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Antimicrobial Activity of Guggul (C. Wightii) Plant in Arid Region of Jhunjhunu

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ABSTRACT:
Guggul plant commonly known as Commiphora wightii. It's belonging to family Burseraceae and its plant belongs to class Magnoliopsida. Use of medicinal plants against disease is not new. We are using herbal plant extracts in our common life from human evolution. So many medicinal plants are available in our environment, which can be used for our benefit in direct or indirectly. Guggul plant (Commiphora wightii) is also contains lot of medicinal values. For checking the quality of these medicinal plants we have to determine the various biochemical tests, for its merits and demerits.

KEY WORDS: - Commiphora wightii , Guggul, Magnoliopsida, Medicinal plants.

INTRODUCTION:

Guggul plant commonly known as Commiphora wightii. It's belonging to family Burseraceae and its plant belongs to class Magnoliopsida. This guggul plant is a Shrub or small tree. It's maximum 4 meter. Its plant branches are throny. This plants leaves are simple and leaves are 1-5 cm. long, 0.5-2.5 cm. broad. The guggul plant prefer arid and Semi arid climate and tolerant of poor soil & unferile soil of Rajasthan, Karnataka and Gujarat. According to Ayurveda five type of guggul name- Krishnan (black), Peet varn (Yellow), Neel (Blue), Kapish (Light Brown) and Rakt (Blood Red). The AtharVaveda, one of the four well known as Holy Scriptures (Veda) the Hindu. (Goyal et al, 2010). A Great discover antibiotic is wide spread emergence of resitance among the pathogenic bacteria against the available antibiotics. The antibiotic chemo therapy is important science achievement of twenty one century. It therapy use practiced for the treatment of various microbiological infections. (Goyal et al, 2010). In recent year, The antimicrobial pathogen such as staphylococcus aureus, micrococcus, Klebsicella, Hemophilus, Neisseria, Moraxella and Enterococcus Faecalis is increasing at alarming rate worldwide (Cohen, 1992, Gold and moellering 1996; Kaushik and Goyal 2008,Qureshi and Chahar 2013,2014). Many antibiotic canrot use longer time. It used for the treafment of infections caused by such as organism and threat to the usage of other drug is steadily increasing (Courvalin 1996; Who 2000).The guggul plant mainly extract by specialize cell for example- Stem bark (guggul plant). It has 6.7% moisture, 0.8 volatile oil, resin 62%, bum 28.7% and Insoluble Substances 2.8%.

MATERIAL AND METHODS:-

PLANT MATERIAL:

Mature and healthy leaves of C. wightii were collected from the Rajasthan (Jhunjhunu) during January and September 2012. Plant samples were collected for the various experiments (microbial activity and other biochemical test), methods are given as under.

COLLECTION OF PLANT SAMPLE AND ITS IDENTIFICATION:

Guggul (C. wightii) plant was collected from January and September, 2012 from different Guggul (C. wightii) growing areas of Rajasthan. Gum resin of C. wightii was collected from Shekhawati region particularly-Jhunjhunu the Kharkara, Gawari and cherani villages near JJT University, Rajasthan (India).

The bark samples were removed use sharp knife and store in clean polythene bags. Every sample was used for the experiment. Sample was stored in freeze for further use.
These medicinal plants identify by botanist Dr. Hanuman Prasad JJT University, Rajasthan (India).

CHEMICALS AND REAGENTS:
Glassware, Nutrient agar, Mueller Hinton broth (MHB), Mueller Hinton agar (MHA), Potato Dextrose broth (PDB), Potato Dextrose agar (PDA), Amoxicillin disc, Penicillin G disc, Polymyxin-B disc and Fluconazole disc were purchased from HI media Pvt. Ltd, Mumbai, India.

PROCESSING OF THE PLANT:
For the experiments, fresh plant leaves of C. wightii were collected from field in polybages with ice packs. These fresh leaves were with tap water and than double distilled water for thrice. The leaves were dried and then grinded into powder form and mixed with distilled water (10 gm of extract in 100 ml of distilled water). The extract was kept under rotary evaporator which was used to dry and concerted, after that these samples were which is kept in air tight container and stored under lower temperature.

