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Antibacterial and Antifungal efficacy of steam distillate of *Moringa oleifera* Lam.

T.R. Prashith Kekuda [1*], N. Mallikarjun [2], D. Swathi [2], K.V. Nayana [1], Meera B Aiyar [1], T.R. Rohini [1]

[1] Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, INDIA

[2] P.G Dept. of Studies and Research in Microbiology, Sahyadri Science College, (Autonomous), Shivamogga-577203, Karnataka, INDIA

Abstract

The present study describes antibacterial and antifungal activity of steam distillate of *Moringa oleifera* Lam, a pan-tropical species known for its nutritional and medicinal properties. A considerable reduction in the growth of test bacteria was observed by distillate suggesting antibacterial effect. Among bacteria tested, more inhibition was observed in case of *E. coli* followed by *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *B. subtilis*. Inhibition of fungi was observed as reduced colony diameter in plates poisoned with distillate as compared to control plates. More inhibition of *A. niger* was observed followed by *A. oryzae*, *A. terreus* and *A. nidulans*. The antimicrobial activity of steam distillate of *M. oleifera* could be attributed to the inhibitory components present in the distillate fraction. Further studies involving separation of active constituents and determining biological efficacy are to be carried out.

Key word: Antibacterial activity, Essential oils, *Moringa oleifera* Lam., Poison food technique, Steam distillate

Introduction

Moringa oleifera Lam, or the horse radish tree, is a pan-tropical species that is known for its nutritional and medicinal properties. *M. oleifera* is the most widely cultivated species of a monogeneric family Moringaceae that is native to the sub Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [1,2]. Various parts of the Moringa tree such as root, root bark, leaves, flowers and unripe pods are used in Ayurvedic system of medicine as antipyretic. Abortificient, galactogogue and anthelmintic. In Siddha and Unani system of medicine the bark is used as an emmenagogue and root decoction is used as gargle, abortifacient, rubefacient, counter-irritant in rheumatic cases [3]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition. This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. Essential oils are essentially obtained by hydrodistillation where the plant material is heated in two to three times its weight of water with indirect steam from outside the still as opposed to steam distillation in which the plant material is extracted by direct steam, produced in the still, or by indirect steam, produced outside and fed into the still [4]. The present study describes antibacterial and antifungal activity of steam distillate of *M. oleifera*.

Materials and Methods

Collection and Identification of plant material

The plant material *M. oleifera* (Voucher no. PK/SRNMN/MB/MO/201) was collected in outskirts of Shivamogga, authenticated in Department of Botany and the voucher specimen was deposited in the department for future reference.

Extraction of plant material

A simple laboratory quick-fit apparatus with a 1000ml distilling flask (to boil the mixture of plant material and water), a condenser (to condense the steam to obtain the steam distillate), and a receiving vessel, was used for the steam distillation. A known weight of (100g) air-dried powdered leaf material was subjected to steam distillation in the assembly for about three hours [5,6]. The distillate obtained was transferred into clean container and stored in refrigerator until use.

Screening for Antibacterial activity

The antibacterial activity of steam distillate *M. oleifera* was tested against

bacteria namely *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in liquid nutrient media. The nutrient broth tubes containing steam distillate (5%) was sterilized by autoclaving and inoculated with standardized volumes of 24 hours old broth cultures of test bacteria followed by incubation at 37^{o}C for 24 hours. A set of nutrient broth tubes inoculated with bacterial cultures was kept as control without adding steam distillate. After incubation, the contents in the tubes were mixed thoroughly and the optical density was measured by spectrophotometer at a wavelength of 490 nm as a guide to microbial growth. The experiment was performed in triplicate to get reliable results [7].

Screening for Antifungal activity

Four species of the genus *Aspergillus* namely *A, niger, A. oryzae, A. nidulans* and *A. terreus* were tested for their sensitivity towards steam distillate of *M. oleifera*. The suspension of spores of the test fungi was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 [8]. The antifungal activity was assessed using Poison food technique [9]. The effect of distillate on fungal growth was determined by measuring the diameter of the colony obtained after incubation on poisoned plate and comparing with control (plates not poisoned with extract). The experiment was done in triplicate and average reading was recorded.

