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Indian medicinal herb: Antimicrobial efficacy of *Mesua ferrea* L. seed extracted 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 seven 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 500 μg/disc. Gram-positive bacteria were most susceptible and yeast was most resistant. The pronounced antimicrobial activity was with the extracts in non-polar solvents than in polar ones. **Results:** The results were compared with the zones of inhibition produced by commercially available standard antibiotics. The lipophilic extracts of *M. ferrea* L. showed more activity towards Gram positive bacteria. **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. These results indicate that *M. ferrea* extract could be useful in fighting emerging drug-resistant microorganisms.

1. Introduction

Herbal medicine represents one of the most important fields of traditional medicine all over the world. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way[1-6].

Recently, infections caused by microorganisms have increased tremendously and antibiotic resistance has become a global therapeutic problem. On account of this, special attention has been paid to extracts and biologically active compounds isolated from plant species[7,8] with a hope to get new promising drugs to treat these resistant stubborn microorganisms. Antimicrobials of plant origin are not only efficient in the treatment of infectious diseases but they are free from many of the side effects that are often associated with synthetic antimicrobials[9]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action[10,11].

Mesua ferrea (*M. ferrea*) L. commonly known as Nagkesar belongs to the family Guttiferae. It is a medium sized glabrous tree found in the North-East and Southern part of India. The trunk is straight, erect and ash colored. The bark is grayish or reddish-brown. The leaves are oblong-lanceolate or acute. Flowers are large, white and fragrant. The fruits are ovoid and the seeds are angular, smooth and chest-nut brown[12]. The principal constituents of *M. ferrea* include mesuaferrone-A & B, mesuaferrol, mesuanic acid, α-& β-amyrin and β-sitosterol present in the stamen[13] while it is reported that seeds contain essential oils, xanthones and coumarins[14-16]. Traditionally this plant is widely used for curing many ailments in India. Decoction of 2-3 flowers with sugarcandy is given twice a day for 3 d to stop bloody stool. Powder of flowers and fruits with butter is applied locally on piles. Essential oil is used to treat skin diseases and its application is recommended in rheumatism[17]. The few scientific reports on the

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biological activities of *M. ferrea* studied in the literature include antiasthmatic activity[18], anti-inflammatory and C.N.S. dependent activities[19], estrogenic and progestational activity[20], antispasmodic activity[21], antimicrobial and anthelmintic activity of essential oil[22], as bactericide from leaf[23] and antimicrobial activity of flower[20-25]. *M. ferrea* is also used in herbal formulation to treat piles[26] and cancer[27]. *In-vivo* antioxidant and immunomodulatory activity[28] as well as disinfection studies of seed kernel oil are reported[29]. According to Mathekga and Mayer[30], *in vitro* antimicrobial screening methods are useful tools to select active extracts for further chemical and pharmacological investigations.

This paper reports the first attempt to study the antimicrobial property of Indian medicinal plant, *M. ferrea* L. seed (Guttiferae) extracts in different solvents against an array of human pathogens.

2. Material and methods

2.1. Plant material

M. ferrea L. seeds were purchased from local market of Rajkot, India. The taxonomic identity of this plant was confirmed by Dr. N. K. Thakrar Department of Biosciences, Saurashtra University, Rajkot. The material was purchased in the form of dried part, in July, 2010 which was then homogenized to fine powder and stored in airtight bottles.

2.2. Extraction of plant material

The air-dried and powdered plant material (10 g of each) was extracted with 100 mL each of petroleum ether, 1, 4-dioxan, chloroform ($CHCl_3$), acetone, N, N, dimethyl formamide, ethanol (CH_3OH) and water (H_2O), kept on a rotary shaker for 24 h. Thereafter it was filtered and centrifuged at 5 000 g for 15 min. The supernatant was collected and evaporated to dryness to give the crude dried extract. The extractive yield (%) of all the extracts is shown in Table 1.

