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Full Length Research Paper

Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengi* Linn

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In order to search for antimicrobial phytochemicals, two antibacterial compounds were detected from the seeds of *Mimusops elengi* Linn. The compounds were extracted by ethyl acetate and purified by column chromatography. Their structures were elucidated through IR, ^{1}H NMR and GC-EL-MS spectral analyses. After characterization, the compounds were identified as 2,3-dihyro-3,3'4'5,7-pentahydroxyflavone and 3,3',4',5,7-pentahydroxyflavone. The compounds showed strong inhibitory activity against Gram positive and Gram negative bacteria.

Key word: Antimicrobial, phytochemical, flavone, seed, ***Mimusops***.

INTRODUCTION

The limited life span of antimicrobials due to resistance because of indiscriminate use necessitates the continuous search for alternatives. Awareness for misuse of antibiotics and also the potential risk of using synthetic form of phytochemicals have been reported (Borris, 1996). The use of traditional plant extracts as well as other alternative forms of medical treatments has been getting momentum since the 1990s (Cowan, 1999). The medicinal use of plant species outnumbered (~10%) its use as food and feed (Moerman, 1996).

Traditionally common people use crude extracts of plant parts as curative agents (Mendoza et al., 1997; Sanches et al., 2005). Medicinal properties of ***Mimusops elengi*** Linn (Family Sapotaceae), a common tree species in India, is not very well known. Phytochemical investigation of the plant reveals that the bark decoction, flower or fruit is used as teeth cleaner and tender twigs are used as a toothbrush (Dymock et al., 1981). However, antimicrobial potential of the seed extract of ***M. elengi*** is yet to be investigated. This paper deals with the isolation, purification and characterization of the extracts from the seeds of ***M. elengi*** (Linn).

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MATERIALS AND METHODS

Extraction and antimicrobial assay

Seed extracts of ***M. elengi*** Linn. (BAKUL), Family Sapotaceae were air-dried, finely ground and extracted with organic solvents (petroleum ether, dichloro methane, ethyl acetate and ethanol) for 48 h in a soxhlet extractor and were tested for antimicrobial efficacy.

Bacterial organisms ***Escherichia coli*** ATCC 25922, ***Bacillus subtilis*** ATCC 6633 and ***Salmonella typhi*** ATCC 6539 were maintained in nutrient agar medium (Hi Media). Antimicrobial assay was done by agar diffusion method (Washington, 1985) on nutrient agar medium using crude extract against test organisms (10^7 cfu/ml). Antimicrobial capability was estimated visually by measureing the inhibition zone.

Purification

Based on antimicrobial efficacy the ethyl acetate extract purified through silica gel (60 - 120; Merck, India) column (58 cm X 5.5 cm). Mixture of hexane-ethyl acetate in various ratios with increased polarity was used as eluting solvent. The flow rate was 2 ml/min and several fractions were collected totaling to 500 ml. The fractions were monitored by thin-layer chromatography on silica gel 'G' (Merck, India) coated (0.5 mm thickness), using benzene-ethyl acetate (1:1) mixture as developing solvent. Fractions of similar R_f value were mixed together and used as apparently purified compound.

Thin layer chromatography (TLC) plates were activated before use. One-dimensional TLC was performed with the purified compounds using two different solvent system; benzene-ethyl acetate (1:9) and chloroform-methanol (1:3) as mobile phase. The bands

Table 1. Physical characteristics of HE-a and HE-b.

Physical characters	HE-a	HE-b
Color	Light orange	Cream
Nature	Crystalline solid	Crystalline solid
Solubility	Hexane-ethyl acetate (60: 40), ethyl acetate and ethanol	Hexane-ethyl acetate (60: 40), ethyl acetate and ethanol
R_f chloroform:methanol =1:3)	0.95	0.93
Melting point	312-3˚C	230-2˚C

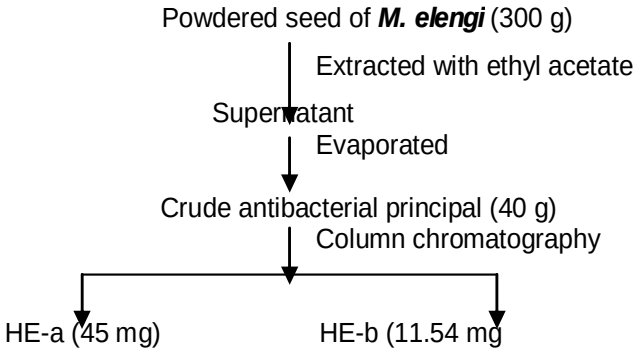

Figure 1. Isolation of active compound from the seed of ***M. elengi*** (Linn).

were detected by keeping the plate in iodine vapor chamber and were eluted with methanol: ethyl acetate (1:1). Confirming homogeneity of compound, the activity was detected by bioassay.