Results

In antibacterial study, a considerable reduction in the growth of test bacteria in distillate containing tubes was observed when compared to control tubes and is suggestive of antibacterial effect of steam distillate. Among bacteria tested, more inhibition was observed in case of *E. coli* (73.43%) followed by *S. aureus* (70.34%), *K. pneumoniae* (51.80%), *P. aeruginosa* (49.16%) and *B. subtilis* (45.67%) by Moringa distillate (Table-1). Results of antifungal activity of steam distillate is given in Table-2. Inhibitory activity was indicated by reduced colony diameter in plates poisoned with distillate as compared to control. More inhibition of *A. niger* (46.51%) followed by *A. oryzae* (26.31%), *A. terreus* (23.07%), and *A. nidulans* (16.21%) was observed.

Discussion

The fragrance of plants is carried in the so called quinta essentia, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure and are called terpenes [10]. Examples of common terpenoids are methanol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids). Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method for extraction is steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential oil normally float on top of the Hydrosol (the distilled water component) and may be separated off [11].

The plant *M. oleifera* is a miracle tree and was found to exhibit several biological activities. It is reported to contain alkaloids, flavonoids, anthocyanins, Proanthocyanidins and cinnamates. It is used in abortion [12,13], Diabetes [14], and as an antiherpes simplex virus type I [15]. All parts of tree are considered to possess medicinal properties and used in the treatment of ascites, rheumatism and venomous bits and as cardiac and circulatory stimulant [16]. *In vitro* antibacterial and antifungal activity of the compound Aglycon of Deoxy-Niazimicineisolated from *M. oleifera* Lam showed more activity against *Shigella boydii*, *Shigella dysenteriae* and *Staphylococcus aureus* and moderate

activity against *Candida albicans* and *Aspergillus flavus* [17]. The Antibacterial activity of methanolic crude extract, purified dichloromethane extract and isolated parts from column chromatography of *M. oleifera* capsules by agar well diffusion was investigated [18]. The purified dichloromethane extract and isolated parts from column chromatography showed activity against the test bacteria. Three fractions from the leaves of *M. oleifera* obtained on sephadex G-25 column chromatograph showed strong inhibitory activity against *E.coli*. Fraction P2 showed significant zone of inhibition against *Aspergillus niger* [19]. The efficacy of *M. oleifera* seed on coliforms in raw water was investigated. About 88 and 97.50% of the total bacteria and coliforms respectively were reduced in the surface water after 24 hr of treatment. The coagulating efficiency and inhibition increased correspondingly with an increase in concentration of the seed extract [20].

Table-1: Inhibition of test bacteria by steam distillate of *M. oleifera*

Test bacteria	Optical density at 490 nm		% inhibition as compared to control
	Control	Distillate	
E.coli	0.606	0.161	73.43
B.subtilis	0.208	0.113	45.67
S.aureus	0.688	0.204	70.34
K.pneumoniae	0.525	0.253	51.80
P.aeruginosa	0.489	0.244	49.16

Results are average of three trials

Table-2: Inhibition of test fungi by steam distillate of *M. oleifera*

Test fungi	Average colony diameter in cm		% inhibition
	Control	Distillate	
A.niger	4.3	2.3	46.51
A.oryzae	3.8	2.8	26.31
A.nidulans	3.7	3.1	16.21
A.terreus	2.6	2.0	23.07

Results are average of three trials

Conclusions

A marked antibacterial and antifungal activity by steam distillate of *M. oleifera* was observed in this study. These activities of steam distillate might be possibly due to the essential oil fraction of the plant material present in the distillate fraction. As the distillate exhibited activity against *S. aureus, K. pneumoniae, E. coli* and *P. aeruginosa*, it can be used in the treatment of many types of infections such as nosocomial infections, food borne illness, gastrointestinal infections, wound infections etc as the bacteria tested were known to cause the above said type of infections. The distillate could be used to treat infections caused by antibiotic resistant bacteria. The distillate could be used against opportunistic mycotic infections caused by some fungi such as species of *Aspergillus*, *Mucor* etc as the study employed *Aspergillus* species which were found to be sensitive. Further studies are to be carried to isolate the active fraction from the steam distillate of *M. oleifera* and determine antibacterial and antifungal efficacy *in vitro* and *in vivo*.

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