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Essential oil extracted from fruits of egyptian *Eugenia jambolana* has antimicrobial activity

Mohamed I.S.Abdelhady[1,2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah, P.O. Box 715, (SAUDI ARABIA)

[2]Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Ain-Helwan, Cairo (EGYPT)

E-mail : mohibrahem@yahoo.com; miabdelhady@uqu.edu.sa

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ABSTRACT

The essential oil obtained by hydrodistillation of the fruits of *Eugenia jambolana* was investigated by GC/MS. It was found that the percentage of volatile oil in fruits afford 1.2 %. The major components of the volatile oil of fruits are methyl eugenol, limonene and alpha-terpineol (22.5%, 14.43% and 12.04% respectively). The antimicrobial activity of the essential oils of *fruit*s was evaluated against gram positive bacteria (*Staphylococcus aureus*), gram negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*) and *Aspergillus flavus* as fungus. The oil had pronounced antifungal activities on all the tested microbes.

INTRODUCTION

The genus *Eugenia* is one of 75 genera (3000 species) belonging to the family Myrtaceae which are native in the tropics, particularly in tropical America and Australia. The *Eugenia* genus is one of the largest within the Myrtaceae family. In Brazil, there are 350 native species from this genus[1, 2]. Several *Eugenia* species are appreciated for their edible fruits; such as *E. uniflora* (pitanga) and *E. involucrata* (cherry) and some are also used in folk medicine as antidiarrhea (*E. uniflora*) and antidiabetic (*E. jambolana*)[3]. Plants of this family are known to be rich in volatile oils which are reported for their uses in medicine. Chemical composition and certain biological studies of essential oils from certain *Eugenia* species[4-25] were studied. Previous studies revealed that the genus *Eugenia* was reported to be used as an anti-inflammatory, analgesic, antipyretic, antifungal and antimicrobial and peptic ulcer treatment. Fruits and seeds of *E. jambolana* used as an antidiabetic. Also anti-inflammatory compounds were characterized from *E. jambos*. The previous studies performed that *E. supraxillaris* fruit contains methyl eugenol 32.8 and eugenol 35.2 % while the leaves contains 21.8 % limonene 17.4 % B-Pinene, Spathulenol was major component in oil of *E. Cuprea* (represents 12.1%) followed by [beta]-caryophyllene (9.2%). *E. arenosa* was dominated by fernesyl acetate (70.4%) and aromadendrene (11.7%) *Eugenia brasiliensis, E. multicostata, E. sulcata and E. xiriricana* oils possessed very similar chemical compositions. In the monoterpene fraction, [alpha]- and [beta]-pinene were the major components (a total of 40.7%, 23.4%, 35.9% and 21.9%, respectively), while for *E. pitanga* (E)-[beta]-ocimene (10.5%) was the major component. For the sesquiterpene fraction, spathulenol and globulol were the major components (a total of 10.5%, 19.4%, 18.8% and 24.0%, respectively). In contrast, the oil of *E. pitanga* was characterised in this fraction by germacrene D and bicyclogermacrene (29.3% and 22.4%, respectively). *E. brasiliensis, E. nudticostata, E. sulcata and E. xiriricana* this fraction was characterized by [alpha]

and [beta]-pinone (ranging 20.7% to 35.9% of both compounds). For *E. pitanga*, (E)-[beta]-ocimene was the major component (10.5%). *E. speciosa* showed a high percentage of [alpha]-pinene (47.3%) and limonene (23.0%). *Eugenia brasiliensis* (β-selinene (17.3%). *Eugenia buxifolia* (51% cineole). *Eugenia caryophyllata* (eugenol 69.8% and acetyl eugenol 20.9%).

EXPERIMENTAL

Plant material

E. jambolana Fruits was collected in July 2011. From El-orman garden, Giza, Egypt. The plant samples were kindly identified by M. Tresa labib, Taxonomist, El-orman garden, Giza, Egypt. Voucher specimens are kept in herbarium, pharmacognosy department, faculty of Pharmacy, Helwan University, Egypt.

Material for testing the antimicrobial activity

Gram positive bacteria (*Staphylococcus aureus*), gram negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*) *Aspergillus flavus* as fungus.

Standard antimicrobial agents

Epinor- Norfloxacin: antibacterial and Amphotricin B antibiotics ready discs (5ug/disc) used as positive control.

METHODS

Preparation of the essential oil

Freshly comminuted leaves of both fruits and flowers (500g) were separately hydrodistilled for 6 hours in a Clevenger type apparatus. The resulting oils were collected, dried over anhydrous sodium sulphate and stored in refrigerator until analysis. Percentage yields were determined according to the Egyptian Pharmacopoeia, 1984[26].

G.C/MS for volatile oil

G.C analyses were performed on a GC/MS system (SHIMADZU GC/MS-QP5050A) with software (Class 5000). Gas chromatograph equipped with a TR-5MS (5% Phenyl Polysil Phenylene Siloxane) column (DB 30m X 0.25 mm i.d X 0.25 um film thickness). The analyses were carried out under the following conditions: Carrier gas: He with flow rate 1 ml/min; 235°C; Detector temp. FID: 250°C; Injector temp.: 235°C; split ratio; 1:10; Oven temp. Program: initial temp.; 40°C (0.5 min) increasing to 150°C (at 7.5°C/min), 150°C (1min) then increasing to 250°C (at 5°C/min)- 250°C (2min). The capillary column was directly coupled with mass spectrometr HP 5973 (Agilent). EI-MS were recorded at 70 ev. The analysis has been done in the Analytical unit, Faculty of Agriculture, Cairo University, Cairo, Egypt. Identification of the components were performed by aid of the computer library search (Class 5000 lab software package) comparison of mass spectra with literature data and by comparison of their retention times and mass fragmentation patterns with those of the library data base (Wiley (Wiley Int. USA))[27-29].

