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A comparative antimicrobial study on *Cordia macleodii*. hook leaf water extract and its ghrita base formulation

Research Article

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Abstract

Cordia macleodii Hook. of family Boraginaceae (Ehretiaceae) is reported for its ethnomedicinal use as a wound healing drug. An attempt has been made to evaluate the antibacterial activity of its leave along with its *ghrita* base preparation against medically important human pathogenic bacteria (two gram positive- *S. aureus, S. pyogenes,* two gram negative- *E. coli, P. aeruginosa)* and fungal strains- *A.niger, C. albicans,* at different concentrations(5, 25, 50, 100, 250 µg/ml), using agar disc diffusion method. Zone of inhibition of these samples was compared with that of different standards (Ampicilline, Ciprofloxacin, Norfloxacin and Chloramphenicol for antibacterial activity and Nystain and Greseofulvin for antifungal activity). Only *ghrita* showed more effective result, at different concentration, in comparison to leave water extract and *ghrita* base formulation.

Key words: Antimicrobial, Boraginaceae, *Cordia macleodii, Ghrita*, Ghee, Leaf

Introduction:

Ayurveda recommends large number of medicinal plants for the management of fresh as well as chronic wounds. (1) These medicinal plants have a wide variety of chemical constituents and some of them have the ability to inhibit the growth of micro organisms.(2) *Cordia macleodii* Hook, is reported for its ethno-medicinal use as a wound healing drug.(3) The antimicrobial and wound healing action of water extract of this plant have been reported. (4) For the management of wound, Ayurveda advocates to use different doses of the drug like *Taila* (oil), *Ghrita* (ghee), *Siktha* (wax) through external application(5). *Ghrita* alone has been also reported for it's wound healing properties.(6) In the present study, an attempt has been made to compare the antimicrobial property of water extract of *C. macleodii* leaf with that of *Ghrita* and *Ghrita* base preparation from its leaves.

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Materials and Methods

Collection of drug:

The drug (Fresh leaf of *C. macleodii)* for the present study was collected by the scholar from its reported habitat (Hoshangabad District of Madhya Pradesh) after proper identification. Cow's *ghrita* was procured from Khadi Gramodyoga Bhandara, Jamnagar and used for preparation of *ghrita* and alone in experimental study being leveled as SG.

Preperation of formulation:-

The test drug i.e. *Ghrita* base formulation of *Cordia macleodii* leaves *was* prepared by following the standard procedure of *Sneha Kalpana* described in *Sharangdhara Samhita* (7) and the prepared drug was leveled as CMG.

Preparation of Leaf extract:-

Water extract from the leaves was obtained following the procedures mentioned in Ayurvedic Pharmacopoeia of India (8) and the leveled as CML.

Selection of microorganisms:

Staphylococcus aureus (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) & fungal strains *Aspergillus niger* (MTCC 282), *Candida albicians* (MTCC 227) were chosen based on their clinical and pharmacological importance. The bacterial strains, obtained from Institute of Microbial Technology, Chandigarh, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24h at 37°C on Nutrient Agar and Potato Dextrose Agar medium (Microcare laboratory, Surat, Gujarat, India) respectively following refrigeration storage at 4°C. The bacterial strains were grown in Mueller–Hinton agar (MHA) plates at 37°C (The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C) whereas the yeasts and molds were grown in Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) media, respectively, at 28°C. The stock cultures were maintained at 4°C. **(9,10,11,12)**

Antimicrobial activity

Determination of zone of inhibition(zoi) method

*In vitro a*ntimicrobial activity testing was carried out by using Agar cup method. Each purified extracts were dissolved in Dimethyl Sulfoxide (DMSO), sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of ZOI, pure Gram positive, Gram negative and fungal strains were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antibacterial and antifungal activities against the *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes* and the fungi *C.albicans* and *A. niger.* The sets of five dilutions (5, 25, 50, 100 and 250 µg/ml) of *Cordia macleodii* leaf extract,*Cordia macleodii ghrita* and *Shuddha ghrita* and standard drugs were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (108cfu) and allowed to stay at 37^{o}C for 3 h. Control experiments were carried out under similar condition by using Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin for antibacterial activity and Nystatin and Greseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 h of in incubation at 37°C for bacteria and 48 to 96 h for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

Results and discussion:-

Microbial load

C. macleodii leaf water extract(CML) , Shuddha *Ghruta*(SG) and *C. macleodii Ghruta*(CMG) were studied for microbial load (Total microbial count, total bacterial count and total fungal count) and was found within prescribed limit. All the tested pathogens were absent in all the three samples before their antimicrobial evaluation (Table -1).

