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Evaluation of antimicrobial studies on the whole plant of *Melothria maderaspatana* Linn

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Abstract:

The main aim and objectives of our present work was to be find the antimicrobial activities of the whole plant of melotheria maderaspatana (family:cucurbitacea) and phytochemical investigation. The antimicrobial study's was determined by agar disc diffusion methods. The antibacterial activities was used in two gram positive organism like staphylococcusaureus, staphylococcusepidermitis, two gram negative organism like escherichia coli, klebsiella pneumonia and ciprofloxacin as standard. Then the antifungal activity was used in aspergillusniger organism and Ketoconazole as standard. The presence of sponines, alkaloids, carbohydrates, phenolic and flavonoids was detected.

Key words: melotheria maderaspatana, phytochemical analysis, agar disc diffusion methods.

INTRODUCTION:

The microbial (bacterial, fungi, virus) are affected in human health & plants. India has tremendous wealth of medicinal plant & its resources which are of different Kinds they grow in different climatic and ecological condition. In ancient time india was not advanced in therapeutic values of medicinal plants. The earliest mention of the use of medicinal plants is found in Rig-Veda(4500-1600 b.c). Considerable work has been carried out on medicinal plants & many new drugs have been brought to the light along with the screening of their phyto-constituents & their biological importance. Out of 45,000 plants species available in India about 15,000-20,000 plants have good medicinal values. However only 7,000-7500 are used their medicinal values by traditional community. Some plants are used in anti-microbials agents. The plants as medicine in different systems of medicine such as in ayurveda, allopathy, unani, & homoeopathy and even in other systems. The melothria maderspatana ayurvedic herb it has beed used in the ayurvedic and allopathic form due to it is great medicinal properties. Melothria maderspatana belonging to the family cucurbitaceae. It has distributed throughout the India, Ceylon, Africa, Malaysia & Australi. The melotheia maderspatanais also called mukia maderaspatana. The synonym of mukia maderaspatana(L) is mukia scabrella Arn, Bryonia scabrella, cucumis moderaspatanus linn. The mukia maderaspatana is also called musumusukkai in tamil. The whole plant of musumusukkai have great medicinal properties.

Leaves: simple alternate ovate or sub deltoid, entire or 3-5 lobed, minutely denticulate, very scabrid abovsinus.

Flower: small, yellow, male in small fascicles on very short peduncles, females almost sessile solitary or sub fasciculate, cordate at the base with awide.

Fruits: Globule, brownish yellow, finally turning red.

Seeds: Ovoid oblong, compressed

MATERIALS AND METHODS:

The plant *Melthria maderaspatana* is widely found throughout India. They mostly found on the rural areas. For our project work the plant was collected from sangiyam and manalurpet which is about 30 km away from tiruvannamalai. The whole plant are collected by hand picking method. The collected whole plant was washed with water to remove the dirt, mud & other plant debris & dried in a room temperature. The dried plant was powderd and used for further studies. The staphylococcus aureus, staphylococcus epidermidis, Escherichia coli, kelebsiella pneumonia, aspergillusniger thous micro-organism are used in antimicrobial activies.

Extraction of Plant Materials:

The 100gm of coarse plant powder was carried out with the following solvent as hydroalcohol(7:3), hexane, ethyl acetate using soxhlet apparatus. The extraction was carried out for about 35 hours. The temperature was maintained at 60^0C to 70^0C. after the extraction process the solvent was completely removed by distillation & concentrated. The concentration extracted were transferred to 100ml beakers to evaporate the remaining solvent on a water bath. Then they was stored in dessicator to remove excessive moisture.

Anti-bacterial studies:

The hydroalcoholic extract of the whole plant of melothria maderaspatana was subjected to Anti-Bacterial studies against various strains of Bacteria. The gram positive organism (Staphylococcus aureus, Staphylococcus epidermitis) and gram negative organism (Escherichia coli, Klebsiella pneumonia) organism was identified & used after the confirmation.The media was used for growth of bacteria in nutrient agar media. The organism were maintained on the nutrient agar slant these were tested using nutrient broth. One loopfulof the respective cultures in each slant, which were maintained below 4^0C was taken and inoculated in the broth & incubated at the 37 ^{0}C for 24 hours and were observed for the growth of the organism with the naked eye for their turbidity nature and compared with sterile broth. The presence of turbidity indicates the growth & suitability of the culture for the further work.

Preliminary Phytochemical Investication of Extract *of* Melothria Maderaspatana

S.No	Group	Hydroalcohol	Hexane	Ethyl acetate
01	GLYCOSIDES	----	--	-
02	ALKALOIDS	----	--	++
03	CARBOHYDRATES	++++	--	--
04	STEROIDES	----	--	-
05	PHENOLIC COMPDS&TANNINS	----	--	+
06	PROTEINS& AMINO ACIDES	++	--	--
07	SAPONINS	---	--	+
08	GUMS& MUCILAGES	+++	--	-
09	FLAVANOIDS & FLAVONES	---	--	++
10	IXEDOIL&FATS	--	--	--

Flourescence analysis:

