tube. The tubes were incubated aerobically at 37°C for 24 hr. Two control tubes were maintained for each test batch that included as antimicrobial control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium and the inoculum). The lowest concentration of the extract that completely inhibited bacterial growth (no turbidity) in comparison to control was regarded as MIC. The MBC was determined by sub culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the organisms was considered as MBC [11].

RESULTS

The preliminary phytochemical screening of the fresh leaf extracts of *C. collinus* revealed the presence of tannins, terpenoids, flavonoids, saponins, glycosides, steroids, phlobatanins and alkaloids were qualitatively screened in all the leaf extracts of the three solvents used for the extraction, methanol was found to be efficient in extracting maximum number of secondary metabolites, followed by ethanol and ethyl acetate hot and cold extracts (Table 1). The hot methanol extract showed profound antibacterial activity (18-30 mm inhibition zone), MIC (0.27- 0.42 mg / ml) and MBC (0.35 – 0.50 mg / ml) values against all tested pathogens followed by other hot extracts, but all cold extract showed only zone of inhibition in *S. typhi* (9-20mm) and other tested pathogens showed resistance at 1 mg concentration (Table 2) for control experiment streptomycin showed activity with the zone of inhibition (30mm-35mm) against tested pathogens.

The MIC values of plant extracts were calculated for all the target pathogens and it ranges from 0.10- 1 mg / ml and represented in Table 3. Among the tested plant extracts, methanol, ethanol, ethyl acetate and aqueous showed the low MIC values (0.27 -0.72 mg/ml). The methanol and ethanol extracts of *C. collinus* showed the best activity (lowest MIC values). The MBC values (Table 4) varied from extract to extract and it was significant at 0.35 – 0.90 mg / ml. The lowest MBC values of 0.35-0.85 mg /ml were found in methanol, ethanol and ethyl acetate extracts of *C. collinus* against *E. coli, S. typhi, K. pneumoniae, P. aeruginosa*, *S. aureus* and *L. monocytogenes* respectively. Among them aqueous extract showed the lowest MBC value of 0.90 mg /ml.

Table 1: Preliminary phytochemical screening from *C. collinus* leaf extracts

Test	Fresh extract	Aqueous extract		Ethanol extract		Ethyl acetate extract		Methanol extract	
		H	C	H	C	H	C	H	C
Tannins	+	+	+	+	-	+	-	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Glycosides	-	+	+	+	+	+	+	+	+
Steroids	-	-	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Phlobatannins	-	-	-	+	+	-	-	+	+
Saponins	+	+	+	-	+	+	-	+	+
Alkaloids	-	-	-	+	+	+	+	+	+

H - Soxhlet extraction; C - Cold extraction; (+) – Present; (-) – Absent

Table 2: Antibacterial activity of *C. collinus* leaf extracts.

Pathogens	Zone of inhibition in mm (Concentration 1mg)								
	Methanol extract		Ethanol extract		Ethyl acetate extract		Aqueous extract		Streptomycin (20µg)
	H	C	H	C	H	C	H	C	
Escherichia coli	26	-	24	-	16	-	12	-	35
Salmonella typhi	30	20	24	14	18	9	14	10	11
Klebsiella pneumoniae	18	-	16	-	-	-	-	-	35
Pseudomonas aeruginosa	22	-	18	-	-	-	-	-	33
Staphylococcus aureus	22	-	17	-	-	-	-	-	32
Listeria monocytogenes	18	-	18	-	-	-	-	-	37

H - Soxhlet extraction; C - Cold extraction

Table 3: Determination of Minimum Inhibitory Concentration (MIC) for the *C. collinus* leaf extracts.

Pathogens	Minimum Inhibitory Concentration (MIC) in mg/ml							
	Methanol extract		Ethanol extract		Ethyl acetate extract		Aqueous extract	
	H	C	H	C	H	C	H	C
Escherichia coli	0.40	-	0.56	-	0.54	-	0.72	-
Salmonella typhi	0.27	0.38	0.40	0.58	0.57	0.76	0. 66	0. 72
Klebsiella pneumoniae	0.42	-	0.52	-	0.52	-	-	-
Pseudomonas aeruginosa	0.36	-	0.44	-	-	-	-	-
Staphylococcus aureus	0.42	-	0.54	-	-	-	-	-
Listeria monocytogenes	0.32	-	0.47	-	0.67	-	-	-

H - Soxhlet extraction; C - Cold extraction

Table 4: Determination of Minimum Bactericidal Concentration (MBC) for the *C. collinus* leaf extracts

