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Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam.

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ABSTRACT

Moringa oleifera known as Moringa is native to north India but is now found throughout the tropics. *Moringa* has an impressive range of medicinal uses with high nutritional value. Having known the bioactive potency of *M. oleifera,* the present study and findings deal with the antimicrobial activities of the organic extract of leaves, seed and flower of *M. oleifera* that contain antibacterial, antifungal, GC-MS chemical profiles and secondary metabolites which are frequently used in traditional medicine references attest to its curative power. The various preparations of *M. oleifera* acts as antibiotic, antitrypanosomal, hypotensive, antispansmoctic, antiulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycemic activities

KEYWORDS: Antibacterial, antifungal, GCMS chemical profiles, *Moringa oleifera,* secondary metabolite

INTRODUCTION

Moringa oleifera Lam. (horseradish tree, drumstick tree, benzolive tree, kelor, marango, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree) is the most widely cultivated species of a monogeneric family, the *Moringaceae*, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. India is the largest producer of *Moringa* with an annual production of 1.1 to 1.3 million tonnes of tender fruits from an area of 380 km². It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses.

All parts of the *Moringa* tree are edible and have long been consumed by humans (Fuglie, 1999). Various parts of *Moringa* acts as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal, and are being employed for the treatment of different ailments in the indigenous system of medicine. *Moringa* seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity (Tsaknis *et al*., 1999). The present study was demonstrated to explore the antibacterial activity and to identify phytochemical constituents present in the *M. oleifera*.

MATERIALS AND METHODS

The *Moringa oleifera* plant samples like *Moringa* leaf 1 (MOL1), *Moringa* leaf 2 (MOL2), *Moringa* Seed (MOS) and *Moringa* Flower (MOF) were collected from the fields and taken to Department for Biotechnology, Karunya University, Coimbatore for identification and evaluation of antimicrobial activity of the plant sample.

Extraction of the sample

Fresh leaves, seed and flower of *M. oleifera* were ground separately in a mortar. Each of the plant tissues was soaked in approximately 400ml of 95% ethanol on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whatman filter paper No. 1. The filtrate was then concentrated on a rotary evaporator at 50^{o}C to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4^{o}C.

Disc preperation and antiomicrobial sensitivity test

The prepared extract was weighed and mixed with known concentration of ethanol. Meanwhile, discs were prepared with Whatmann no. 1 filter paper. Discs were added with different concentration (50,100,150 and 200mg) of the extract. The standard disc diffusion method by Kirby Bauer, 1996 was followed to evaluate the microbial

activity of clinical isolates like *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp*, Proteus mirabilis, Pseudomonas aeroginosa, Salmonella typhi A, Staphylococcus aureus, Streptococcus* and *Candida albicans* of fungal groups.

Phytochemical constituents

Preliminary phytochemical studies on *M. oleifera* were determined by the standard method of Odebiyi and Sofowora, 1999. The secondary plant metabolites were tannins Saponin, Flavanoids, Steroids, Terpenoids and Glycosides. The quantitative estimation of secondary metabolites was compared with nutritional indices. Ethanolic extract of leaves, seed and flower of *M. oleifera* was injected on to a MD 800 FISONS interfaced with the GC/MSD Chemstation of Clarus 500 Perkin Elmer model at SPIC, Tuticorin, Tamil Nadu containing Wiley and NIST mass spectral library in order to analyse the chemical nature and molecular structure of bioactive compounds present in the extract.

RESULTS AND DISCUSSION

The antimicrobial activities of *M. oleifera* leaves, flower and seeds were investigated in vitro against fungus, gram negative and gram positive bacteria (Table 1). The antibacterial activity of MOL1 was checked over the most often isolated clinical pathogens like *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp*, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus,* and *Streptococcus.* It showed antibacterial activity against most of the organisms like *E. coli, K. pneumoniae, Enterobacter, P. aeruginosa* and *S. aureus* MOL2 samples showed antibacterial activity only against *P. aeruginosa* and *Staphylococcus*. Similarly ethanolic extracts of *M. olifera* demonstrated the highest activity, while the aqueous extracts showed the least activity against *S. typhi,* causative agent of typhoid fever (Doughari *et al.,* 2007). The activities of these extracts were comparable to those of antibiotics, ciprofloxacin, cotrimoxazole and chloramphenicol, commonly used for treating typhoid fever. MOS showed antibacterial activity against *K. pneumoniae*, *S. aureus,* and *Streptococcus*. Caceres, 1991 demonstrated that the fresh leaf juice and aqueous extracts

