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ANTIMICROBIAL SCREENING OF METHANOLIC EXTRACT FROM VARIOUS PARTS OF *CEROPEGIA BULBOSA* AND *CEROPEGIA ATTENUATA*

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ABSTRACT

Ethnomedicine provides avenues for identification of compounds with antimicrobial properties and potential new antibiotics. The present studies focus on the antimicrobial activities of the crude methanolic extract of Ceropegia spp collected from Jaipur and Ajmer region of Rajasthan (India). Methanolic extracts of *Ceropegia bulbosa* and *Ceropegia attenuata* were tested against four common bacteria and two fungus as *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443*), Bacillus subtilis (*MTCC 121), *Fusarium solani* (MTCC 9667), *Trichophyton rubrus* (MTCC 3272), *Aspergillus niger* (MTCC 3376) of medical importance using a quantitative agar well diffusion test and tube dilution assay. All plant extracts showed antimicrobial activities against the selected microorganisms; the methanol extracts in callus and tuber were most effective. The effectiveness against bacteria is more than fungus. These results support the ethnomedicinal claim that *Ceropegia spp* is an effective treatment for microbes.

KEYWORDS: Quantitative, Extract, antibiotics, Ethanomedicine.

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INTRODUCTION

India has a rich history in the use of diverse medicinal flora for traditional healing. Medicinal plants are generally used to treat various medical conditions. Plants are the source of many chemical compounds. The presence of active antimicrobial compounds in plants represents a useful area for development of natural products that can be used as substitutes for antibiotics resistant to pathogenic bacteria and fungi. Furthermore, they provide the foundation for the development of new antimicrobials. The study will also confirm if there is a biological basis to the claim that the ethnomedicinal plant has useful medicinal purposes 1. Medicinal plants were used by people of an ancient culture without knowledge of their active ingredients 2. Bioactive substance lead to the discovery of new compounds that could be used to formulated new and most potent antimicrobial drugs to overcome the problem of resistance to the currently available medicines. Antimicrobial activity of various plant parts has also been observed by various workers viz. 3,4,5,6. Anti-microbial activity of plants can be detected by observing the growth response of various microorganisms to plant tissue or extracts which are in contact with them. Fungal related diseases may not be as common as other microbial infections, but when present, they are difficult to treat especially in immunosupressed persons. According to the W.H.O important progress has been made in controlling major infectious diseases. However, about 43% of total deaths occurred in developing countries due to the infectious diseases in recent years 7. The search for new antimicrobial agents is necessary due to the appearance of microbial resistance and occurrence of fatal opportunistic infections 8. Present study was undertaken to investigate the antimicrobial activity or *Ceropegia bulbosa* and *Ceropegia attenuata* .

MATERIALS AND METHODS

Collection of Plant material

Plant material for antimicrobial screening was collected from The whole plant material of *Ceropegia bulbosa and Ceropegia attenuata* was collected from Jaipur and Ajmer region of Rajasthan state of India, in the month of July - August 2012. Botanical identification and authentication was done by herbarium incharge Department of botany University of Rajasthan Jaipur, where a voucher specimen was deposited with the herbarium file number RUBL21159 and RUBL21160 respectively.

Preparation of extract

Samples of plant were washed and dried at room temperature in the dark for several days. Dried material crushed into powder form and extracted by soxhlet extraction method using methanol as solvent. Afterwards, the solvent was distilled under reduced pressure in a rotatory vacuum evaporator until the extract become dry. The crude evaporated plant extracts were dried at room temperature for 5-10 days. Then 50 mg of each crude plant extract dissolved in 1mL of the solvent to give a final concentration of crude extract in solvent of 50mg/ml. Then, this extract is used in the antimicrobial test.

Test microorganisms

Six different strains of microorganism were used in the screening process including *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443*), Bacillus subtilis (*MTCC 121), *Fusarium solani* (MTCC 9667), *Trichophyton rubrus* (MTCC 3272), *Aspergillus niger* (MTCC 3376). They were collected from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacteria were grown on nutrient broth media and fungi were grown on PDA (potato dextrose agar) media.

