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ANTIMICROBIAL EFFICACY OF *MESUA FERREA* L. SEED EXTRACTS AND SOME ISOLATED COMPOUNDS IN DIFFERENT SOLVENTS AGAINST INFECTION CAUSING PATHOGENIC STRAINS

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ABSTRACT

Objective: To study the antimicrobial potential of Mesua ferrea (M. ferrea) L. seed extracts employed for antimicrobial assay. **Methods:** The plant powder was extracted in several different solvents of increasing polarities against a wide spectrum of microbial strains. Agar disc diffusion method was employed for antimicrobial assay at the concentration of 300 µg/disc. Gram-positive bacteria were most susceptible and yeast was most resistant. **Results:** The results were compared with the zones of inhibition produced by commercially available standard antibiotics. The hexane and methanol extracts of M. ferrea L. showed more activity towards Gram positive bacteria along with compound A9. **Conclusion:** These results indicate that activity could be attributed to the presence of essential oil, xanthones and coumarines present within the seed of this plant.

KEYWORDS: Antimicrobial activity; *Mesua ferrea L.*; Seed; Organic solvents; Microorganism.

INTRODUCTION

The plant kingdom comprises many species of plant containing substances of medicinal value, which are yet to be explored. A large number of plants are constantly being screened for their possible medicinal value.[1] The use of plant extracts in traditional medicine has been going on from ancient time.[2] In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of modern antibiotics.[3-9] Therefore the demand for new and effective antimicrobial agents with broad

spectrum activities from natural sources is increasing day by day. An antimicrobial is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi or protozoans. Antimicrobial drugs either kill microbes or prevent the growth of microbes.

In the recent years, research on medicinal plants has attracted a lot of attention globally. Large body of evidences have accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternative systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavenoidsetc. which have been found invitro to have antimicrobial properties.[10-11] Numerous drug resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.[12] Ethno-pharmacologists, botanists and natural product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases especially in the light of emergence of drug resistant micro-organisms and the need to produce more effective antimicrobial agents.[13] The rising incidence in multi-drug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Different extracts from traditional medicinal plants have been tested to identify the sources of the therapeutic effect. As a result, some natural products have been approved as new antimicrobial drugs. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians, several are already being tested upon humans. It is reported that, on average, two to three antibiotics derived from micro- organisms are launched every year.[14] Second the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds are readily available over the counter from herbal suppliers and natural food stores and self medication with these substances is commonplace. The use of plant extracts as well as other alternative forms of medical treatment is enjoying great popularity from the late 90's.

PRESENT WORK

For present study, extracts and compounds isolated from the seeds of Mesuaferrea were taken. Plant extracts showed significant antibacterial and antifungal activity.[24] Thus it was

planned to screen the antimicrobial activity of extracts and compounds isolated from the seeds of Mesuaferrea. The following extracts and compounds were taken for screening of antimicrobial activity.

EXTRACTS

1. Hexane extract of Mesuaferrea(Seed).
2. Benzene extracts of Mersuaferrea(Seeds).
3. 3 Chloroform of Mesuaferrea(seeds).
4. Acetone extract of Mesuaferrea(Seeds).
5. Ethanol extract of Mesuaferrea(SEEDS).
6. Methanol extract of Mesuaferrea(SEEDS).
7. Aqueous extract of Mesuaferrea(SEEDS).

COMPOUNDS

(a). A9

(b). A13

(c).SD4

(d) SD5

(e) SD6

Antimicrobial activity

Agar diffusion technique was used for the screening of anti microbialactivity, using paper disc method.[25] The antibacterial and antifungal activities will be determined by oxoid broth and saboruds broth using agar diffusion technique, followed by filter paper disc method. The filter paper disc moistened with extracts is placed over seeded medium. The test is simultaneously compared with specific control microbial testing biodisc of Ciprofloxacin for antibacterial and Itraconazolefor antifungal activity. It is incubated at 37 degree centigrates for 24 hours and 72 hours at 26 degree centigrates for bacteria and fungi respectively. The experiments are performed in triplicate and their average zone of inhibition is taken and recorded.[26,30]

Following antimicrobials(Bacteria and Fungi) were taken for study.

