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Chemical Composition and Antimicrobial Activity of Flower Essential Oil of *Jacaranda acutifolia* Juss. against Food-Borne Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Author NMM prepared the oil sample, performed the antimicrobial activity, interpretation of the volatile constituents and wrote the manuscript. Author OAE shared in interpretation of the volatile components, writing and revising the manuscript. Author ANBS designed the study, supervised the whole work and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the chemical composition and antimicrobial activity of the hydrodistilled flower essential oil of *Jacaranda acutifolia* Juss. (Bignoniaceae) to validate some of its ethnopharmacologial uses such as treatment of wounds and dermatitis.
Study Design: Volatile oil isolation, component identification and antimicrobial activity.
Place and Duration of Study: Faculty of Pharmacy, Ain Shams University, and the flowers were collected on 15 April 2012 and the study is completed within four months.
Methodology: The essential oil of the flowers of *Jacaranda acutifolia* was extracted by hydrodistillation, analysed by capillary gas chromatography (GC/FID) and gas chromatography–mass spectrometry (GC/MS). Antimicrobial activity was studied *in vitro*; this included both antibacterial activity against food-borne pathogens and antifungal activity using agar diffusion method.

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Results: Forty six components, comprising almost 81.36 of the total peak area, were identified in the analysis. The main components were *n*-dodecanoic acid (17.48%), *n*-tetradecanoic acid (15.59%), *n*-hexadecanoic acid (10.98%), hexahydrofarnesyl acetone (8.2%), *n*-decanoic acid (7.9%), and nonacosane (7.71%). According to the observed inhibition zone, which ranged from 13 to 26.5 mm, the oil showed significantly high *in-vitro* antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* with minimum inhibitory concentration (MIC) values ranging from 0.09 up to 1.09 mg/ml, and moderate antimicrobial activity against *Salmonella typhimurium* and *Shigella flexneri*. The potency of the oil was calculated as compared to standard antibiotics, and 1 mg oil was equivalent to 1.05 µg penicillin against *S. aureus*; 2.72 µg nystatin against *C. albicans;* 13.55, 1.3 and 272 µg gentamicin against *E. coli, Samonella* and *Shigella* species respectively.
Conclusion: The essential oil of *Jacaranda acutifolia* exhibited promising antimicrobial activity, and this makes its local traditional uses rational.

Keywords: Jacaranda acutifolia; essential oil; chemical composition; antimicrobial activity; GC/MS.

1. INTRODUCTION

Jacaranda is a member of Bignoniaceae Juss., it contains 49 species around the world [1]. The Bignoniaceae is especially common in the tropics of South America and occurs in habitats consisting of mainly woody trees, shrubs, lianas and rarely herbaceous plants. Many woody representatives of Bignoniaceae as *Jacaranda* are well known for their use in the timber industry [1]. Chemical constituents recognized in the family are naphthoquinones of the lapachol type, iridoid glucosides, alkaloids, flavones, triterpenes, polyphenols, tannins and seed oils [2].

Chemical studies on the constituents of *Jacaranda* have only been reported for six species: *Jacaranda acutifolia*, *Jacaranda caucana*, *Jacaranda copaia*, *Jacaranda decurrens*, *Jacaranda filicifolia* and *Jacaranda mimosifolia*. The compounds have been identified as triterpenes, quinones, flavonoids, fatty acids, acetosides and, recently, a novel phenylethanoid dimmer [1]. Members of the genus *Jacaranda* possess significant pharmacological potential and promising activities of extracts in the context of ethnomedicinal knowledge, especially in the field of tropical diseases, skin problems and venereal illnesses. *Jacaranda* species might serve as an important source of medicine among people living in tropical regions [1].

Though antimicrobial activity of other species such as *Jacaranda cuspidifolia* Mart. has been reported, [3] yet, to our knowledge, nothing could be traced regarding the chemical composition and the biological activity of the essential oil obtained from *Jacaranda acutifolia*. We present here results of GC-MS analysis of the essential oil of flowers of *Jacaranda acutifolia* Juss. (Bignoniaceae), for the first time, to acquire a comprehensive knowledge on the volatile composition of the leaves. Also, in this study, the antimicrobial activity was evaluated against fungal and food-borne bacteria, to validate some of its ethnopharmacologial uses in the aforementioned disorders.