Antimicrobial activity

Well Diffusion Assay- Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial or anti-fungal activities of the plant sample 9. A sterile swab was used to evenly distribute bacterial or fungal culture over the appropriate medium as nutrient agar medium containing (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptones 5 gm in one liter distilled water) at 37o c for 24 h. the suspension was to inoculate 90 mm diameter of petriplates. Well (6mm diameter) were punched

in the agar and filled with the test samples (crude methanolic extract of various plant parts) to get different concentrations viz. 5, 10, and 15mg of the extract. Amoxilline was used as a standard for anti bacteria assay and Cyclohexamide for antifungal assay. Plates were incubated at37ºC for 24 h after which they were examined for inhibition zone. A caliper was used to measure the inhibition zones. The same method was followed for antifungal activity using potato dextrose agar medium.

$$\text{Activity Index}=\frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Determination of minimum inhibitory concentration (MIC)

To measure the Minimum inhibitory concentration values, various concentrations of the stock, 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 mg/ml were assayed against the test pathogens. Plant extracts were re-suspended in acetone (which has no activity against test microorganisms) to make 15 mg/ml final concentration and then two fold serially diluted; 1 ml of each extract was added to test tubes containing 1 ml of sterile Nutrient Agar media (for bacteria) Sabouraud Dextrose Agar media (for fungi). The tubes were then inoculated with standard size of microbial suspension (for bacteria 1×10^8 CFU/ml and 1×10^7 cell/ml for fungi) and the tubes were incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h for fungi in a BOD incubator and observed to change in turbidity after 24 h and compared with the growth in controls{1} A tube containing nutrient broth and inoculum but no extract was taken as control. Bacterial and fungal suspensions were used as control.

RESULTS AND DISCUSSION

The nature has provided a storehouse of natural remedies to cure all malfunctioning of human body. There is no doubt that plants are a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis 10. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials 11. The antimicrobial activity of methanolic extract of tuber, stem, leaves and callus against bacteria and fungus examined in the present study and its potency was quantitatively assessed by the presence or absence of inhibition zone and zones diameter(in mm). The methanol aqueous extracts of *Ceropegia bulbosa and Ceropegia attenuata* exhibited varying degree of inhibitory effect against all tested pathogenic strains (Table 1, 2, 3 and 4 in zone of inhibition). The most susceptible bacterium and fungi are *Bacillus subtilis Aspergillus niger* and respectively. The inhibition zones (IZ) were in the range of 3.6±0.52 to 11.3±0.92 mm for most of the tested strains. The MIC of crude extract of samples were determined by the concentration ranging from 0.039mg/ml to 20mg/ml.The results of the antimicrobial activity are present in tables. antimicrobial screening of methanolic extract of *Ceropegia attenuata* and *Ceropegia bulbosa* revealed that tuber and callus of *Ceropegia attenuata* and *Ceropegia bulbosa* shows better antimicrobial activity against microorganism against other parts of plants. Zone of inhibition increased along with increasing extract concentration. Maximum zone of inhibition was observed in the tuber of *Ceropegia attenuata* (12mm, Fig I) and stem of *Ceropegia bulbosa* (12mm Fig I) against *Bacillus subtilis* bacteria. And in tuber of *Ceropegia attenuata* (7.6mm,Fig II) against *Trichophyton rubrus* fungus and callus of *Ceropegia bulbosa* (9.6mm, Fig II) against *Aspergillus niger* fungus. Minimum zone of inhibition found in leaves of *Ceropegia attenuata* (7.3) against *Proteus vulgaris* bacteria and and stem of *Ceropegia bulbosa* against *Staphylococcus aureus* and leaves of *Ceropegia attenuate*(3) against *Fusarium solani* and stem of *Ceropegia bulbosa* (3) against *Fusarium solani* also Among the pathogens bacterial strains were to be more susceptible Than fungus . The MIC method was used to further investigate extracts that showed broad spectrum activity against microorganism. The highest dilution of a plant extract that still retained an inhibitory effect against the growth of microorganism (above of zone of inhibition) was reported as the MIC. In this study methanolic extract of tuber of *ceropegia attenuate* sample showed lowest promising MIC of 0.039mg/l in *Bacillus subtilis* for bacteria and

1.25mg/l in callus of C*ceropegia bulbosa* against fungus *Fusarium solani* . The crude methanol extract of *Ceropegia tuberosa* stem was active against two bacterial strains. Zones of inhibition; 14 mm and 13.3 mm; were observed against *Bacillus subtilis* and *Micrococcus luteus,* respectively 12. *Bacillus subtilis* was found more susceptible among other tested strains as all fractions except methanol fraction exhibited activity against it. This observation contradicts previous findings that *Bacillus subtilis* was found least sensitive among other strains against different plant extracts 13. *Bacillus subtilis* has also been reported most sensitive among different stains 14.The fractions of *Ceropegia tuberosa* indicated the distribution of active constituents in chloroform and ethyl acetate fraction. Chloroform fraction was most active fraction as it showed considerable activity against all bacterial strains. Maximum zone of inhibition was observed against *Micrococcus luteus* that is 17 mm (Table 2). Distribution of activity in less polar fractions indicates that active compounds of the plant are non polar to slightly polar in nature. Aqueous fraction was mildly active only against *Pseudomonas aeruginosa* with mean zone of inhibition 12.6 mm. Flavonoids and phenols isolated from *Ceropegia tuberosa* have shown leuchorroeal properties 15.