1. Bacillus subtilis
2. Staphylococcus aureus
3. Citrobacterfruendii

4. Escherichia coli
5. Klebsiellapneumoniae
6. Salmonella typhimurium
7. Proteus vulgaris
8. Pseudomonas aeruginosa

(C). Fungi

1. Candida albicans
2. Candida tropicalis
3. Aspergilluscandidus
4. Aspergillusflavus
5. Aspergillusniger

EXPERIMENTAL

The antimicrobial activity was screened by filter paper disc method.[31-32]

Requirements

Muller-Hinton Agar

Formulla

I

Ingredients	(gms/lit)
Beef, heart infusion form	300.00
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.5
Distilled water	1litre

Final PH(at 25C) 7.3+_ 0.2

Yeast Malt Agar (Medium)

Ingredients	gms/litre
Yeast malt broth	21.0
Agar	20.0
Distilled water	1 litre

Final PH (at 25 C) 6.2+-0.2

Potato Dextrose agar

Ingredients	gms/litre
Potato infusion form	200
Dextrose	20
Distilled water	1 litre

Final PH(at 25C) 5.6+_ 0.2

Nutrient broth

Ingredients	gms/litre
Peptone	5.0
Beef extract	3.0
Distilled water	1 litre

PROCEDURE

Preparation of inoculums

Inoculums of bacteria were prepared in nutrient broth and that of yeast in yeast malt agar and fungi in potato dextrose agar slant. The cultures were inoculated and incubated for 48 hours in case of bacteria and yeast and for 5 days in case of fungi.

Preparation of seeded agar plates

The Molten Muller Hinton agar was poured in sterile petridish to get a depth of 4mm. The medium was left to solidify. There after it was seeded with respective test organisms. For the purpose of seeding, 5ml sterile water was added to each agar slant culture at yeast and fungi. The culture was scrupt to get suspension of yeast cell of fungal spore. A sterile cotton swab was dipped in the suspension/culture and lightly rubbed over the solidified medium. The plate was left for few minutes and then used for the test.

Preparations of the sample

10 mgs of each sample to be tested was dissolved in 1 ml of respective solvent.

Determination of activity

4 mm discs of Whatmann filter paper no.42 were cut and sterilized. The filter paper discs were immersed in the solution of sample, after socking, the disc was removed and left in a petridish to permit the solvent to evaporate. After about 10 minutes the paper discs were transferred to seeded agar plate. Near the periphery of the petridish 4 discs were kept on the seeded agar plates. in the centre, the fifth disc was also placed which was soaked with the

standard solution. After the discs were transferred to the seeded plates the petridishes were incubated at 30^0C for about 24 hours. At the end of incubation each plate was observed for the zone of inhibition. Each distinct inhibition zone was measured as diameter in millimeter. The experiment was performed in triplicate and average zone of inhibition was reported.

RESULTS AND DISCUSSION

Mesuaferrea extracts(Seeds)

The result has shown that hexane and methanol extract showed maximum sensitivity towards B. subtilis and P. vulgaris where as the aqueous extract showed least sensitivity. M. ferrea did not show any activity against E. coli. Aqueous extract showed slight activity against A. candidus while as it was ineffective against all other studied moulds. Methanolic extract of M. ferrea exhibit remarked antifungal activity against A. niger and A. flavuswhere as hexane extract did not exhibit any antifungal activity against any of the tested fungal strains.

A9 showed maximum activity against B. subtilis, P. vulgaris and coud not exhibit any activity against all other tested bacteria. A13 showed moderate activity against A. candidus, B. subtilis, S aureus and could not exhibit any activity against all other tested bacteria and fungi. SD4, SD5 and SD6 showed moderate activity against B. subtilis, S. aureus and K. pneumoniaecould not exhibit any activity against all other tested bacteria and fungi.

Antimicrobial activity of Mesuaferrea L. (Seed) Extracts against microbial strains given in terms of mean zone of inhibition (mm).

Inhibition Zone(mm) Mesuaferrea L. (seed) extracts

G +ve & G –ve Bacteria	Hexane	Benzene Chloroform	Acetone	Ethanol	Methanol	Aqueous	S	
Bacillus Subtilis	21	- 21	22	14	22	9		18
Staphylococcus aureus	20	- 13	11	13	17	10		21
Citobacterfruendii	-	- -	-	-	-	-		-
Escherchia Coli	-	- -	-	-	-	-		22
KlebsiellaPneumoniae	-	- 12	11	12	-	8		-
Salmonella typhimurium	-	- -	-	-	-	-		-
Proteus vulgaris	22	- -	-	-	23	-		20
Pseudomonas aeruginosa	-	-	-	-	-	16	-	23

Fungi

Candida albicans	-	-	-	-	-	-	-	17
Candida tropicalis	-	-	-	-	-	-	-	-
Aspergilluscandidus	-	-	14	12	-	-	11	-
Aspergillusflavus	-	-	12	-	-	21	-	20
Aspergillusniger	-	-	-	-	-	23	-	-

Disc diameter= 4mm++++=21-25+++ = 16-20 mm.++ = 11-15 mm+ = 6.0-10 mm - = no activity.

Inhibition values beyond control are + = 6-10mm,+++= 16-20mm and - = not active(the values are including disc diameter) S= Itraconazole(antifungal agent) and Ciprofloxacin (antibacterial agent).

The standards are in the form of sterile Hi-disc cartridges, each disc containing 10mg of the drug.