2. MATERIALS AND METHODS

2.1 Plant Material

Flowers of *Jacaranda acutifolia*, family Bignoniaceae were collected from El-Merryland Botanical Garden, Cairo, Egypt. Identification of the plant was verified by Prof. Dr. Abd El Salam Mohamed Al-Nowiahi, Department of Taxonomy, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt. A voucher specimen of authenticated *Jacaranda acutifolia* Juss. flower (JAF-2013) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo, Egypt. Fresh flowers were used for the extraction of the volatile oil, the intact flowers (kept at 20-25ºC) were used within 4 hours after picking. Care was taken that the flowers were not crushed or otherwise damaged.

2.2 Isolation of Volatile Components

Fresh plant materials (800 g) were hydrodistillation in a Clevenger-type apparatus [10], using *n*-hexane as a collecting solvent, until there is no significant increase in volume of oil collected within 4 hours. The yield of the pale

yellow oil was 0.01%. The oil was dried over anhydrous sodium sulfate and kept in separated sealed vials at -30ºC for analysis.

2.3 GC/FID Analysis

The GC analyses were carried out on a Varian 3400 equipped with an DB-5 fused bonded column (30 m × 0.25 mm × 0.25 µm) (Ohio Valley, Marietta, USA) and FID detector; carrier gas was helium (2 ml/min); the operating conditions were: initial temperature 45ºC, 2 min isothermal, 300ºC, 4ºC / min 300ºC, then 20 min isothermal. Detector and injector temperatures were 300 and 250ºC, respectively. The split ratio was 1:20. PeakSimple 2000 chromatography data system (SRI Instruments, Torrance, USA) was used for recording and integrating of the chromatograms. Average areas under the peaks of three independent chromatographic runs were used for calculating the % composition of each component.

2.4 GC/MS Analysis

The analyses were carried out on a Hewlett-Packard gas chromatograph (GC 5890 II; Hewlett-Packard GmbH, Bad Homburg, Germany) equipped with the same column and conditions as for the GC/FID. The capillary column was directly coupled to a quadrupole mass spectrometer (SSQ 7000; Thermo-Finnigan, Bremen, Germany). The injector temperature was 250ºC. Helium carrier gas flow rate was 2 ml/min. All the mass spectra were recorded with the following conditions: filament emission current, 100 mA; electron energy, 70 eV; ion source, 175ºC; diluted samples (0.5% v/v) were injected with split mode (split ratio, 1:15). Compounds were identified by comparison of their spectral data and retention indices with Wiley Registry of Mass Spectral Data 8th edition, NIST Mass Spectral Library (December 2005), our own laboratory database and the literature [4,5].

2.5 Microbial Strains

The essential oil of *Jacaranda acutifolia* flowers was tested against *Staphylococcus aureus* ATCC 2821 (Gram positive bacteria); Escherichia coli ATCC 25922, clinically isolate microorganisms; *Shigella flexneri* and *Salmonella typhimurium* (Gram negative bacteria); and *Candida albicans* ATCC 60193 (fungus). The microorganisms were obtained from the stock cultures of the Department of Microbiology, Faculty of Pharmacy, Ain Shams University.

2.6 Screening for Antimicrobial Activity

Antimicrobial activity was assayed via the agar diffusion method [6]. Bacterial and fungal inocula in nutrient agar slants were directly suspended in 10 ml saline. Aliquots (0.25 ml) of bacterial or fungal inocula were spread on the surface of nutrient agar (Lab M, England) using sterile rod to obtain uniform microbial growth on the plates. Small cups (10 mm diameter) were taken out of the inoculated agar surfaces. Each cup was filled accurately with 100 µl solution (30 mg oil was dissolved in 1 ml dimethylsulphoxide (DMSO, Sigma Aldrich, Germany)) as well as DMSO as a control. The plates were incubated overnight at temperature 37ºC for bacteria and 28ºC for fungi. Clear zones of inhibition were developed and diameter of zones of inhibition were measured (in mm) and compared to standard antibiotics [penicillin for Gram (+), gentamicin for Gram (-), nystatin for yeast]. Each test was performed in duplicate and the mean values were calculated.