Characterization

Chemical characterization of purified active compound was done through IR, and EL-MS, ^{1}H-NMR spectral analyses. For IR-analyses, the sample was kept in vacuum desiccators over KOH pellets for 48 h, and then IR-spectral analyses were done with 1 mg sample in a FTIR (FT/IR-420 Jasco). The mass spectra (EL-MS) were analyzed from available library data.

RESULTS

Isolation and purification

For isolation of the active principal (outlined in Figure 1) air-dried and finely ground seeds of ***M. elengi*** was extracted with ethyl acetate and supernatant was assayed for antimicrobial efficacy. The crude antimicrobial principal was loaded to silica gel column for purification. A mixture of hexane and ethyl acetate (3:2) showed best result as eluting solvent mixture. After evaporation, the homogeneity was tested by TLC using a mixture of chloroform and methanol (1:3) as developing solvent that separated the mixture into two spots (HE-a and HE-b). Both spots area were eluted and showed good antimicrobial activity. After repeat TLC, both spots areas pooled separately, tested for their antimicrobial activity and purified by column chromatography, used hexane and ethyl acetate (3:2) as eluting solvent mixture. The eluents were evaporated to dryness. Dried fractions were designated as HE-a (45 mg) and HE-b (11.5 mg). Physical characteristics of both fractions of active compound are given in Table 1, and they responded to specific tests for flavonoids compounds as recommended by Stahl (1969).

Spectral analyses

Fraction HE-a: IR: V_{max} (HBR) 3419 (OH group), 1614 (chelated CO) 1477 (unsaturated, due to '=' bond) (Figure 2A). ^{1}H-NMR δ_H (300 MHz, Acetone-d_6): HE-a spectral analyses show close similarity with that of HE-b, only exception is the presence of 4.73 of a H attachment to C-3 and appearance of a peak (s, δ_H 11.78) for enolic OH at position 3 (Figure 3A). From the IR-spectra, ^{1}H-NMR and mass spectra of HE-a, it could be identified as dihyro-3,3'4'5,7-pentahydroxy flavone and molecular formula is $C_{15}H_{12}O_7$ (M^+ 304). Mass (EIMS): m/z, 302 (M^+, 100%) (Figure 4A).

Fraction HE-b: IR: V_{max} (HBR) 3418 (OH group), 1635 (chelated CO) cm^{-1} 1477 (Figure 2B). ^{1}H-NMR δ_H (300 MHz, Acetone-d_6) 6.86 [d,1H(C-8,H), J1.9], 6.73(d, 1H(C-6,H), J1.9], 6.76 - 6.67[m,1H(C-2',H)], 5.82 - 5.77 [dd,2H(C-5',H&C-6',H) J11.8 and J10.4], 4.78[s, 5H,(OH-50], 4.80[d,1H(C-2,H)], 4.39[d,1H(C-3,H), J11.4] (Figure 3B). Compilation of spectral data of HE-b suggests it as 2,3-dihyro-3,3'4'5,7-pentahydroxy flavone and molecular formula is $C_{15}H_{12}O_7$ (M^+ 304). Mass (EIMS): m/z, 304 (M^+, 55%), 153 [$C_8H_8O_3$ (152) + H or $C_7H_4O_4$ (152) +H Base peak, 100%)], 137 (M-167, 23%), 123 [$C_8H_8O_3$ (152) – HCO] (Figure 4B).