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Table 1 : Microbial load reported in *C macleodii* Leaf Ghrita, *C macleodii* Leaf water extract and Shuddha Ghrita

S.No.	Test parameter	SG	CML	CMG	Prescribed Limit
1.	Total microbial count	20 CFU per gm	40 CFU per gm	30 CFU per gm	100 CFU per gm
	Total bacterial count	10 CFU per gm	30 CFU per gm	20 CFU per gm	
	Total fungal count	10 CFU per gm	10 CFU per gm	10 CFU per gm	
2.	**Pathogens**				
	E.Coli	Absent	Absent	Absent	Should be absent per 10g
	Salmonella spp.	Absent	Absent	Absent	
	Pseudomonas aeruginosa	Absent	Absent	Absent	
	S. aureus	Absent	Absent	Absent	

Table No.2 Antibacterial activities (zone of inhibition) of test drugs on gram negative bacteria

Coded test drugs	***E. coli* MTCC 443**					***P. aeruginosa* MTCC 424**				
Diameter of zone of inhibition in mm										
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
CMG	Nil	15	17	19	20	Nil	14	16	17	18
SG	Nil	16	18	19	21	Nil	15	17	18	20
CML	Nil	14	16	17	19	Nil	13	19	16	18

Table No.3 Antibacterial activities (zone of inhibition) of test drugs on gram positive bacteria

Coded test drugs	***S. aureus* MTCC 96**					***S. pyogenus* MTCC 442**				
Diameter of zone of inhibition in mm										
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
CMG	Nil	17	18	20	21	Nil	12	14	17	19
SG	Nil	17	18	21	22	Nil	14	16	17	20
CML	Nil	17	18	20	22	Nil	15	17	19	22

Table No.4:-Antibacterial activities (zone of inhibition) of standard drugs on gram negative bacteria

Standard drugs	***E. coli* MTCC 443**					***P. aeruginosa* MTCC 424**				
Diameter of zone of inhibition in mm										
	5 µg/m	25 µg/m	50 µg/m	100 µg/m	250 µg/m	5 µg/m	25 µg/m	50 µg/m	100 µg/m	250 µg/m

	l	l	l	l	l	l	l	l	l	l
Ampicilline	14	15	16	19	20					
Chloramphenicol	14	17	23	23	23	14	17	18	19	21
Ciprofloxacin	20	23	28	28	28	20	23	24	26	27
Norfloxacin	22	25	26	26	29	18	19	21	23	23

Table No.5:-Antibacterial activities (zone of inhibition) of standard drugs on gram positive bacteria

Standard drugs	***S.aureus* MTCC 96**					***S.pyogenus* MTCC 442**				
Diameter of zone of inhibition in mm										
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg ml	250 µg/ml
Ampicilline	10	13	14	16	18	11	14	16	18	19
Chloramphenicol	12	14	19	20	21	10	13	19	20	20
Ciprofloxacin	17	19	21	22	22	16	19	21	21	22
Norfloxacin	19	22	25	26	28	18	19	20	21	21

Table No.6: Antifungal activities (zone of inhibition) of test drugs

Coded test drugs	***A. niger MTCC 282***					***C. albicans* MTCC 227**				
Diameter of zone of inhibition in mm										
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
CMG	-	13	15	17	20	-	14	16	19	22
SG	-	14	15	18	20	-	15	16	18	20
CML	-	13	14	17	19	-	15	16	17	21

