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Antibiotic potential in Indian Herb *Hemidesmusindicus*

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Abstract: India has one of the world's richest flora with about 120 families of plants comprising 1,30,000 species and about 119 secondary plant metabolites are used globally as drugs. *Hemidesmusindicus,* an Indian herb which has potential of medical importance, which is supported by the present study . In this study bioactive compound2-Hydroxy-4-methoxybenzaldehyde (2H4MB) was isolated which has shown potential of antibacterial and antifungal activities.2-Hydroxy-4-methoxybenzaldehyde (2H4MB) was the major chemical entity (99.41%) amongst 10 identified compounds. Due to bioactive property *Hemidesmusindicus*could be utilized to control infectious diseases caused by the testpathogens and *Candida albicans* in the human system. The *in vitro* antibacterial activity of 3 different extracts (Haxane, Methanol and Aqueous) and Bioactive compound 2-Hydroxy-4-methoxybenzaldehyde (2H4MB) as well as MIC values of isolated compound (2H4MB) were performed. The most active extract was found to be the hexane extract showing the maximum zone of inhibition of 22 mm against *Staphylococcus aureus*.

Keywords: *Hemidesmusindicus,* antibacterial/ anticandidal activity, bioactive compound

INTRODUCTION

Thousands of people are killed every day due to Infectious diseases which are the prominenet cause of premature deaths.The most common pathogens for infections are*Escherichia coli,Salmonella* spp., *Staphylococcus aureus* .Drug resistance to common pathogens is the world major problem.(Piddock and Wise,1989;Singh et al 1992 and

Mulligen et al;1993).The microorganisms are now resistant due to continous and indiscriminate use of antibiotics.Adverse reactions hypersensitivity, immunosuppressant and allergic reactions are also reported from antibiotics (Lopez et al.2001 and Idsoe et al.1968) which may associated with many clinical problems to treat infections(Davis 1994).Now this is a high time to discover and develop new antimicrobial drugs to control infectios diseases caused by drug resistant microbes. Searching of new chemical entities from plants with antimicrobial properties taking advantage of recent biological and medical technologies is a new and developing field .Since a long times, medicinal herbs and their bioactive constituents like essential oils have been known for their antimicrobial properties (Shelef 1983;Zaika 1988;Beuchat and Golden 1989 and Juven et al.1994).One possible approach is to screen/unexplored Indian medicinal bioactiveplants extracts for their potential to be used against multiresistant bacteria. India has one of the world's richest flora with about 120 families of plants comprising 1,30,000 species and about 119 secondary plant metabolites are used globally as drugs. The WHO reported that 80% of world population rely chiefly on traditional medicines/herbs for primary healthcare have steadily increased worldwide in the recent years. Keeping in view this study is designed to evaluate the antimicrobial potentials of Hemidesmusindicus and bioactive phytochemicals present in it.

MATERIAL AND METHODS

Collection of plant materials

The roots of*Hemidesmusindicus*were obtained from the The Himalaya Drug Company Dehradun India .The collected plant material was identified by the department of Pharmacognosy, The Himalaya Drug Company Dehradun.Roots were washed with potable water 2-3 times and once with sterile distilled water and then dried,a homogenous fine powder was made and stored in air tight container till further use.

Preparation of solvent root extraction- For the preparation of the plant extract ,the modified method of Alade and Irobi,(1993)was used.The powdered root(25g)were soaked in 100 ml each of the organic solvents(Hexane,methanol) and water in separate flasks and kept on rotating shaker for 72 hours,filtered using

Whatman filter paper No.1.The extracts were concentrated to half its volume using rotary evaporator while water extract was concentrated on water bath.

Culture media-Soyabean casein digest agar/broth of Hi Media Pvt.Ltd.Bombay,Indiawere used for this study

Inoculum-The Soyabean casein digest Agarwere inoculated with approximately 10^5 CFU/ml of 4 h growth that was incubated at 37 °C in Soyabean casein digest broth .

Isolation of Volatile bioactive component of of*H. indicus* through Clevenger apparatus

The method of Peyson& Richard(1992) was used with little modification**.**The dry root (1Kg) wastaken in a round bottom flask of a Clevenger type apparatus for 4 hrat 100°C.Thedistillate obtained was washed with diethylether and dried over anhydrous sodium sulphate. After filteration, the yield of essential was 1.2 g(0.12% w/w) and then it was stored in aairtight glass vessel at 4°C until required.

