Identification of Essential Oils Composition and Antifungal Activity of *Eugenia Singampattiana* Fruits

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Summary

Essential oil of *Eugenia singampattiana* fruits were hydrodistillation by Clevenger apparatus and analysed by gas chromatography-mass spectrometry (GC-MS). The major constituents, α-terpineol (59.57%), camphene (12.08%), *O*- methyl eugenol (11.52%), and α- pinene (4.68%) were found to be *E. singampattiana* fruits. Minimum inhibitory concentration at 0.2µl/ml of essential oils was completely percentage of inhibition active against *Candida albicans*.

Keywords: *Eugenia singampattiana*; fruits; Essential oils; GC-MS; antifungal activity

Introduction

Plant extracts and essential oils, as well as their constituents, are used in the food, cosmetics, and pharmaceutical industries (1). Many essential oils and their ingredients have been shown to possess diverse biological activities, including anti-bacterial, antifungal, and antiviral effects (2-4).

Photo: *Eugenia singampattiana* fruit

Myrtaceae consists of approximately 129 genera and 4620 species (5). *Eugenia* is a one of the larger genera with around 500 species (5). *Eugenia singampattiana* Bedd. is a small tree found in evergreen forests. Flowering and fruiting season is February- August. It is an endemic, vulnerable and aromatic tree found in Karaiyar, Southern Western Region, Tirunelveli. Tribal communities of Kanis known as "Jungle Guava". They were used as edible in ripe fruits. No work done on phytochemicals and antimicrobial activity of whole plant. Therefore, the present work was to evaluate the anti-fungal activity of the essential oils of *E. singampattiana* fruits active against growth of human pathogenic fungi.

Materials and Method

Plant materials

Fresh fruits of *Eugenia singampattiaya* were collected from Karaiyar Region, Tirunelveli District, South India. A voucher specimen SXCK1 was deposited in St. Xavier's College (Autonomous), Palayamkottai-627002, Tamil Nadu.

Isolation of essential oil

Oil was obtained by hydrodistillation method using a Clevenger apparatus for 4h. An yield of the essential oil was dried over anhydrous Na_2SO_4 and stored under refrigeration.

Instrumentation

GC analysis was carried out on a Shimadzu GC 15A chromatograph equipped with a FID detector, using a SE-30 column (3m/3mm). Oven temperature was held at 60^{o}C for 5min, programmed at 2^{o}C/min to 225^{o}C and then held for 10 min. Helium was used as carrier gas at a flow rate of 2ml/min. The quadrupole mass spectrometer was scanned, over the range at 1scan/s, with an ionizing voltage of 70 eV and then ionization current of 150μA. An identification active constituent was based on a comparison of retention time and mass spectra with those authentic samples, Wiley 275L library and with literature values (6).

Strains and growth

Aspergillus flavas (moult), *Aspergillus niger* (moult), *Penicillium notatum* (moult) and *Candida albicans* (Human pathogen) were provided by IMTECH. All strains were grown on Sabouraud dextrose agar (SDA), supplemented with chloramphenicol at 28^{o}C.

Agar Diffusion methods

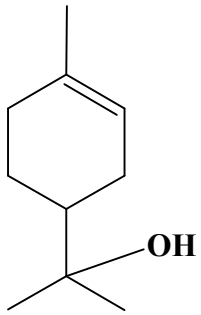
The fungal growth inhibitory potential of the essential oil of *Eugenia singampattiaya* fruits was determined by using the agar disc diffusion method as described by Collins and Lyne, (7). Inocula were prepared by mixing a few microbial colonies with sterile ringer's solution and comparing the turbidity with that of the standard 0.5 McFarland solution, which is equivalent to 10^6-10^8 CFU/ml. One hundred microlitres of inocula of all tested microorganisms were inoculated on Sabouraud Dextrose Agar medium for *Aspergillus flavas* (moult), *Aspergillus niger* (moult), *Penicillium notatum* (moult) and *Candida albicans* were used.

Determination of MIC of essential oils against human fungi

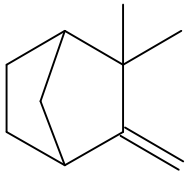
Minimal inhibitory concentration (MIC) of essential oils was determined using a modification of the CLSI micro-dilution test using yeast nitrogen base (YNB) (8). One hundred microliters of fungi suspension at a concentration of 1-5x10^3 ml was added to microtiter wells containing 100ml essential oils double diluted to concentrations ranging from 1 to 50 µl/ml. MIC was determined after 48 h incubation at 37^{o}C, as the minimal concentration that inhibits visible growth of the fungus, by spectrophotometry at 530 nm in an ELISA reader.

Results and Discussion

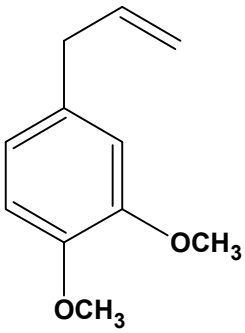
Hydrodistillation of the fresh fruits were produced in pleasant smelling yellow oil at an yield of 0.98% (V/W). Twelve components representing 100% of the fruit essential oil was identified (Table-1). The major constituents of the essential oils were α-terpineol (59.57%), camphene (12.08%), *O*- methyl eugenol (11.52%), and α- pinene (4.68%) Fig-1. To the best of our knowledge this represents the first complete GC-MS analysis of *Eugenia singampattiaya* from India. Major constituent of α-terpineol was previously reported these plants *Eugenia austin*-smith and *E. haberi* (9).