Table 1
Antibacterial screening (zone of inhibition) of methanolic extract from Ceropegia attenuata

Bacterial Species Plant Parts	***Bacillus subtilis***	***Staphylococcus aureus***	***Proteus vulgaris***
CAT IZ	12	11	8.3
AI	.8	.64	.59
CAL IZ	9.3	10	7.3
AI	0.6	.85	.52
CAS IZ	8.5	10	10
AI	0.5	.58	.71
CAC IZ	11.3	10.6	8.6
AI	.75	.62	.61

Zone of inhibition = in mm, IZ = Inhibition Zone; AI = Activity Index
CAT= Ceropegia attenuate tuber
CAL= Ceropegia attenuata leaves
CAS= Ceropegia attenuata stem
CAC= Ceropegia attenuata callus

Table 2
Antifungal screening (zone of inhibition) of methanolic extract from Ceropegia attenuate

Fungal Species Plant Parts	***Fusarium solani***	***Trichophyton rubrus***	***Aspergillus niger***
CAT IA	6.6	7.6	7.3
AZ	.41	.42	.38
CAL IA	03	6.3	5.6
AZ	.18	.35	.29
CAS IA	7.6	03	2.6
AZ	.47	.16	.14
CAC IA	07	05	07
AZ	.43	.27	.36

Zone of inhibition = in mm, IZ = Inhibition Zone; AI = Activity Index
CAT= Ceropegia attenuata tuber
CAL= Ceropegia attenuata leaves
CAS= Ceropegia attenuata stem
CAC= Ceropegia attenuata callus

Table 3
Antifungal screening (zone of inhibition) of methanolic extract from Ceropegia bulbosa

Fungal Species Plant Parts		*Fusarium solani*	*Trichophyton rubrus*	*Aspergillus niger*
CBT	IZ	3.3	09	03
	AI	.14	.50	.15
CBL	IZ	8,6	2.6	8.3
	AI	.54	.14	.43
CBS	IZ	03	5.3	6.6
	AI	.18	.29	.35
CBC	IZ	9.6	7.3	9.6
	AI	.57	.40	

Zone of inhibition = in mm, IZ = Inhibition Zone; AI = Activity Index
CBT= Ceropegia bulbosa tuber
CBL= Ceropegia bulbosa leaves
CBS= Ceropegia bulbosa stem
CBC= Ceropegia bulbosa callus

Table 4
Antibacterial screening (zone of inhibition) of methanolic extract from Ceropegia bulbosa

Bacterial Species Plant Parts		*Bacillus subtilis*	*Staphylococcus aureus*	*Proteus vulgaris*
CBT	IZ	7.3	8.6	11.3
	AI	.48	.50	.80
CBL	IZ	7.3	10	7.3
	AI	.48	5.8	.53
CBS	IZ	6.6	6.3	9.3
	AI	.40	.37	.66
CBC	IZ	12	10.3	6.6
	AI	.80	.78	.47

Zone of inhibition = in mm, IZ = Inhibition Zone; AI = Activity Index
CBT= Ceropegia bulbosa tuber
CBL= Ceropegia bulbosa leaves
CBS= Ceropegia bulbosa stem
CBC= Ceropegia bulbosa callus

Table 5
Antimicrobial activity (mic) of Ceropegia bulbosa and Ceropegia attenuata

Name Of The Test Sample	*Bacillus subtilis*	*Staphylococcus aureus*	*Proteus vulgaris*
CAT	2.5	10.00	1.25
CAL	0.62	05.00	2.50
CAS	-	-	0.039
CAC	0.78	1.56	0.01
CBT	1.56	0.625	-
CBL	0.41	-	0.15
CBS	2.50	-	5.00
CBC	0.039	0.72	0.15