from the seeds inhibit the growth of *P. aeruginosa* and *S. aureus*. The ethanolic extract of *M. oleifera* was found to have better inhibition of *S. typhi* than any other organic extracts. A water-soluble polysaccharide was isolated from the aqueous extract of pods of *M. oleifera* was found to has effect on urinary tract infection causing agents and have the potency to clear it (Shaw and Jana, 1982). MOF was showing activity against *E. coli, K. pneumoniae* and *P. mirabilis* (Table 4) but not against *S. typhi A.* All the extracts except the MOF extract were showed antifungal activity against *C. albicans*. Das *et al.,* (1957) have isolated a compound name Pterygospermin from flowers of *M. oleifera* which was known to possess antifungal activity. Various sub species of *M. olifera* are also known to exhibit antimicrobial activity (Spiliotis *et al.,* 1998).

Results of phytochemical analysis demonstrated the presence of the common phytoconstituents (Table 2) like tannins, saponins, flavanoids, glycoside, terpenoids in both the MOL1 and MOL2 extracts. Tannins, saponins, flavanoids and terpenoids have been identified from MOS extract. Tannins, phlobatannin, steroid, glycoside, saponin and flavanoid were identified from MOF extract. The presence of these constituents has been reported to account for the exertion of antimicrobial activity by plants (Clark, 1981; Gonzalel and Mather, 1982; Lutterodt *et al.,* 1999; Pretorius and Watt, 2001).

Ethanolic leaf extract of *M. oleifera* (MOL1), when analysed by GC-MS found to have fifteen major components (Fig 1). The retention time varied from 11.075 to 12.635. compounds like hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate, Palmitic acid ethyl ester, 2,6- Dimethyl-1, 7-octadiene-3-ol, 4-Hexadecen-6-yne, (z)-(CAS) etc has been identified. MOL2 extract showed (Fig 2) the retention time between 11.083 and 19.783. 2-hexanone, 3- cyclohexyliden-4-ethyl - E2- Dodecenylacetate, Hi-oleic safflower oil (CAS), Safflower oil etc compounds has been identified from MOL2 extract (Table 11). MOS extract showed (Fig 3) the retention time between 12.667 and 21.375 fifteen compounds were identified in MOS extract. The major compounds found were Roridin E, Veridiflorol, 9-Octadecenoic acid etc. MOF extract showed (Fig 4) the retention time between 9.542 and 12.650. 9- Octadecen – 1- ol, (Z) – (CAS) cis - 9 – Octadecen – 1 –

ol, Oleol, Satol, Ocenol, Sipo, Decanoic acid, Dodecanal etc has been identified in MOF extract.

CONCLUSION

In the present scenario of emergence of multidrug resistance to human pathogenic infections, it has become very necessary to search for new antimicrobial substances from other sources such as plants. *M. oleifera* is highly valued plant, with impressive range of medicinal uses and high nutritional value. A plethora of traditional medicine references attest to its curative power, and scientific validation of these popular uses is developing to support at least some of the claims *Moringa* preparations known to have antibiotic, antitrypanosomal, hypotensive, antispansmoctic, antiulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycemic activities as cited in the scientific literature. Further purification of compounds can be done and the compounds may be subjected for animal studies.

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Table 1: Antimicrobial activities of leaf, seed and flower extracts of *M. oleifera*

S.No.	MICROORGANISM	MOL 1	MOL 2	MOS	MOF
Antibacterial activity					
1.	*E. coli*	+	-	-	+
2.	*K. pneumoniae*	+	-	+	+
3.	*Enterobacter*	+	-	-	-
4.	*P. mirabilis*	-	-	-	+
5.	*P. saerogenosa*	+	+	-	-
6.	*S. typhi A*	-	-	-	-
7.	*S. aureus*	+	+	+	-
8.	*Streptococcus*	-	-	+	-
Antifungal activity					
1.	*C. albicans*	+	+	+	-

(+) Susceptible (-) Resistance

Table 2: Preliminary phytochemical analysis of leaves, seed and flower of *M. oleifera*

S. No	PARAMETERS	MOL 1	MOL 2	MOS	MOF
1.	Tannins	+	+	+	+
2.	Phlobatannin	-	-	-	+
3.	Saponin	+	+	+	+
4.	Flavanoid	+	+	+	+
5.	Steroid	+	+	+	+
6.	Terpenoids	+	+	+	-
7.	Glycoside	+	+	-	+