α - terpineol



camphene



***O* - methyl eugenol**

Fig-1: Structure of essential oils

In the present study of antifungal activity of the essential oils isolated from the *E.singampattiana* fruits showed that *Candida albicans* was completely inhibited at 200ppm and *A. niger* at 400ppm. Both *A. flavas* and *P. notatum* were inhibited active higher concentration at 600ppm (Table-2). Reference drug of fluconazole was totally inhibited active against *C. albicans* and no active against *A. flavas* and *P. notatum*. Minimum inhibitory concentrations of the essential oils of *E. singampattiana* fruits are represented in Fig-2. With these fungal cultures, *Candida albicans* showed the highest activity. Volatile oil is their hydrophobic nature and the cell membrane has been proposed as the primary target of their antimicrobial action. Essential oils appear to accumulate in the cell membrane causing the leakage of ions, enzymes, and metabolites (10). Above the effective results, concluded that the antifungal activity may be acted as major constituents of α- terpineol, Camphene, O- methyl eugenol and α- pinene were found to be essential oils composition of *E. singampattiana* fruits.

Table-1: Identification of essential oil composition of *Eugenia singampattiana* fruits

Peak No	Rt. Time	Composition	%
1	4.2	α- thujene	2.40
2	4.7	α-pinene	4.68
3	5.1	myrcene	1.94
4	11.5	camphene	12.08
5	11.7	terpineol	1.02
6	13.1	α-terpineol	59.57
7	13.4	O-Methyl eugenol	11.52
8	13.6	Safrole	0.76
9	14.3	γ- elemene	1.11
10	14.4	β- caryophyllene	2.19
11	15.9	Germacrene D	1.66
12	17.2	β- selinene	1.05

Table-2: Antifungal activity of essential oils of *Eugenia singampattiana* fruits

Fungal strains	% of inhibition				
	100ppm	200ppm	400ppm	600ppm	Fluconazole 100ppm
Candida albicans	52±0.01	100	-	-	100
Aspergillus niger	18±0.02	52±0.25	100	-	-
Aspergillus flavas	15±0.002	41±0.003	85±0.001	100	-
Pencillium notatum	18±0.002	56±0.022	75±0.21	100	-

Values are expressed as triplicate (Mean± SD)

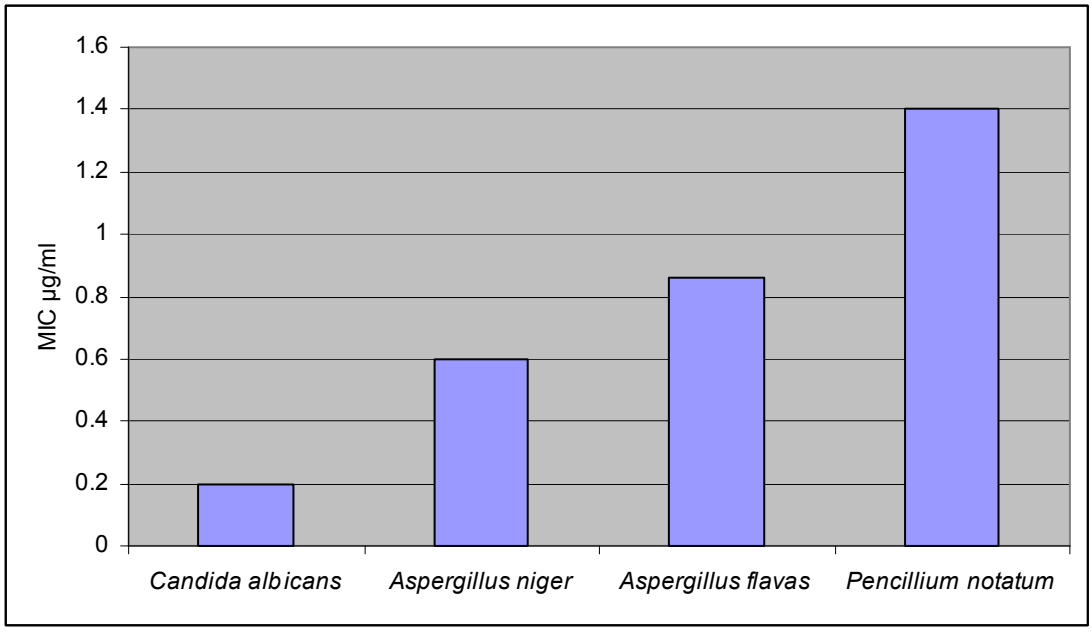


Fig-2: MIC Concentrations of essential oils of *E. singampattiana* fruits against human pathogenic fungi

Conclusions

The GC/MS analysis of *E. singampattiana* fruits essential oil revealed the presence of major constituents were identified as *α*- terpineol, camphene, and *O*-methyl eugenol. As evident from Table-2, the strongest antifungal effect of essential oils of *E. singampattiana* fruits was detected. Further study, therapeutic potential of essential oils of *E. singampattiana* fruits should be isolated and tested for antifungal activity of undergone all the needed stages of modern drug development process.

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