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in vitro Evaluation of Antimicrobial and antifungal Activity of *Cordia macleodii* Bark. (HOOK.F. & THOMSON)

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Abstract: This study was carried out with an objective to investigate the antibacterial and antifungal potential of Bark of *Cordia macleodii*. Antibacterial activity of Methanolic extracts of the Bark was carried out against two Gram negative bacteria – *Escherichia coli*, and *Pseudomonas aeruginosa* and two Gram positive bacteria –*St. Pyogenes* and *Staphylococcus aureus*. The antifungal activity of the extracts was evaluated on three common pathogenic fungi – *Aspergillus niger* and *Candida albicans*. The testing was done by the agar plate method. Zones of inhibition of extracts were compared with that of different standard like Ampicilline, Ciprofloxacin, Norfloxacin and Chloramphenicol for antibacterial activity and Nystatin and Greseofulvin for antifungal activity. The extracts showed antibacterial and antifungal activities comparable with that of standard against the organisms tested. The results showed that the inhibition of the bacterial growth was more pronounced on *E. coli* and *S. aureus* as compared to the other tested organisms. The extract showed the antifungal activity against *C. albicans* and *A.niger*.
Keywords: Antibacterial, antifungal, In *Vitro, Cordia macleodii*, Gram positive, gram negative.

Introduction:

Infectious diseases are the second leading cause of death world wide.[1] In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns.[2] The emergence of multidrug-resistant bacteria has created a situation in which there are few or no treatment options for infections with certain microorganisms.[3] Along with bacterial infections, the fungal infections also are a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents.[4]

Although the need for new antimicrobials is increasing, development of such agents faces significant obstacles.[5] A number of factors make antimicrobial agents less economically attractive targets for development than other drug classes.[6] Pharmaceutical research and development costs, which are estimated to be $400–$800 million per approved agent,[7] pose a considerable barrier to new drug development in general. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many

pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine.[8, 9] The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the.[10,11] Historically, plants have provided a good source of anti-infective agents; emetine, quinine, and berberine remain highly effective instruments in the fight against microbial infections. Phytomedicine derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections. Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and garcinia biflavonones used in traditional African system of medicine, have been found to be active against a wide variety of micro-organisms.[12] Many plants have been reported to have antifungal activity.[13,14]

Cordia macleodii (Boraginaceous) commonly known as Dahiphal (Hindi). It is an 8-10 m high tree with a corky grey bark. The leaves are broad ovate, 5 – 10 cm as long as broad, scabrous, base cordate and crenate-serrate margins. They are arranged alternate to subopposite. The flowers are white in color and polygamous, in short terminal axillary corymbs. The calyx is densely tomentosus the corolla lobes are oblong in shape and 0.6 to 0.8cm long. The drupes are 1.2 to 1.9 cm long, ovoid, acuminate at apex, seated at persistent calyx. The flowers and fruits appear in February – August. [15]. Preliminary Phytochemical analysis of *C. macleodii* bark indicated the presence of relatively high levels of flavonoids, Alkaloides, steroides and terpenoides. Hence the present investigation was undertaken to determine the Antimicrobial and antifungal potential of *C. macleodii* Bark.

Materials and Methods

Plant Materials:

Fresh Bark of ***Cordia macleodii*** was collected in Orissa, India- in 2009 and was authenticated by Pharmacognosy Department of Gujarat Ayu University-Jamnagar, India.

Preparation of Extracts:

The Bark was shade dried and crushed to make coarse powder. The powder (660 g) was extracted with 3 L of Methanol (95%v/v) by continuous extraction method for 48 h. Solvent was distilled off and the extract was concentrated and dried under reduced pressure, which yielded a brownish green mass. The extract was preserved at 2–4 _C. This crude extract was used for further investigation for potential Antimicrobial properties

Preliminary Phytochemical screening of extract

The methanolic extract was testing to detect for the presence of different chemical groups of compounds as per the methods described in API. Preliminary Phytochemical screening Shows the presences of relatively high levels of flavonoids, Alkaloids, steroids terpenoides, Tannin, Coumarines, etc. Isolation and Identification of marker compound from bark and further investigation was under study.

Microorganism:

The microorganisms employed in the current study were procured from the Microcare Laboratory, Surat (Gujarat) Standard cultures of different species of Two Gram positive and two Gram negative bacteria including pathogenic and nonpathogenic and A.niger, C.albicans, and A.Clavatus for antifungal activity strains were used.

Media:

Nutrient broth, Nutrient agar, Malt extract broth and Sabouraud dextrose agar, all product of Himedia Laboratories Mumbai (India) were used in this study.

Antimicrobial and Antifungal agents:

Ampicilline, Ciprofloxacin, Norfloxacin, Chloramphenicol for antibacterial activity and Nystatin and Greseofulvin for antifungal Activity.