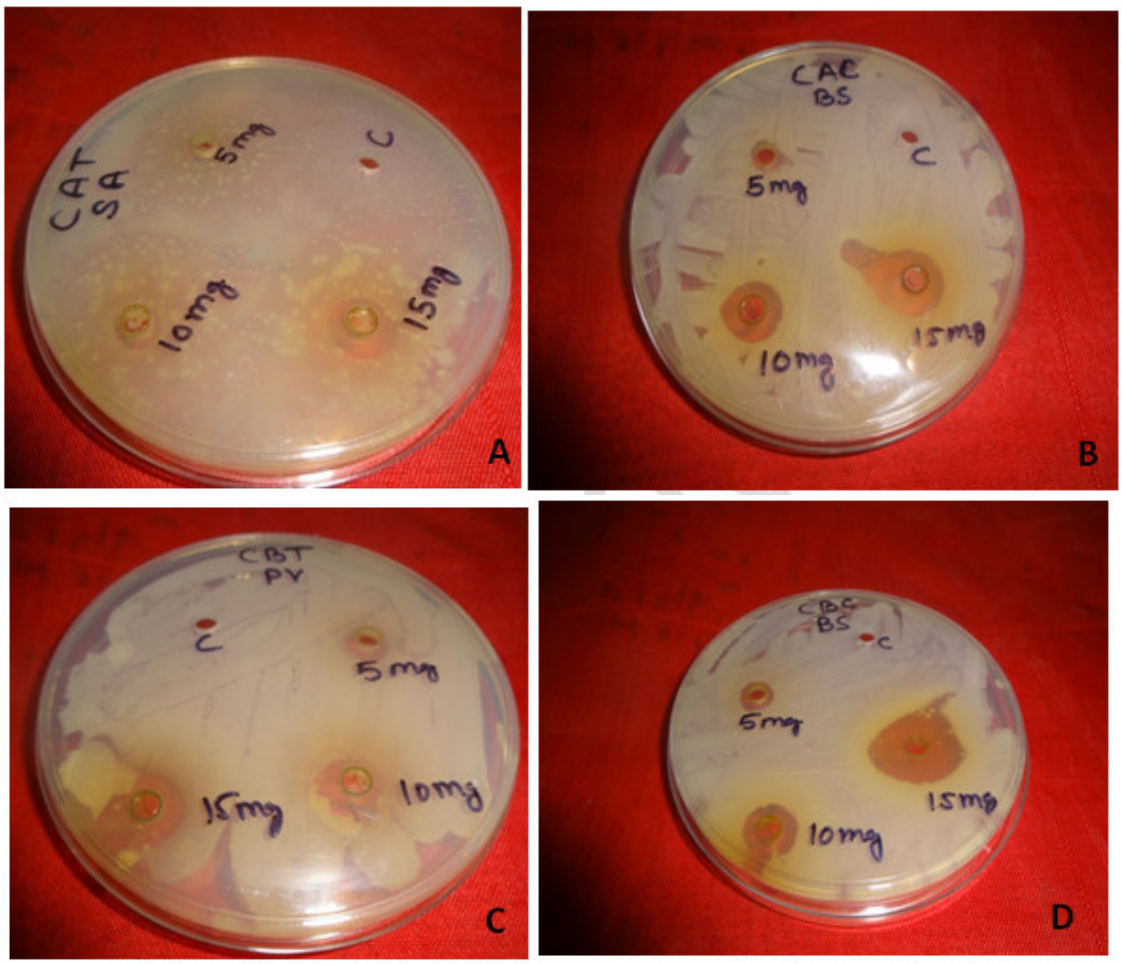
CAT= Ceropegia attenuata tuber
CAL= Ceropegia attenuata leaves
CAS= Ceropegia attenuata stem
CAC= Ceropegia attenuata callus
CBT= Ceropegia bulbosa tuber
CBL= Ceropegia bulbosa leaves
CBS= Ceropegia bulbosa stem
CBC= Ceropegia bulbosa callus

Table 6
Antifungal activity (MIC) of Ceropegia bulbosa and Ceropegia attenuate

Name of the test organism	*Fusarium solani*	*Trichophyton rubrus*	*Aspergillus niger*
CAT	-	10	10
CAL	-	-	-
CAS	-	6.25	20
CAC	-	-	-
CBT	-	-	5
CBL	5.0	-	-
CBS	-	-	
CBC	1.25	5.0	10

CAT= Ceropegia attenuata tuber
CAL= Ceropegia attenuata leaves
CAS= Ceropegia attenuata stem
CAC= Ceropegia attenuata callus
CBT= Ceropegia bulbosa tuber
CBL= Ceropegia bulbosa leaves
CBS= Ceropegia bulbosa stem
CBC= Ceropegia bulbosa callus