TEST MICROORGANISM:
The guguul extract was used for testing anti microbial assay and some bacterial strains were used, these were also: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, A. flavus, Aspergillus niger, C. tropicalis and Candida albicans activity. Microbial cultures were grown on nutrient agar and potato dextrose agar for bacteria and for fungi respectively and cultures were maintained at 4°C in refrigerator.

POSITIVE AND NEGATIVE CONTROL:
Penicillin G disc (10 U/disc) was used for S. aureus; Erythromycin (10 µg/disc) for M. luteusand; Polymyxin-B (300 U/disc) for P. aeruginosa and Chlorampinacol (30 µg/disc) for K. pneumonia; Amoxycillin (10 µg/disc) was used as positive control for B. cereus; Fluconazole disc (10µg/disc) was used for the fungal cultures. For negative control sterilized distilled water was used.

ANTIBACTERIAL ASSAY:
Plant crude extract was prepared for the checking of antibacterial activity against various bacterial strains by the agar well diffusion method (Kumar et al., 2010). For the antibacterial activity all test organisms were inoculated on MHB for 8 hours. Selected organisms were seeded on MHA plates with the help of sterile cotton swabs. Wells were prepared with help of sterilized gel borer on agar surface (7 mm diameter). 100 µl of sterilized distilled water (negative control) and 100 µl of the test extract were poured in to separate wells. For the test of positive control, various antibiotics (slandered) were placed with plant extract on the agar surface. These cultured Petri plates were put for two days (48 hours) into incubator at 37^0C. All these antibiotics tests were performed in triplicates.

ANTIFUNGAL ASSAY:
Antifungal activity was examined by agar well diffusion method, against plant extract (Kumar et al., 2010). All test organisms were inoculated PDB medium for 12 hours at 28^0C. Fugal isolates were spread on PDA medium with the help of cotton swabs (sterilize). Wells were prepared with help of sterilized gel borer on agar surface (7 mm diameter). 100 µl of sterilized distilled water (negative control) and 100 µl of the test extract were poured in to separate wells. For the test of positive control, various antibiotics (slandered) were placed with plant extract on the agar surface. These cultured Petri plates were put for three days (72 hours) into incubator at 28^0C. All these antibiotics tests were performed in triplicates.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC):
DETERMINATION OF RELATIVE % INHIBITION:
The relative % inhibition of the examined plant extract with contrast to the +ve control was calculated by using this formula. Formula given as under (Ajay et al., 2002).

Relative percentage inhibition of the test extract =100 x (x-y)

(z-y)

Where,

z: total area of inhibition of the standard drug
y: total area of inhibition of the solvent
x: total area of inhibition of the test extract

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC):

Minimum inhibitory concentration of extract plant was examined by agar well diffusion method. For making various concentration of extracts (range from 0.01-10 mg/ml), sterile distilled water was used and all extract was prepared by serial dilution method. Test cultures were inoculated in MHB and PDB for bacteria and fungi respectively. Microbial suspensions were seeded on MHA and PDA for bacteria and fungi respectively for spreading organisms on plate Sterilized cotton swab was used. In each of these plates, four wells were cut out using a standard cork borer (7 mm). Using a micropipette, 100 µl of each dilution was added in to wells. Bacterial plates were incubated at 37ºC for 24 hours and fungal plates were incubated at 28°C for 72 hours. The minimum concentrations of each extract were showing a clear zone of inhibition, which was considered to be MIC (Rios et al., 1988, Okunji et al., 1990).