GC analysis of essential oil

The essential oil of *Hemidesmusindicus*was analysedby GC/MS technique using an GCMS-QP2010 Ultra(Shimadzu Company) GC system ,mass Spectrometer for composition.The identification of volatile oil component was done by comparison of their spectra with with NIST 11 lib./Wiley 8 lib. library data of the GC-MS system and also compared their retention indices(IR) with available relevant data.The percentage of peak area relative total peak area represent to relative amount (RA) of each individual component of the essential oil.Determination of RI value of each component relative to the retention time (RT) of series C8-C40 4-alkanes with linear interpolation on the Rtx-5 MS(30 meter x 0.25 i.dx0.25 Um film thickness)-column was done.

HPLC analysis of 2hydroxy4-methoxybenzaldehyde (2H4MB)

The fine powder of the root was poured into a glass vessel containing 75% of ethanol and then was filtered and evaporated. The residue of obtained was mixed with n-butanol and water in the ratio of (2:1) and both the layer of n-butanoland water were separated and evaporated under vacuum. The residues were washed with first with petroleum ether then with methanol. Themethanolic extract was concentrated and analyzed using HPLC as per standard method of (Shimizu *et al.*,1997) with slight

modifications. The extract was passed through Sartorius RC-membrane syringe filter (0.20 m) and 20 µl of filtrate was used for analysis in the HPLC. Shimadzu HPLC (Model SPD-10A UV-VIS Detector) and supelcosil LC-18 column (25 cm x 4.6 mm, 5 m) with mobile phase prepared with water, acetonitrile and acetic acid in the ratio of (50:50:0.1) was used into performing the chromatography.The flow rate and back pressure was consistently retained at 1.0ml/minute and 250 psi respectively . UV detector at 210 nm was used to read the compound. The initial total run time was 20min but after that it was preferably extended up to 40min (Shimizu *et al*., 1997). The resultsobtained were compared with standard.

Microorganisms

The antimicrobial activity of the plant extract and the essential (bioactive compound) were tested individually on G+ve and G-ve bacterial strains .All bacterial test strains were received from IMTECH,ChandigarhIndia.TheG+ve strain used was *Staphylococcus aureus MTCC 737 and* G-vebacterial strains were *E.coli MTCC 1687;Psuedomonas aeruginosa MTCC 1688* and *Salmonella enteric MTCC 3858.and Candida albicans MTCC 3017.*

Evaluation of antimicrobial activity

The method of (Perez et al; 1990) was modified.Soyabean casein digest agar plates were inoculated with test cultures in SCD broth. Wells of 8mm diameter were made on the inoculated plate through cork borer and filled with test samples and blank of distilled water,hexane and methanol and positive control of standard antibiotic was simultaneously used.The plates were kept for incubation at 37°C for 18 h.The antibacterial/anticandidal activity was determined by measuring the diameter of zone of inhibition that was observed. Wells were filled with 0.1 ml of 20 mg/ml concentration of each sample (2 mg/well).Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm.

Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration Determination of Bioactive compound(MBC) (2H-4-MB)

MTT staining method of (Scudiero et.al.1988; Marshall et al 1995 and Stevens and Olsen, 1993was used to determine the minimum Inhibitory Concentration (MIC) value which is defined as the lowest concentration of the sample that inhibited growth of microorganisms. MTT could convert to Formazan only by living organisms and a blue colour appeared in the well. To determined the Minimum Bactericidal Concentration (MBC)approximately10µl of the sample from the minimum Inhibitory Concentration assay was spread onto

freshly prepared and sterile LB plates and incubated at 28 °C over night.The MBC were taken as the lowest concentration that did not allow bacterial growth on the surface of agar plates.

RESULTS AND DISCUSSION

The yield of the essential oil obtained by hydrodistillation of the root of *Hemidesmusindicus* was 0.12% .The essential oil was evaluated by GC-MS. 10 compounds were identified(Table-1). The major compound in the oil was found to be 2-hydroxy-4-methoxybezaldehyde (99.41%).

The isolated compound 2-hydroxy 4-methoxy benzaldehyde (2H4MB) from *Hemidesmusindicus* was also analyzedby HPLC on the basis of their standard retention time 6.598min**.**The *Hemidesmusindicus* root extract was also analyzed through HPLC the results showed almost the same Retention time (Rt) in both root extract (6.881 min) and 2H4MBcompound*Standard* (6.598min). This revelaed the presence of 2H4MBcompoundin root extract of *Hemidesmusindicus*(Table-2).