DISCUSSION

Chemical properties of a compound facilitate its separation from a mixture (Urekawa et al., 1993; Chandra and Nair, 1995). Isolation of plant flavonoids by solvent extraction and subsequent purification by silica gel column chromatography are well documented (Pepljnak et al.,

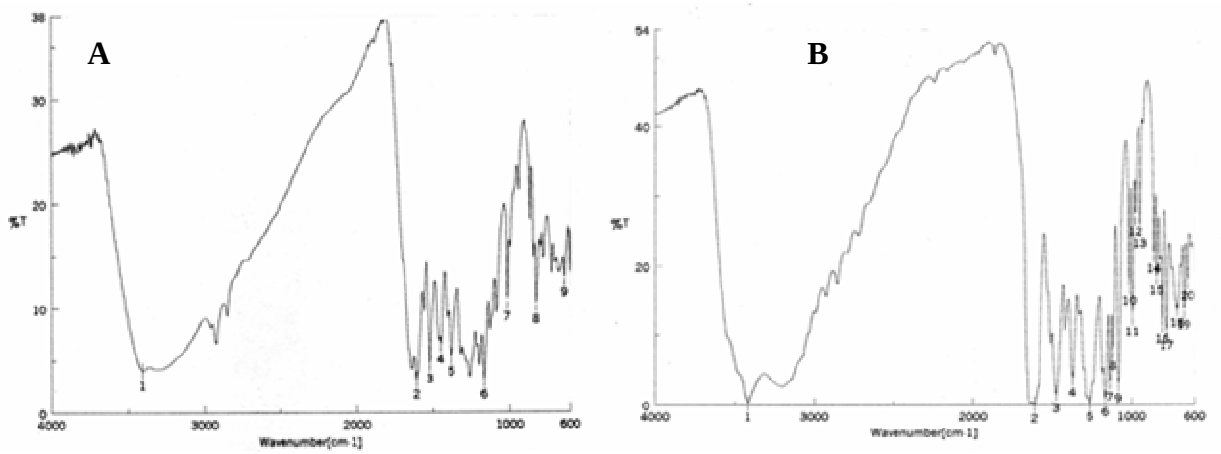

Figure 2. IR Spectra of HE-a (A) and HE-b (B).

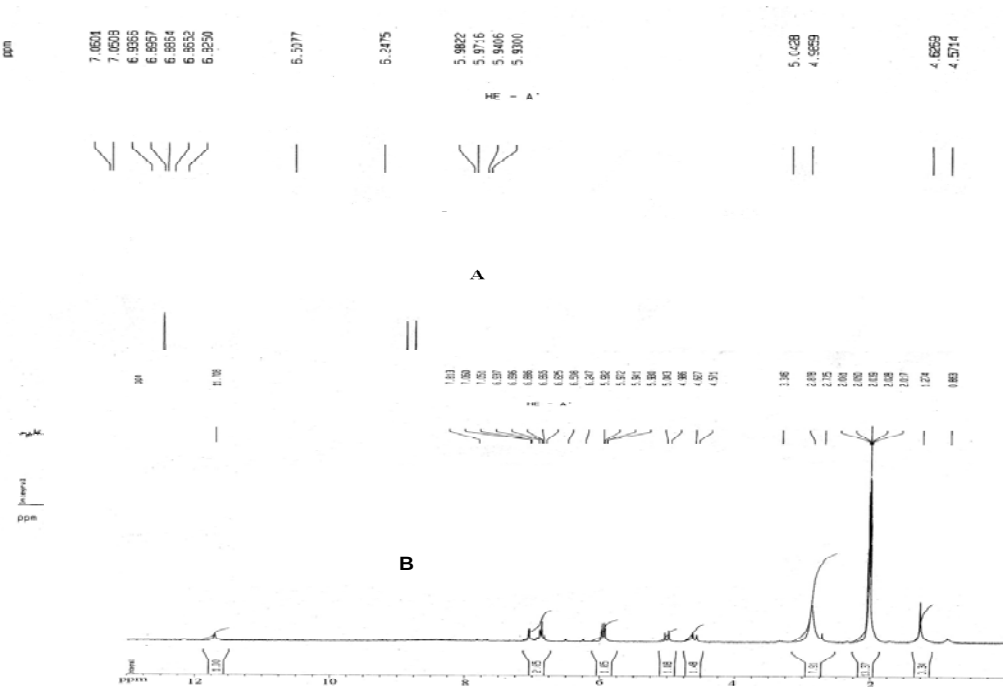

Figure 3. ^{1}H NMR spectra (200 MHz) of HE-a (A) and HE-b (B).