Antibacterial and Antifungal Activity:

The antibacterial activity was evaluated on four common pathogenic bacteria viz *Escherichia coli MTCC 96*, *Pseudomonas aruginosa MTCC 424, Staphylococcus aureus MTCC 96 and S.pyogenes MTCC 442*. While the antifungal activity of the extracts was evaluated on three common pathogenic fungi viz. *Aspergillus niger MTCC 282*, *Candida albicans MTCC 227 and A. Clavatus MTCC 1323*.

For evaluation of antibacterial and antifungal activities of alcohol extract of *C. macleodii bark*, Agar diffusion assay method was used [16]. For investigation of antibacterial activity, Sterile Muller Hinton agar media (Hi-media) were prepared in Petri dishes. The bacteria (1×108 bacteria/ ml) were inoculated separately in the media. In each Petri dish four wells (diameter 6mm) were prepared under aseptic conditions. In these various concentrations of the extracts were prepared (i.e.5µg/ml, 25µg/ml 50µg/ml, 100µg/ml & 250µg/ml) with DMSO. Same procedure applies in standard drug. All the dishes were incubated at 35ºC for24 Hrs.

For investigation of antifungal activity, Sterile Potato dextrose agar media (Hi-media) were prepared in Petri dishes. The fungal spores (1×106 spores/ ml) were inoculated separately in the media. In each Petri dish four wells (diameter 6mm) were prepared under

aseptic conditions. In these various concentrations of the extracts were prepared (i.e.5µg/ml, 25µg/ml 50µg/ml, 100µg/ml & 250µg/ml) with DMSO. Same procedures apply in standard drug. All the dishes were incubated at 35ºC for seven days.

At the end of the incubation period, the media were observed for zone of inhibition. The zones of inhibition were measured in millimeter using Vernier Calipers.

Result & Discussion

The results of investigation of antibacterial and antifungal activities of *C. macleodii bark* summarized in tables 1 and 3 respectively. Methanolic extract of *C. macleodii* bark shows antibacterial activity against the *Escherichia coli*, *Pseudomonas aeruginosa ,St. Pyogenes* and *Staphylococcus aureus.* It was clear that *C. macleodii* extract shows maximum antibacterial effect on *E. coli* and *P.Aeruginosa* (Table 1) with comparison of standard drug (Table 2) .

Methanolic extract of *C. macleodii* and Standard drug Nystatin and Greseofulvin shows antifungal activity against the *Aspergillus niger* and *Candida albicans*.(Table 3). Extract Shows maximum antifungal effect on *Candida albicans*. (Table 3). It means it is active against Fungies.

Preliminary Phytochemical analysis showed that the Bark extracts of *C. macleodii* possess phenolics compounds, saponins, Tannin, Coumarines**.** Phytoconstituents such as saponins, phenolics compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections. [17, 18] Further studies are under investigation to be carried out to isolate the various classes of phytoconstituents and determine their antimicrobial potential.

Table 1: Antibacterial activity of alcohol extracts of *C. macleodii* Bark against organisms: (Zone of inhibition of different bacteria (mm))

Microorganism	**Cordia Macleodii Bark (MeOH Extract)**				
	Concentration				
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
Escherichia coli	@	13	15	16	19
P.aeruginosa	@	13	14	16	20
St. Pyogenes	@	12	15	17	18
S.aureus	@	12	13	14	17

Table 2: Antibacterial activity of Standard Drug against organisms:

Drug	**Concentration µg/ml**	**(Zone of inhibition of different bacteria (mm))**			
		***Escherichia coli*,**	***Pseudomonas aeruginosa***	***St. Pyogenes***	***S.aureus***
Ampicilline	5	14	14	11	10
	25	15	15	14	13
	50	16	15	16	14
	100	19	18	18	16
	250	20	20	19	18
Chloram-phenicol	5	14	14	10	12
	25	17	17	13	14
	50	23	18	19	19
	100	23	19	20	20
	250	23	21	20	21
Ciprofloxacin	5	20	20	16	17
	25	23	23	19	19
	50	28	24	21	21
	100	28	26	21	22
	250	28	27	22	22
Norfloxacin	5	22	18	18	19
	25	25	19	19	22
	50	26	21	20	25
	100	27	23	21	26
	250	29	23	21	28

@ = No zone of inhibition , ## Control Sample(DMSO) has not shown any Zone of Inhibition.

Table 3: Antifungal activity of alcohol extracts of *C. macleodii* Bark and Standard drug Against Fungi's.

Drug	Concentration µg/ml	(Zone of inhibition of different bacteria (mm))	
		A. Niger	C. Albicans
Cordia Macleodii	5	@	@
	25	15	17
	50	16	18
	100	18	20
	250	20	21
Greseofulvin	5	19	18
	25	23	21
	50	25	22
	100	25	22
	250	28	24
Nystatin	5	18	18
	25	19	21
	50	24	24
	100	29	25
	250	29	26

@ = No zone of inhibition , ## Control Sample(DMSO) has not shown any Zone of Inhibition.

Conclusion

In the current investigation, the Methanolic extract of *C. macleodii* bark was found to be active on most of clinical isolates Microorganism and fungi's in compare to standard drug. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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