S.NO:	**Treatment**	**Day Light**	**U.V. Regium**
1	Powder as such	Green	Green
2	Powder + 1N Hcl	Brownish Green	Brownish Green
3	Powder +1N NaoH	Dark Green	Dark Green
4	Powder +1N NaoH + Methanol	Pale Green	Dark Green
5	Powder + 50% HNO3	Brownish	Greenish Orange
6	Powder + 50% H2SO4	Pale Green	Dark Green
7	Powder + Methanol	Dark Green	Light Green
8	Powder + Ammonia soln	Dark Green	Brownish Green

Preparation Of Drug Solution:

The drug solution was prepared by dissolving the hydroalcoholic extract of *melothriamaderaspatans* in DMF(DiMethylFormamide) solvent. The DMF was removed from the refrigerator one hour prior to its use & allowed to warm up to the room temperature. The solution of the test drug at the concentration of 25, 50, 100 microgram/ml was prepared. The solubility of the drug was facilated by warming the drug solution to 60^0C for 15 minutes while continuously shaking it. *Ciprofloxacin*with the concentration of 50 microgram/ml was used as standard for above organism.

Disc Diffusion Method:

A suspension of organism was added to steril nutrient agar at 45^0C in aseptic environment. The mixture was transferred to strilepetridishes & allowed to solidity. Sterile discs of 5mm in diameter (made from whatmann filter paper which is previously sterilized in uv lamp) dipped in the solution of sample (hydroalcoholic extract).

All the plates were allowed to stand at room temperature for 1 hour as a period of preincubation diffusion to minimize of the effect of variation in time between the application of the different solutions. Then the plates were incubated at 37^0c ± 1^0C for 18 hour and observed for antibacterial activity. Then the diameter of zone of inhibition was measured in the plates. A similar procedure was carried out for studying the antibacterial activity of compounds against *staphylococcus epidermitis, Escherichia coli &Klebsiella pneumonia.* The average area of zone of inhibition was calculated & compared with standard.

Anti fungal studies:

The hydroalcoholic extract of the whol plant of *melothria maderaspatana* was subjected to antifungal activity studies against following strains of fungi. Sample was used in the concentration of 25,50,100 microgram/ml using ethanol as a solvent. The standerd used was *ketoconazole* (50microgram/ml)against both the organisma.The disc diffusion method was employed for the screening of antifungal activity.The media used for the growth of fungi was dextrose agar medium formula.

Disc Diffusion Method:

A small inoculam of aspergillus niger was added to sterile sabour& dextrose. Agar at 45^0C in an aseptic environment the medium was transferred to sterile petri dish & allowed to solidity. Sterilized discs of 5mm in diameter (made from whatmann filter paper previously sterilized in uv lamp) was dipped in solution of compound, standard & blank were placed on the surface of agar plates. All the plates was allowed to stand at room temperature for 1hour as a period of pre-incubation diffusion to minimize the effect of variation in time between the applied of the different solution. Then the plates were incubated at 37 ± 1^0C for 18 hours & observed for antifungal activity. The diameter of zone of inhibition was measured in the plates in the zone of inhibitions was observed. The zone of inhibition was calculated & and compared with that of standard.

RESULTS AND DISCUSSION

Antibacterial activitys:

The hydroalcoholic extracts of *melothria maderaspatana* was subjected to the antibacterial studies with the following strains gram positive organism (Staphylococcusaureus, Staphylococcusepidermitis) and gram negative organism (Escherichia coli, Klebsiella pneumonia) The hydroalcoholic extract of melothria maderaspatana showed antibacterial activity against staphylococcus aureus, staphylococcus epidermitis,

Gram Positive Response:

Name of Organism	Zone of inhibition(mm)			
	Standard (ciprofloxacin	Dilution concentration of Hydro alcoholic extract		
		25µg/ml	50µg/ml	100µg/ml
Staphylococcus aureus	32	15	21	24
Staphylococcus epidermidis	29	14	17	20

Gram Negative Response:

Name of Organism	Zone of inhibition(mm)			
	Standard (ciprofloxacin	Dilution concentration of Hydro alcoholic extract		
		25µg/ml	50µg/ml	100µg/ml
Escherichia coli	32	12	26	18
Klebsiella Pneumoniae	29	16	19	21

Antifungal activity

Name of Organism	Zone of inhibition(mm)			
	Standard ((Ketoconazole)	Dilution concentration of Hydro alcoholic extract		
		25µg/ml	50µg/ml	100µg/ml
Aspergillus niger	29	13	16	19

Escherichia Coliand Klebsiella Pneumonia compared with *ciprofloxacin*(50microgram/ml) as a standard.The antibacterial activity of hydroalcoholic extract was found to have more in 100 µ/ml than 50µ/ml and 25µ/ml.

Antifungal activities:

The hydro alcoholic extract of melothria maderaspatana showed antifungal activity against Aspergillus Niger compared with ketaconazole(50microgram/ml)as a standard. The antifungal activity of hydro alcoholic extract was found to have more in 100µ/ml than 50µ/ml & 25µ/ml.

CONCLUSION;

This study was showed that the extract of melatheria maderaspatana contain phytochemical constituent such as alkaloids, flavonoid, phenolic and saponins are responsible for the anti-microbial activities of the plant. The hydroalcoholic extract showed maximum antimicrobial activities against gram negative and gram positive organism in compare to other extract.

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