The antibacterial and antifungal activities of the root extracts of *Hemidesmusindicus* and 2-hydroxy-4-methoxybenzaldehyde compound were evaluated against 5 test microorganisms including one G+ve bacteria,three G-ve bacteria ad one fungi. Their potency were assessed by diameter of zone of inhibition and MIC/MBC values. Among all the tested extracts hexane extract was found to have maximum zone of 22mm against *Staphylococcus aureus* (Table-3) followed by *E.coli* (18mm),*Candida albicans*(18mm),*Pseudomonas aeruginosa*(16mm) and *Salmonella enteric*(15mm).The isolated bioactive compound 2H4MB showed the highest diameter of zone of inhibition of 23mm against *Staphylococcus aureus followed by E.coli 16mm(Table-3).*.The Minimum Inhibitory Concentration (MIC) values of the Bioactive compound 2H-4-MB on test microorganisms ranged from 80µg/ml to 250µg/ml, MBC values from 150µg/ml to 250µg/ml. The Minimum Fungicidal Concentration (MFC) values and MIC values of 2H-4-MB on *Candida albicans* were 200µg/ml and 150µg/ml respectively.(Table-4). The significant antimicrobial effect of *Hemidesmusindicus* against all the pathogen confirmed that the compound present in the crude extract are responsible for the effective antimicrobial activity.

Table-1.List of compound isolated in the study as per the GC-MS Peak Report TIC

Peak#	R. Time	Area	Area%	Name of the isolated compounds
1	11.531	53636	0.01	S)-3-Ethyl-4-methylpentanol
2	24.855	128396	0.02	4-UNDECADIENAL
3	25.672	25430106	99.41	2-Hydroxy-p-anisaldehyde/2-Hydroxy-4-methoxybenzalde
4	26.657	1947873	0.37	Phenol, 2-methoxy-3-(2-propenyl)-
5	29.181	515809	0.10	Caryophyllene<(E)->
6	31.801	51357	0.01	BENZENE, 1-(1,5-DIMETHYL-4-HEXENYL)-4-METHY
7	32.322	112830	0.02	Funebrene<alpha->
8	32.855	118869	0.02	Bisabolene<beta->
9	33.453	100649	0.02	5.ALPHA.-CADINA-4,9-DIENE, (-)-
10	35.776	51565	0.01	-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRI
		528541090	100.00	

Table: 2 .Minimum Inhibitory Concentration (MIC)of 2-hydroxy-4-methoxy-benzaldehyde.

S.No.	Test microorganisms	2-Hydroxy-4-methoxy-benzaldehyde(µg/ml)	
		MBC*/MFC**	MIC***
1.0.	*Staphylococcus aureus MTCC 737*	150	80
2.0.	*E.coli MTCC 1687*	200	200
3.0.	*Pseudomonas aeruginosa MTCC 1688*	250	250
4.0.	*Salmonella enterica MTCC 3858*	200	150
5.0.	*Candida albicans MTCC 3017*	200	150

Table: 3.Antibacterial and antifungal activity of *Hemidesmusindicus*root extract and isolated compounds (2H-4-MB)

S.No.	Bacterial/Fungal strains	Inhibition zone diameter(mm)					
		Hexane extract	Methanol extract	Aqueous extract	ISOLATED BIOACTIVE COMPOUND(IBC) 50 mg/ml	REFERENCE COMPOUND(RC) 50 mg/ml	-VE Control Ciprofloxacin 30µg/ml
1.0.	*Staphylococcus aureus MTCC 737*	22	16	NAD	23	27	25
2.0.	*E.coli MTCC 1687*	18	14	NAD	16	17	21
3.0.	*Pseudomonas aeruginosa MTCC 1688*	16	12	NAD	14	15	22
4.0.	*Salmonella enteric MTCC 3858*	15	13	NAD	16	17	21
5.0.	*Candida albicans MTCC 3017*	18	16	NAD	20	20	---

Table-4 : HPLC analysis Hemidesmusindicus extract and the standard bioactive compound

Sample	Retention Time min)
2H4MB Compound standard	11.03
Hemidesmusindicus	11.14

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