2.7 Determination of the Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was calculated through agar diffusion method [7]. Cups were made in inoculated agar surfaces. Serial two fold dilution of the oil in DMSO was carried out. Each cup is filled with a different concentration of the oil to obtain final concentration range 0.5-3.2 mg oil in each cup. The plates were incubated overnight at temperature 37ºC for bacteria and 28ºC for fungi. Clear zones of inhibition were developed and diameter of zones of inhibition were measured (in mm) and plotted in y-axis against log concentration in x-axis to obtain a standard calibration curve from which log MIC was determined by extrapolation at cup diameter (10 mm). Same method was applied with the above mentioned antibiotics.

2.8 Determination of the Antimicrobial Potency

Using agar diffusion method [8], four cups were made in the inoculated agar and removed with the help of sterilized loop. The two opposite wells were filled with the working standard of 1:2 dilutions and marked as S_1 and S_2, respectively. The remaining two were filled with the sample

whose potency was to be determined in the same dilution (1:2) and marked T_1 and T_2 respectively. One hundred micro liters of standards as well as samples were poured with the help of micropipette in the digged holes. The plates were then placed carefully (to avoid spreading of solution due to tilting of the plates) in incubator for 18-24 hours at temperature 37°C for bacteria and 28°C for fungi. Clear zones of inhibition were developed and diameter of zones of inhibition were measured and compared with the known standard.

The % potency of the antimicrobial potency of the tested sample was calculated by the following formula:

i) Difference due to doses: $E = ½ [(T_2+S_2) – (T_1+S_1)]$
ii) Difference due to sample: $F = ½ [(T_2+T_1) – (S_1+S_2)]$
iii) Log ratio of doses: I = log 2
iv) Slope: B = E/I, M = F/B
v) Potency ratio: Antilog M

S_2 = Standard High (in concentration)
S_1 = Standard Low (in concentration)
T_2 = Test High
T_1 = Test Low

3. RESULTS AND DISCUSSION

The flower oil (0.01% yield) of *Jacaranda acutifolia* had a pale yellow color, and pleasant odor. Most of their components could be identified unambiguously by direct comparison (mass fragmentation and retention index) with published data as well as computer library search. As shown in Table 1 and Fig. 1, forty six compounds were identified, representing about 81.36 % of the total peak area.

The oil consisted mainly of fatty acids (52.18%) rich in dodecanoic acid (17.48%), tetradecanoic acid (15.59%), hexadecanoic acid (10.98%) and decanoic acid (7.9%); followed by aliphatic hydrocarbons (11.61%), of which nonacosane (7.71%) was identified. While terpene related compounds reached 8.98%, of which Hexahydrofarnesyl acetone (8.2%), represented almost all of terpene related compounds content. The essential oil showed the presence of many methyl and ethyl esters such as methyl linoleate (3.81%), methyl hexadecanoate (1.65%), methyl tetradecanoate (0.24%) and trace amounts of esters of phenylacetate, salicylate and dodecanoate.