Pathogens	Minimum Bactericidal Concentration (MBC) in mg/ml							
	Methanol extract		Ethanol extract		Ethyl acetate extract		Aqueous extract	
	H	C	H	C	H	C	H	C
Escherichia coli	0.45	-	0.65	-	0.65	-	0.85	-
Salmonella typhi	0.35	0.45	0.45	0.65	0.70	0.85	0.75	0.80
Klebsiella pneumoniae	0.50	-	0.55	-	0.60	-	-	-
Pseudomonas aeruginosa	0.45	-	0.55	-	-	-	-	-
Staphylococcus aureus	0.50	-	0.60	-	-	-	-	-
Listeria monocytogenes	0.40	-	0.55	-	0.75	-	-	-

H - Soxhlet extraction; C - Cold extraction

DISCUSSION

Preliminary phytochemical screening revealed the presence of major phytochemicals in both hot and cold leaf extract of *C. collinus*. Aqueous extracts phytochemical profile indicates that it is better than fresh leaves extract but poor recovery than organic solvents because of the pigments and other constituent. This is in concerned with the finding of Govindachari *et. al.,*[12]. The hot and cold extracts of *C. collinus* showed higher antibacterial activity against *E. coli, S. typhi, K. pneumoniae, P. aeruginosa, S. aureus,* and *L. monocytogenes*. Among them, methanol hot extracts of *C. collinus* shows higher activity against those pathogens but the cold methanol, ethanol, ethyl acetate and aqueous extract failed to control tested pathogens except *S. typhi*. Secondary metabolites present in plants are known as active principles and these serves to protect the plants themselves against microbial (bacteria, fungi, viruses) infections as well as predation by pests and animals [13]. The inhibitory activities exhibited by the extracts tend to agree with the previous reports [14-15], all of whom linked antimicrobial properties of plants to the presence of bioactive secondary metabolites. For example, Mbuh *et. al.,*[16] investigated the antibacterial activity of leaf extracts of *Psidinum guajava* (Linn) and reported that the bioactive compounds found in the plant inhibited the growth of *S. aureus, S. typhi, E. coli*, *B. subtilis, Shigella* spp, *Proteus mirabilis* and *K. pneumoniae* and that the methanol extract was most active.

The earlier report of Maji *et. al.,*[17] revealed that acetone , aqueous and benzene extract of *C. collinus* leaf showed profound antimicrobial activity (> 11 mm inhibition zone) against *E. coli* (MDR), *S. aureus* (MDR), *K. pneumoniae, Bacillus cereus, Vibrio cholerae* and *Candida albicans* in 0.40-0.60 mg/ml. So these results are reconfirming the use of solvents for the extraction of chemical compounds from the natural materials may help to derive of one or more active principles from the crude extracts. The activity of the plant extracts against bacteria is an indication of the presence of broad or narrow spectrum antibiotic compounds or simply metabolic toxins in the plant [18-19]. Our preliminary investigation showed that all methanol, ethanol, ethyl acetate and aqueous extracts of *C. collinus* plant (leaves) were active against few human pathogens. Though, the mechanism of the action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of solvent used for extraction. The organic extracts provided more significant antimicrobial activity as compared to aqueous extract. This observation clearly indicates that the existence of non-polar residues in the extracts which have higher both bactericidal and bacteristatic abilities. Mukherjee [20] mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can be easily solubilized in organic solvents.

The MIC value of the active plant extracts obtained in this study were lower than the MBC values suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration. There are several evidences on the presence of antimicrobial metabolites like tannins, flavonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides, alkaloids, etc in the studied plant species [21-22]. Antibacterial activity of the methanol, ethanol, ethyl acetate and aqueous crude extracts of *C. collinus* showed activity against all tested organisms. The methanol and ethanol shows higher activity compared to others. It is believed that fractionation of the solvent based extract may yield quite number of high value and low volume active principles, because of the synchronized activity of various polarity properties bearing solvents in a mixture [23]. The antibacterial activity of medicinal plants and drugs varies in their inhibitory effect, depending on the concentration in crude extracts or synthetic drug, size of inoculums, temperature, nature of organism, and rate of diffusion [24]. The results obtained in this study has shown that the leaf extract of *C. collinus* possess antibacterial properties.

CONCLUSION

Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. The remarkable contribution of plants to the drug industry is highly possible, because of the extraction of large number of phytochemical and biological studies globally. From this present study it is obvious that *C. collinus* leaf extracts prepared by soxhlet method showed profound antimicrobial activity against human pathogens rather than cold extracts. The results of the present study support the folkloric usage of the plant and proved that the plant extracts could be effectively used against microbes causing infectious diseases. And also this study gives leads to the pharmaceutical industry to identify and characterize the active principles of promising fractions which may be exploited for drug preparation.

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