2005; Sanches et al., 2005). Active compound of seeds of ***M. elegans***, extracted by ethyl acetate and purified by silica gel column, resulted into two compounds (HE-a and HE-b). After spectral analyses, HE-a was identified as 2,3-dihyro-3,3'4'5,7-pentahydroxy flavone $C_{15}H_{10}O_7$ (M^+ 302) and HE-b as 3,3',4',5,7-pentahydroxy flavone $C_{15}H_{12}O_7$ (M^+ 304). Both of them exhibited wide spectra of antibacterial activity.

Flavonoids are a group of natural compounds known to have various pharmacological properties such as antioxidative, antiinflammatory and diuretic (Havsteen, 2002). However, antiviral (Cowan, 1999) and antimicrobial (Pepaljnjak, 2005) activities of flavonoids are quite well known. In plants, such compounds are commonly synthesized in response to microbial infection (Dixon et al., 1983). It is not surprising that they have been found ***in vitro*** as effective antiviral, antimicrobial substances against a wide array of microorganisms. Probably, the activity lies in their ability to form complexes with soluble proteins and also with bacterial cell walls, as well as by disruption of membrane particularly with lipophilic flavonoids (Tsuchiya et al., 1996).

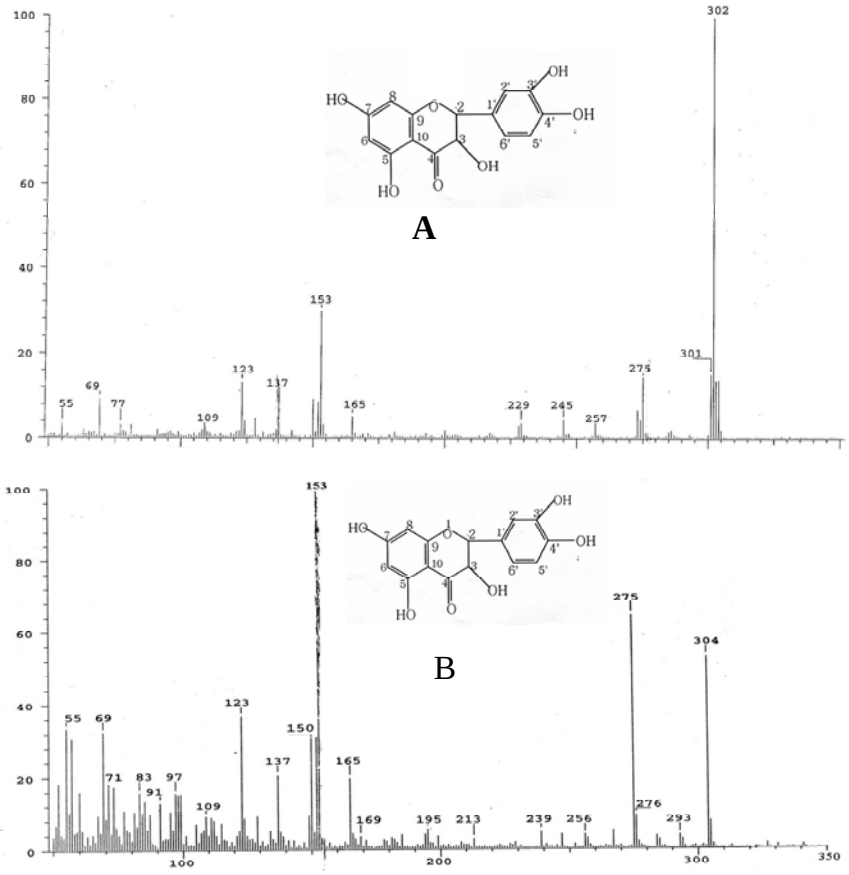

Figure 4. Electron impact mass spectra of HE-a (A) and HE-b (B).

Conclusion

M. elegans is a valuable plant source for traditional drug preparations. But the antimicrobial activity of the plant has not been properly established. Two of our solvent-derived extracts showed good antimicrobial activity. This study may be a lead for further ethnopharmacognostic investigation to identify new compounds with therapeutic promise.

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