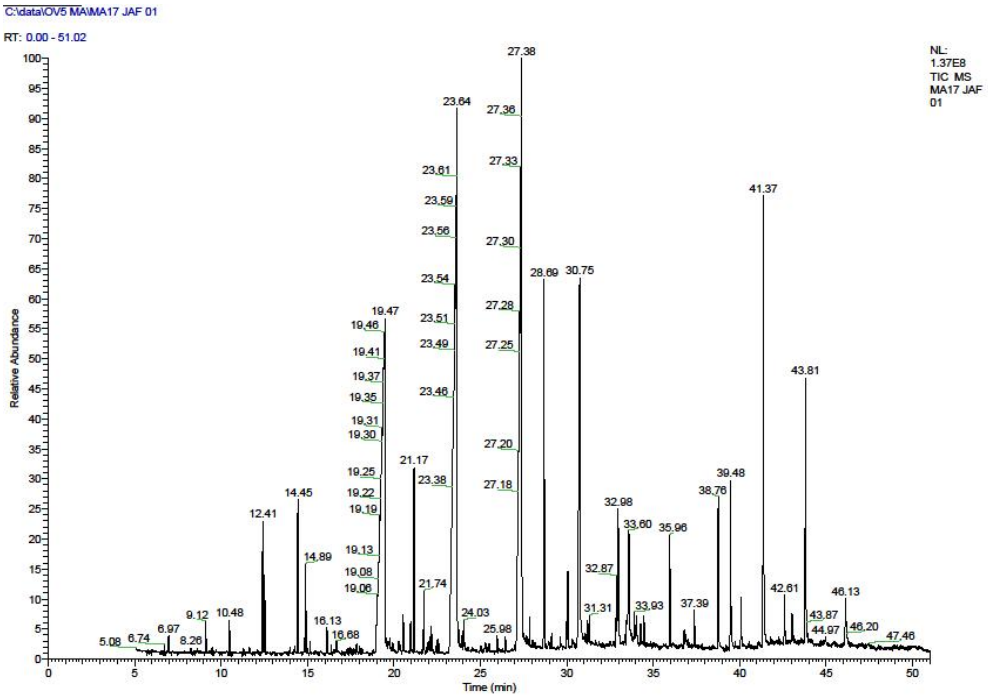

Fig. 1. GC chromatogram of *Jacaranda acutifolia* flowers essential oil.

Table 1. Chemical composition of the essential oil of *Jacaranda acutifolia* flowers

	Compound[a]	RI*	Percentage composition	Method of identification
1	2-Heptanol	893	tr	RI, GC-MS
2	α-pinene	928	tr	RI, GC-MS
3	1-Octen-3-ol	977	tr	RI, GC-MS
4	Limonene	1029	tr	RI, GC-MS
5	β-Linalool	1101	1.4	RI, GC-MS
6	*n*-Nonanal	1106	tr	RI, GC-MS
7	Methyl phenylacetate	1178	tr	RI, GC-MS
8	α-Terpineol	1191	tr	RI, GC-MS
9	Methyl salicylate	1194	tr	RI, GC-MS
10	Decanal	1205	tr	RI, GC-MS
11	Ethyl phenylacetate	1246	tr	RI, GC-MS
12	*trans*-Geraniol	1256	tr	RI, GC-MS
13	Geranyl vinyl ether	1257	tr	RI, GC-MS
14	***n*-Decanoic acid**	**1382**	**7.9**	**RI, GC-MS**
15	1,3-Dimethylnaphthalene	1423	tr	RI, GC-MS
16	4-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-butanone	1424	tr	RI, GC-MS
17	*trans*-Geranylacetone	1455	tr	RI, GC-MS
18	Germacrene D	1492	1.19	RI, GC-MS
19	Pentadecane	1502	tr	RI, GC-MS
20	ç-Elemene	1507	tr	RI, GC-MS
21	α-Farnesene	1512	0.3	RI, GC-MS
22	Methyl dodecanoate	1527	tr	RI, GC-MS
23	ë -Cadinene	1532	tr	RI, GC-MS
24	***n*-Dodecanoic acid**	**1572**	**17.48**	**RI, GC-MS**
25	Spathulenol	1588	tr	RI, GC-MS
26	*n*-Hexadecane	1600	0.28	RI, GC-MS
27	*tau*-Muurolol	1652	tr	RI, GC-MS
28	α-Cadinol	1666	tr	RI, GC-MS
29	Heptadecane	1701	tr	RI, GC-MS
30	Methyl tetradecanoate	1726	0.24	RI, GC-MS
31	***n*-Tetradecanoic acid**	**1769**	**15.59**	**RI, GC-MS**
32	Octadecane	1799	0.54	RI, GC-MS
33	**Hexahydrofarnesyl acetone**	**1847**	**8.2**	**RI, GC-MS**
34	Pentadecanoic acid	1862	tr	RI, GC-MS
35	Farnesyl acetone	1922	0.78	RI, GC-MS
36	Methyl hexadecanoate	1926	1.65	RI, GC-MS
37	***n*-Hexadecanoic acid**	**1964**	**10.98**	**RI, GC-MS**
38	Eicosane	1998	tr	RI, GC-MS
39	Ethyl hexadecanoate	1993	tr	RI, GC-MS
40	Methyl linoleate	2100	3.81	RI, GC-MS
41	α-Linolenic acid	2149	0.23	RI, GC-MS
42	Ethyl linoleate	2169	tr	RI, GC-MS
43	*n*-Pentacosane	2503	2.06	RI, GC-MS
44	*n*-Hexacosane	2605	0.23	RI, GC-MS
45	*n*-Octacosane	2798	0.79	RI, GC-MS
46	***n*-Nonacosane**	**2890**	**7.71**	**RI, GC-MS**
Aliphatic HC			11.61	
Oxygenated monoterpenes			1.4	
Sesquiterpene HC			1.49	
Terpene related compounds			8.98	
Fatty acids			52.18	
Fatty acid ester			5.7	
Total			81.36	