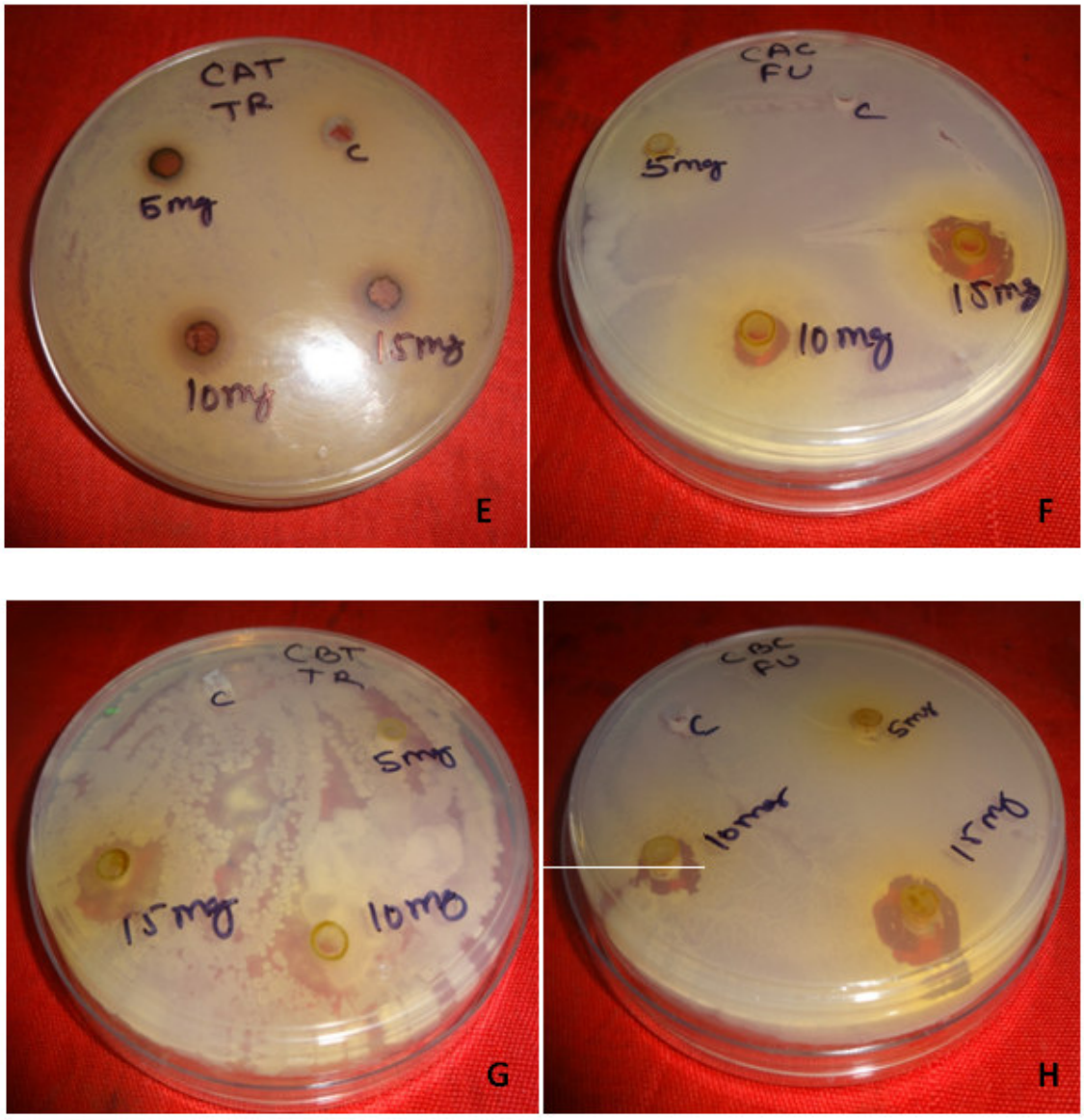
Figure 1
Zone of inhibition against bacterial species



Plant samples
CAT= Ceropegia attenuata tuber
CAC= Ceropegia attenuata callus
CBT= Ceropegia bulbosa tuber
CBC= Ceropegia bulbosa callus

Bacterial species
SA = Staphylococcus aureus
BS = Bacillus subtilis,
PV = Proteus vulgaris

Figure 2
Zone of inhibition against fungal species



Plant samples

CAT= Ceropegia attenuata tuber
CAC= Ceropegia attenuata callus
CBT= Ceropegia bulbosa tuber
CBC= Ceropegia bulbosa callus

Bacterial species

TR = Trichophyton rubrus
FS = Fusarium solani

CONCLUSION

The present investigation revealed that the methanolic extract from callus and tuber of *Ceropegia bulbosa* and *Ceropegia attenuata* exhibited antimicrobial properties which explain the basis for its use in traditional medicines. Presence of important metabolites viz.,carbohydrate, tannins, flavonoids, alkaloids, triterpenoids and saponins in extracts were confirmed after performing specific qualitative test which are responsible for antimicrobial activity.

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