2.3. Microbial strains

The test microbial strains investigated are listed in Table 1. All the bacterial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4 ℃while fungal strains were grown in Sabouraud broth and maintained on MGYP slants (yeast) and potato dextrose agar slants (mould) at 4 ℃

2.4. Antimicrobial assay

A modified agar disc diffusion method[31,32] was used to determine the antimicrobial activity. Molten Mueller Hinton agar No. 2 (HiMedia) was inoculated with microbial cell suspension (100 μL) and poured into sterile Petri dishes. Sterile filter paper discs of 7 mm diameter were impregnated with 20 μL extract solution equivalent to 500 μg of the each dried extract in 100% DMSO (Dimethylsulphoxide) and air dried. Thereafter the discs were placed on the surface of the seeded agar plates. Piperacillin (100 μg/disc), Gentamicin (10 μg/disc) and Amphotericin B (100 units/disc) were used as positive controls. Paper discs loaded with 20 μL of DMSO served as negative control. The plates were incubated at 37 ℃ for 24 h for all the bacterial strains while that of fungal strains were incubated at 28 ℃ for 48 h. The experiment was done three times to minimize error. After incubation period the antibacterial activity was evaluated by measuring the inhibition zones. An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antibacterial activity.

3. Results

The presence of antifungal and antibacterial substances in the higher plants is well established. Plants have provided a number of novel drugs and they have made significant contribution towards health. The extractive yield of different solvents is given in Table 1. Maximum yield was obtained in DMF (23.18%) while minimum was in petroleum ether (3.42%) (Table 1).

In the present study an array of solvents with different polarity were used to study the antibacterial activity of *M. ferrea* seed extract. The antibacterial activity was definitely better when the plant was extracted in organic solvents than in aqueous solution (Table 1). Out of the 9 Gram positive strains studied, significant antibacterial activity was shown against *M. flavus* followed by *B. subtilis* and *S. subflava*. Lowest antibacterial activity was shown against *S. epidermidis*. *M. ferrea* leaf extract in all the organic solvents and aqueous showed activity against the Gram positive bacteria studied. An entirely different trend was obtained with Gram negative bacteria. Here only 35% of the strains were active and maximum antibacterial activity was shown against *P. putida* followed by *P. mirabilis* and *K. aerogenes*; all the other strains viz. *A. fecalis*, *C. freundii*, *E. aerogenes*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, *P. pseudoalcaligenes*, *P. testosteroni* and *S. typhimurium* were resistant.

In antifungal study, except *T. beigelli*, none of the yeast showed antifungal activity; on the other hand, 50% of the moulds studied showed antifungal activity. Only, chloroform, DMF and ethanol extracts showed activity

Table 1
Antimicrobial efficacy of *M. ferrea* L. seed extracts against microbial strains.

	Microbial strains	Inhibition zone (mm)a							Antimicrobicsd		
		Mesua ferrea L. Extracts (500 μg/disc)b [Extract yield in %c]									
		MPe [3.42]	MDi [5.36]	MCl [10.6]	MAc [5.01]	MDf [23.18]	MEt [7.29]	MAq [13.59]	Pc(100 g/disc)	G (10 μg/disc)	Ap (100 μg/disc)
G−	*Bacillus cereus* ATCC 11778	18	17.5	15.5	15	12.5	16	9	18	14	NT
	Bacillus megaterium ATCC 9885	16.5	16	18	17	13	13	9	12	32	NT
	Bacillus subtilis ATCC 6633	21	21.5	21	22	14	14	9.5	20	13	NT
	Corynebacterium rubrum ATCC 14898	15	15	13	12.5	11	13.5	–	24	21	NT
	Micrococcus flavus ATCC 10240	26	27	25	26	22	25	11	35	37	NT
	Staphylococcus aureus ATCC 25923	14	15	13	11	10	14	10	28	17	NT
	Staphylococcus aureus ATCC 29737	14	17.5	18	15	12	13	8	27	15	NT
	Staphylococcus epidermidis ATCC 12228	10	12	15	25	11.5	11	11	11	22	NT
	Staphylococcus subflava NCIM 2178	21	20	20	18	16	18	10	22	15	NT
G−	*Alcaligenes fecalis* ATCC 8750	–	–	–	–	9.5	–	–	10	18	NT
	Citrobacter fruendii ATCC10787	–	–	–	–	–	–	–	12	12	NT
	Enterobacter aerogenes ATCC13048	–	–	–	–	–	–	–	12	10	NT
	Escherichia coli ATCC 25922	–	–	–	–	–	–	–	13	25	NT
	Klebsiella aerogenes NCTC 418	18.5	17	17	15	13.5	17	9	17	17	NT
	Klebsiella pneumoniae NCIM 2719	15	16	12	11	10	13	9	25	22	NT
	Proteus mirabilis NCIM 2241	20	21.5	22.5	21	18	18	10	25	23	NT
	Proteus morganii NCIM 2040	11	11.5	12	12	10	9.5	9	19	28	NT
	Proteus vulgaris NCTC 8313	–	–	–	–	–	–	–	20	25	NT
	Pseudomonas aeruginosa ATCC 27853	–	–	–	–	–	–	–	23	20	NT
	Pseudomonas putida ATCC 12842	30	29	31.5	27	27	26	13	45	35	NT
	Pseudomonas pseudoalcaligenes ATCC 17440	–	–	10	–	–	–	–	25	27	NT
	Pseudomonas testosteroni NCIM 5098	–	–	–	–	–	–	–	–	15	NT
	Salmonella typhimurium ATCC 23564	–	–	–	–	–	–	–	19	25	NT
Yeast	*Candida albicans* ATCC 2091	–	–	–	–	–	–	–	NT	NT	13
	Candida albicans ATCC 18804	–	–	–	–	–	–	–	NT	NT	17
	Candida glabrata NCIM 3448	–	–	–	–	–	–	–	NT	NT	18
	Candida tropicalis ATCC 4563	–	–	–	–	–	–	–	NT	NT	12
	Cryptococcus luteolus ATCC 32044	–	–	–	–	–	–	–	NT	NT	15
	Cryptococcus neoformans ATCC 34664	–	–	–	–	–	–	–	NT	NT	16
	Trichosporon begelii NCIM 3404	9	9	10	9	9	10	11	NT	NT	15
Mould	*Aspergillus candidus* NCIM 883	–	–	14	12	15	–	12	NT	NT	20
	Aspergillus flavus NCIM 538	–	–	12	–	–	–	–	NT	NT	18
	Aspergillus niger ATCC 6275	–	–	–	–	–	–	–	NT	NT	20
	Mucor hiemalis wehmer NCIM 873	9	–	12	10	9	–	–	NT	NT	17