Table No.7: Antifungal activities (zone of inhibition) of standard

Standard drugs	***A. niger MTCC 282***					***C. albicans* MTCC 227**				
Diameter of zone of inhibition in mm										
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
Greseofulvin	19	23	25	25	28	18	21	22	22	24
Nystatin	18	19	24	29	29	18	21	24	25	26

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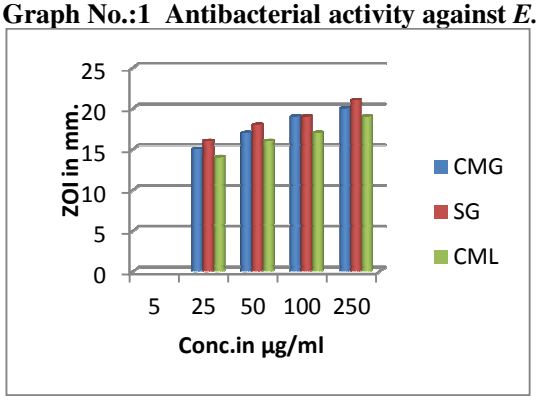
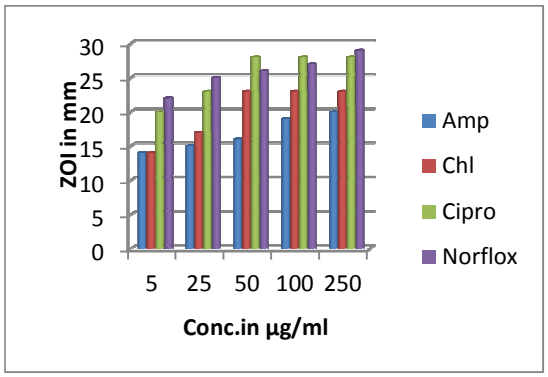
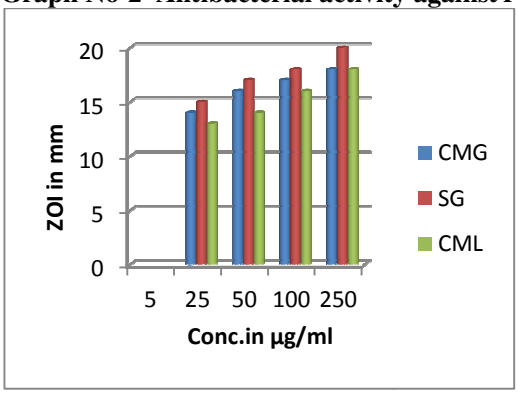
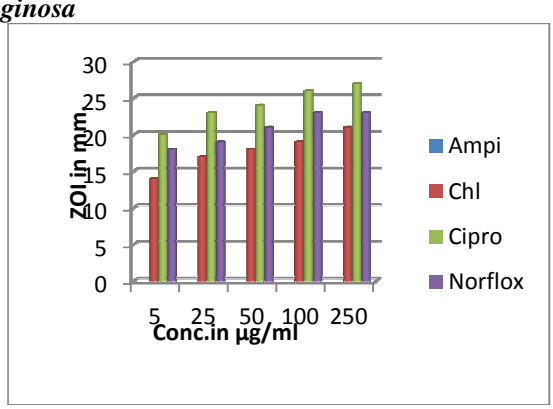
Graph No.:1 Antibacterial activity against *E. coli*