Result: -

Antibacterial activity of aqueous extract of c. wightii

Test Organism	Zone of inhibition (mm)		
	C. wightii	PC	NC
Staphylococcus aureus	11.0±1.73	18.3±1.15	0±0
E. coli	12.3±1.52	34.3±1.52	0±0
Pseudomonas aeruginosa	12.0±1.0	15.3±0.7	0±0
Bacillus cereus	15.6±1.15	14.0±1.0	0±0

Note: - PC (Positive control)
NC (Negative Control)
Value is express mean ± Standard deviation of the three replicate
Zone of inhibition not include the diameter of the well

ANTIFUNGAL ACTIVITY OF AQUEOUS EXTRACT OF C. WIGHTII:

Test Organism	Zone of inhibition (mm)		
	C. wightii	PC	NC
Candida albicans	5.3±3.21	14.66 ± 1.52	0±0
Candida tropicanis	17.6±0.57	23.6 ± 1.52	0±0
Aspergillus niger	0±0	17.3 ± 0.57	0±0
Aspergillus flavus	15.3±1.15	19.5 ± 0. 57	0±0

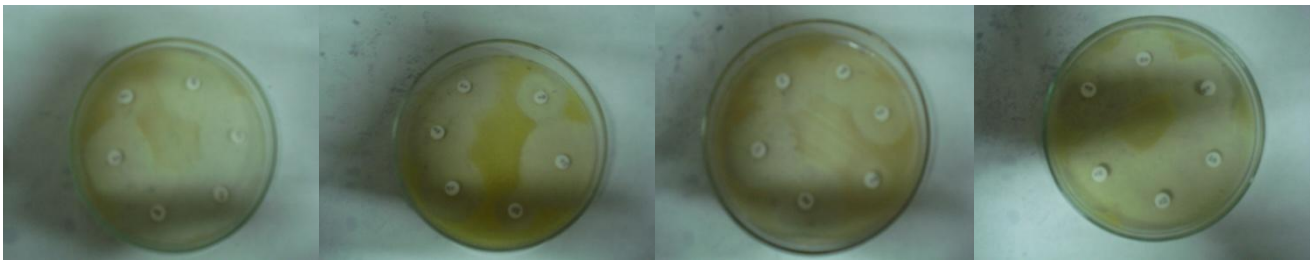
Note: - PC-(Positive control)
NC (Negative Control)
Value is express mean ± Standard deviation of the five replicates.
Zone of inhibition not include the diameter of the well

RPI AND MIC OF AQUEOUS EXTRACT OF C. WIGHTII:

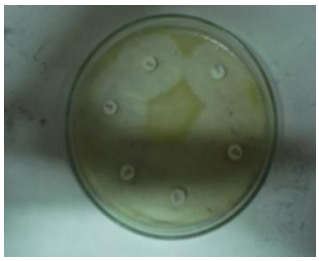
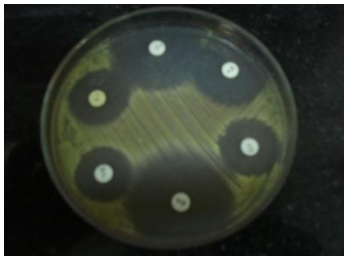
Test Organism	Aqueous extract of C. wightii	
	RPI (%)	MIC(µg/ml)
Staphylococcus aureus	36.13	2000
E. coli	12.85	500
Pseudomonas aeruginosa	61.27	500
Candida albicans	13.07	1000
Candida tropicalis	55.61	500
Aspergillus Niger	-	-
Aspergillus flavus	78.21	125
Bacillus cereus	124.16	125

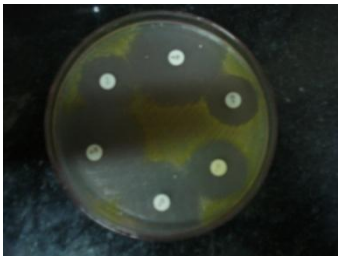
Note: - RPI - Relative percentage inhibition
MIC – Minimum inhibitory concentration

ANTIBACTERIAL ACTIVITY:
(A) Staphylococcus aureus
(B) E. coli
(C) Pseudomonas aeruginosa
(D) Bacillus cereus



ANTIFUNGAL ACTIVITY
(A) Candida albicans
(B) Candida tropicanis
(C) Aspergillusniger
(D) Aspergillus flavus



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