−: no activity, NT: not tested, Negative controls did not show any activity, aInhibition zones are mean of three replicates including the diameter of the paper disc (7 mm), bMPe−petroleum ether, MDi−1,4 dioxan, MCl−chloroform, MAc−acetone, MDf−dimethylformamide, Met−ethanol, MAq−aqueous. cPercentage extract yield (w/w) was estimated as dry extract weight/dry material weight×100, dPc−Piperacillin, G−Gentamicin, Ap−Amphotericin−B.

against *A. candidus* and *M. hiemalis* wehmer, while the other three organic solvent extracts (petroleum ether, 1, 4−dioxan and ethanol) did not show any antifungal activity. Aqueous extract showed slight activity against *A. candidus* while it was ineffective against all the other studied moulds. There is no antifungal activity found due to the fungal cell wall is a complex structure and extensive cross−linking between chitin, glucans and other polymers.

The results of the antibacterial and antifungal activity of the different extracts of *M. ferrea* were compared with the standard antimicrobics (Table 1). The extracts showed significant activity against more than 50% of the investigated microbial strains, which is infect a promising result, which was also comparable with standard antimicrobics, which suggest that the plant extracts contain certain constituents with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It is interesting to note that the extracts are not pure compounds and in spite of it good results were obtained which only suggests the potency of these extracts.

4. Discussion

From the results it is observed that the antibacterial activity was more pronounced with the extracts in non-polar solvents than in polar ones. This suggests that the polarity of the solvent plays an important role in exhibiting antibacterial activity[33]. The Gram negative bacteria were more resistant than Gram positive bacteria as also reported by many researchers[34-36]. Also the organic solvents extracts of *M. ferrea* seed were found to have good activity against bacteria than yeast or moulds. This differences in the activity may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure and the yeast cell wall is quite complex[37,38]. These results are in good agreement with the results reported earlier by Ali *et al*[39].

Antifungal activity is not common in medicinal plants. Marting *et al*[40] screened 23 extracts of 12 Cuban plants but they did not inhibit the growth of yeast. The potential for developing antimicrobial from higher plants is rewarding as it will lead to the development of a phyto-medicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects as compared to synthetic compounds. The results obtained are encouraging as the organic solvent extracts have shown considerable antibacterial activity[41,42].

The present work has demonstrated the antimicrobial potential of *M. ferrea* L. seed extracted in various solvents. This is the first report on the antimicrobial activity of *M. ferrea*. The above observations have clearly demonstrated *M. ferrea*, as a folk remedy which confirms its folkloric utilization. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties. Hence, *M. ferrea* seed extract should be taken up for further bioactivity guided isolation of active compound and carry out pharmaceutical studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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