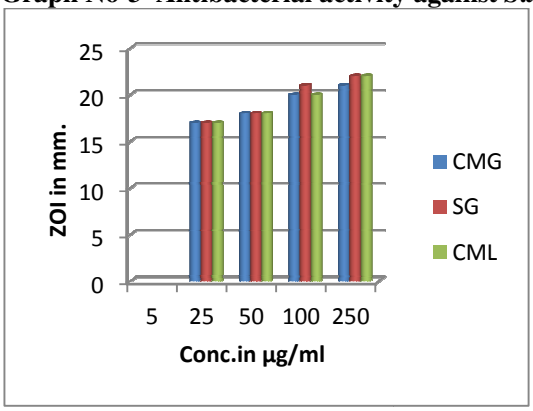


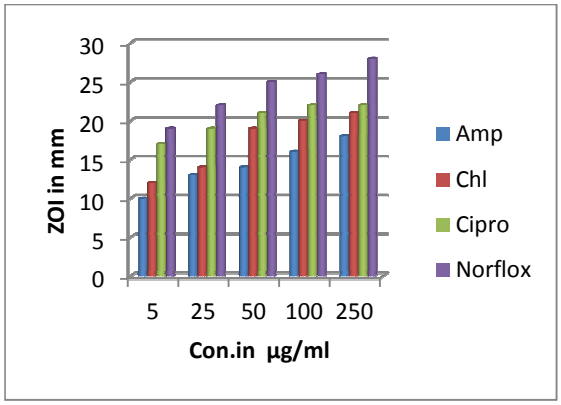
Graph No-2 Antibacterial activity against *P. aeruginosa*



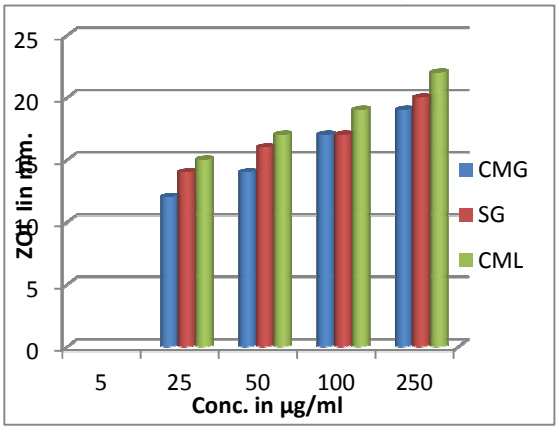


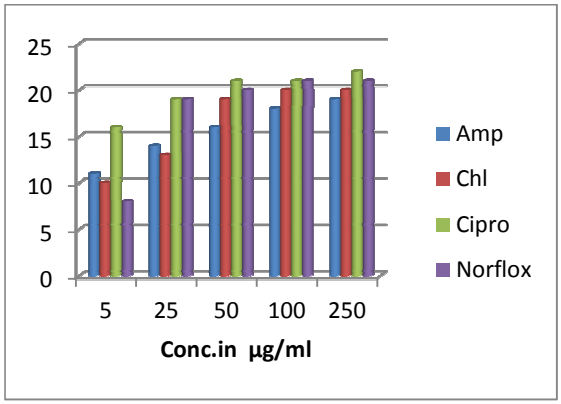
Graph No-3 Antibacterial activity against *S.aureus*



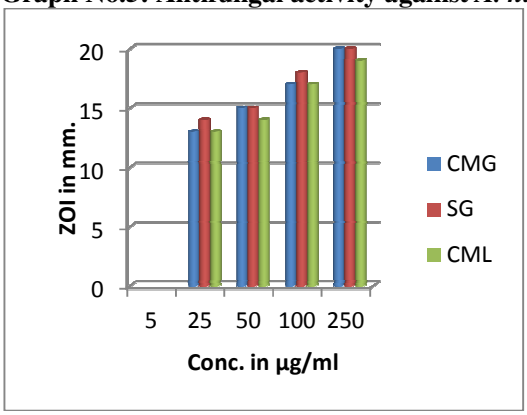


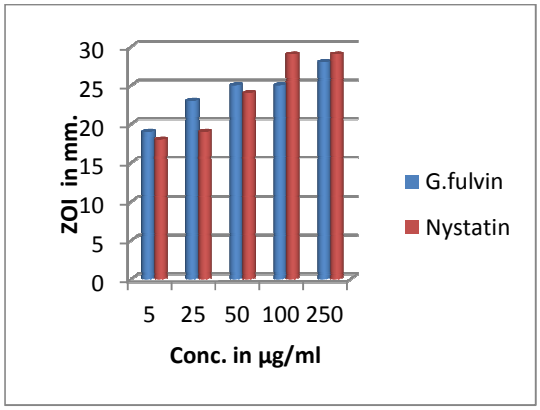
Graph No-4 Antibacterial activity against *S.pyogenus*