Antimicrobial activity of the Mesua Ferrea (Seeds) extracts (in present work) Microbial Strains

Mesuaferrea L. (seed) extracts

	Hexane	**Benzene**	**Chloroform**	**Acetone**	**Ethanol**	**Methanol**	**Aqueous**	**S**
G +ve & G –ve Bacteria								
Bacillus Subtilis	++++	-	++++	++++	++	++++	+	++++
Staphylococcus Aureus	+++	-	++	++	++	+++	+	++++
Citobacterfruendii	-	-	-	-	-	-	-	-
Escherchia Coli	-	-	-	-	-	-	-	++++
Klebsiella Pneumoniae	-	-	++	++	++	-	+	-
Salmonella typhimurium	-	-	-	-	-	-	-	-
Proteus vulgaris	++++	-	-	-	-	++++	-	+++
Pseudomonas Aeruginosa	-	-	-	-	-	+++	-	++++
Fungi								

Candida albicans	-	-	-	-	-	-	-	+++
Candida tropicalis	-	-	-	-	-	-	-	-
Aspergilluscandidus	-	-	++	++	-	-	++	-
Aspergillusflavus	-	-	++	-	-	+++	-	++++
Aspergillusniger	-	-	-	-	-	++++	-	-

Disc diameter= 4mm++++ =21-25+++ = 16-20 mm.++ = 11-15 mm+ = 6.0-10 mm - = no activity.

Inhibition values beyond control are + = 6-10mm,+++= 16-20mm and - = not active(the values are including disc diameter) S= Itraconazole (antifungal agent) and Ciprofloxacin (antibacterial agent).

The standards are in the form of sterile Hi-disc cartridges, each disc containing 10mg of the drug.

Antimicrobial activity of compounds isolated from the hexane, aqueous and ethanol extracts of Mesua ferrea L. (Seeds).against microbial strains given in terms of mean zone of inhibition (mm).

Microbial Strains	**Inhibition Zone(mm) Mesuaferrea L. (seed) extracts**						
	A9	**A13**	**SD4**	**SD5**	**SD6**	**S**	
G +ve & G –ve Bacteria							
Bacillus Subtilis	21	7	11	12	15		18
Staphylococcus aureus Citobacterfruendii Escherchia Coli	17 - -	9 - -	14 - -	13 - -	12 - -		21 - 22
Klebsiella Pneumoniae	-	8	12	14	13		-
Salmonella typhimurium	-	-	-	-	-		-
Proteus vulgaris	22	-	-	-	-		20
Pseudomonas aeruginosa	-	-	-	-	-		23

Fungi

Candida albicans	-	-	-	-	-	17
Candida tropicalis	-	-	-	-	-	-
Aspergillus Candidus	-	12	-	-	-	-
Aspergillusflavus	-	-	-	-	-	20

Aspergillusniger	-	-	-	-	-

Disc diameter= 4mm++++ =21-25+++ = 16-20 mm.++ = 11-15 mm+ = 6.0-10 mm - = no activity.

Inhibition values beyond control are + = 6-10mm,+++= 16-20mm and - = not active(the values are including disc diameter) S= Itraconazole (antifungal agent) and Ciprofloxacin (antibacterial agent).

The standards are in the form of sterile Hi-disc cartridges, each disc containing 10mg of the drug.

Antimicrobial activity of compounds isolated from the hexane, aqueous and ethanol extracts of Mesuaferrea L. (Seeds). (in present work).

Microbial Strains

G +ve & G –ve Bacteria	**A9**	**A13**	**SD4**		**SD5**		**SD6**		**S**	
Bacillus Subtilis	++++	+		++		++		++		+++
Staphylococcus aureus	+++	+		++		++		++		++++
Citobacterfruendii	-	-		-		-		-		-
Escherchia Coli	-	-		-		-		-		++++
Klebsiella Pneumoniae	-	+		++		++		++		-
Salmonella typhimurium	-	-		-		-		-		-
Proteus vulgaris	++++	-		-		-		-		+++

Pseudomonas aeruginosa	-	-	-	-	-	++++

Fungi

Candida albicans	-	-	-	-	-	+++
Candida tropicalis	-	-	-	-	-	-
Aspergillus Candidus	-	++	-	-	-	-
Aspergillusflavus	-	-	-	-	-	++++
Aspergillusniger	-	-	-	-	-	-

Disc diameter= 4mm++++ =21-25+++ = 16-20 mm.++ = 11-15 mm+ = 6.0-10 mm - = no activity.

Inhibition values beyond control are + = 6-10mm,+++= 16-20mm and - = not active(the values are including disc diameter).

S= Itraconazole (antifungal agent) and Ciprofloxacin (antibacterial agent).

The standards are in the form of sterile Hi-disc cartridges, each disc containing 10mg of the drug.

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