Biological study

The antimicrobial test

The antimicrobial activity of extract was determined using modified Kirby-Bauer disc diffusion method. Briefly, 100 ml of the test bacteria/fungi were grown in 10 µl of fresh media until they reached a count of approximately 108 cells/ml for bacteria or 105 cells/ml for fungi. 100 ml of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method[30-32].

Firstly, Disc diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by the[33] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the NCCLS, 2003.

Plates inoculated with filamentous fungi at 25^{0}C for 48 hours; Gram(+) bacteria and Gram(-) bacteria were incubated at 35-37^{0}C for 24-48 hours and yeast as *Candida albicans* incubated at 30^{0}C for 24-48 hours and, then the diameters of the inhibition zones were measured in millimeters.

Standard discs served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as a negative control.

When a filter paper disc impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disc into the agar. This diffusion will place the chemical

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TABLE 2 : results of antimicrobial activity of the essential oil of the fruits and flowers of *E.jambolana*.

	Sample	Inhibition Zone diameter (mm/sample)			
		E. coli	Staphylococcus aureus	Asparagillus flavus	Candida albicans
standard	EPINOR- Norfloxacin (antibacterial agent)	22 (100%)	24 (100%)	-	-
	Amphotericin B (antifungal agent)	-	-	17 (100%)	21 (100%)
Oil fruit		16 (72.73%)	19 (79.17%)	19 (111.7%)	19 (90.48%)

TABLE 1 : Results of GC/MS analysis of the volatile oils of fruits and flowers of *Eugenia supraxillaris*.

Identified compound	RT*	M+	*E. jambolana* fruits
Alpha- Phellandrene	10.98	136	0.82
Limonene	11.23	136	14.43
1,8- Cineole	11.56	154	5.06
Alpha terpinolene	11.92	136	4.62
Linalool	12.66	154	0.91
Linalyl propanoate	13.31	210	6.23
Terpinen-4-ol	13.61	154	0.64
Alpha terpineol	14.79	154	12.04
Citronellal	15.94	156	1.42
Maltol	16.16	126	2.74
Geraniol	18.16	154	2.88
Alpha pinene	20.03	136	2.54
Citronellyl acetate	20.83	206	0.40
Neryl acetate	21.37	196	1.43
Eugenol	22.25	164	0.54
Geranyl acetate	23.12	194	1.76
Methyl eugenol	24.72	178	22.5
Aromadenderene	25.7	204	0.67
Leden	26.61	204	0.94
Geranylisobutyrate	28.24	224	1.23
Epigalobulol	28.72	222	3.21
Alpha- Farnesene	30.47	204	0.72
Spathulenol	31.26	220	6.32
Globulol	32.43	222	3.52
Virdiflorol	32.82	222	3.26
Identified Hydrocarbons			19.45%
Identified Oxygenated compounds			76.86%
Total Identified compounds			96.31%
Unidentified compounds			3.69 %

RT= Retention time ; M+= molecular ion peak

in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar, it will not grow in the area around the disc; if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition" or "clear zone". The zone diameter were then measured with slipping calipers.

RESULTS AND DISCUSSION

Essential oils were obtained by hydrodistillation of the fruits of *E. jambolana* yielded 1.2 % and was analyzed by GC/MS. Qualitative and quantitative variations of the components in the fruits are complied in TABLE (1). GC-MS analysis of the oil under the experimental conditions revealed the presence of 25 components in *fruits* (representing 96.31% of the total oil composition).

The oil is dominated by oxygenated compounds 76.86%. In the same time, the hydrocarbon contents 19.45% for fruits. It is dominated by methyl eugenol, limonene and alpha-terpineol (22.5%, 14.43% and 12.04% respectively).

The fruits oil sample could find valuable applications in aromatherapy and fragrance industry, due to their high content of oxygenated constituents.

The antimicrobial bioactivity evaluated for the fore mentioned oil (TABLE 2) revealed valuable and significant efficacy and potency for the two samples in comparison with standards. The antimicrobial screening showed that oil under investigation exhibited broad spectrum effect against gram-positive, gram-negative bacteria and yeast (TABLE 2). The highest antimicrobial activity was observed against *Asparagillus flavus* (111.7 % that of amphotericin B) and *Candida albicans* (90.48% that of amphotericin B) for volatile oil of fruits. The oil of the fruit possessed a moderate activity against E. coli and *Staphylococcus aureus* (72.73% and 79.17% of that Epinor-Norfloxacin respectively). From the previous data it is observed that the activity of Fruits against the tested microorganisms is very high and that

is supposed to be associated to the high content oxygenated compounds and specially of methyl eugenol in the *fruits*. The oil sample of the fruit containing the highest relative percentage of phenolic compound methyl eugenol exhibited significant antimicrobial activity (TABLE 2) against gram +ve bacteria, gram -ve bacteria, hence, can offer a valuable antibacterial phytomedicine. These effects support the use of these oils as mucous membrane stimulant. So more study has to be done to investigate if the oil sample can be used in gargles and mouth washes preparations.

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