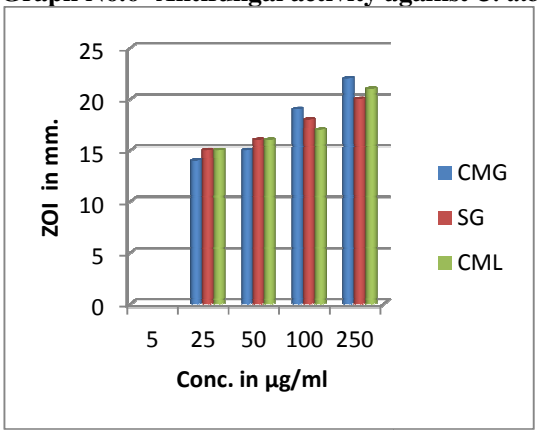


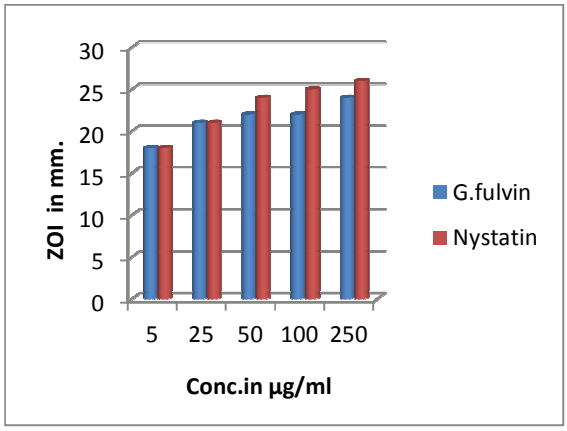
Graph No.5: Antifungal activity against *A. niger*





Graph No.6- Antifungal activity against *C. albicans*





Antibacterial activity of test drugs against *E. coli*..

Test drugs did not affect any of the pathogens at 5 µg/ml concentration where as all the standard drugs affect at all the tested levels of concentrations. At concentration of 25µg/ml SG showed maximum effect i.e. 16, in standard group Norfloxacin showed maximum.effect 25; at the concentration of 50 SG shows maximum effect,while at concentration of 100 and 250 CMG and SG shows maximum effect respectively.

Antibacterial activity of test drugs against *P. auregenosa*

Test drug shows no effect against *P.aeruginosa* at concentration 5 but the standard drugs shows the zone of inhibition on same concentration. At concentration of 25and 50 SG shows relatively more activity and in standard group Ciprofloxacin is more effective.

Antibacterial activity of test drugs against *S. auresus.*

Test drug shows no effect against *S.auresus* at concentration 5 but the standard drugs shows its effect .At concentration of 25 and 50 all three test drugs shows similar effect but in standard group Norfloxacin has maximum strength. At concentration of 250 the potency of CMG is equal to Chloremphenical.At this concentration, the potency of *SG* and *CML* is equal to the standard drug Ciprofloxacin.

Antibacterial activity of test drugs against *S. pyogenus.*

Test drug shows no effect against *S.pyogenus* at concentration 5 but the standard drugs shows its effect .At concentration of 25 and the zone of inhibition is equal to Ampicilline i.e.14 and concentration of 25, 50, 100 and250 CML showed maximum inhibition than other two.

Antifungal activity of test drugs against *C. albicans* and *A. niger.*

Test drugs did not affect any of the pathogens at 5 µg/ml concentration where as all the standard drugs affect at different levels against *A.niger* . At concentration of 250 SG and CMG both have equal zone of inhibition but against *C.albicans* almost similar zone of inhibition was found at different concentration levels.

Conclusion:-

The three tested drugs (*Cordia macleodii ghrita, Shuddha ghrita* and *Cordia macleodii leaf)* showed antibacterial activity against the tested organisms. The effectiveness is more when the concentration is more. Among all the tested drugs *Shuddha ghrita* showed better result.

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