[a]*Compounds are listed in order of elution; *in DP-5 column; tr, traces <0.1. The major components are highlighted in bold.*

The oil showed significantly high *in-vitro* antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* with inhibition zones of 22-26.5 mm and

minimum inhibitory concentration (MIC) values (as shown in Table 4) ranging from 0.9 up to 10.9 mg/ml, while it showed moderate antimicrobial activity against *Shigella flexneri* and *Salmonella typhimurium* with inhibition zones of 13 and 14.5 mm, respectively. Only one (*E.coli*) of the tested strains showed sensitivity to the essential oil in biologically relevant concentrations (MIC of 0.9 mg/ml). Mean inhibition zone diameter (mm) is shown in Table 2 and presented in Fig. 2.

The antimicrobial potency of the oil was calculated as compared to standard antibiotics, and 1mg oil was equivalent to 1.05 µg penicillin against *S. aureus*; 2.72 µg nystatin against *C. albicans*; 13.55, 1.3 and 2.72 µg gentamicin against *E. coli*, *Salmonella typhimurium and Shigella Flexner* respectively (Table 3).

The strong antibacterial activity of flower oil of *Jacaranda acutifolia* may be due to their high content of fatty acids, which constitute 52.18% in content. Fatty acids have been shown to possess antibacterial activities and Gram-negative bacteria are generally more resistant than Gram-positive bacteria due to antagonistic effects of fatty acids with their cell wall lipopolysaccharides [9]. This might explain the moderate antibacterial effect seen on *Shigella flexneri* and *Salmonella typhimurium*. Dodecanoic acid, decanoic and hexadecanoic acids possess antibacterial activity [9,10]. Also, Hexahydrofarnesyl acetone had proven to demonstrate antimicrobial activity [11]. It must be pointed out, however, that minor compounds may also importantly contribute for the antimicrobial activity of essential oils [12]. Probably, components such as β-linalool and α-linolenic acid detected in our oil could be responsible for this activity as they have shown to be antibacterial [13,14].

Table 2. Antimicrobial activity of *Jacaranda acutifolia* Juss. by Agar Well Diffusion Method

Microorganisms	Mean of inhibition zone diameter (in mm)			
	EO*	Penicillin (25 µg/ml)	Gentamicin (400 µg/ml)	Nystatin (200 µg/ml)
Gram (+)				
Staphylococcus aureus	25.5	24	NT	NT
Gram (-)				
Escherichia coli	26.5	NT	20	NT
Salmonella typhimurium	14.5	NT	22	NT
Shigella flexneri	13	NT	23	NT
Yeast				
Candida albicans	22	NT	NT	24

EO, 30 mg of the essential oil is dissolved in 1 ml DMSO, 100 µl of the resulting solution is used in this assay; NT, not tested. Results are the mean value of duplicate determinations.*

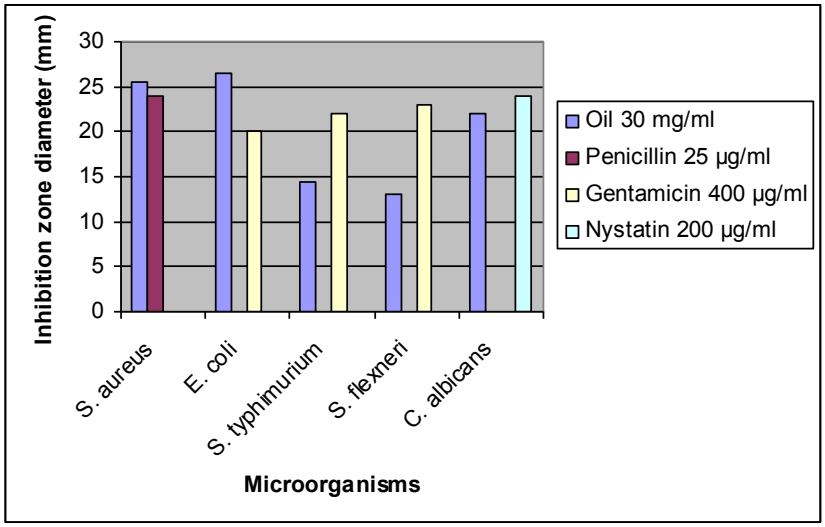

Fig. 2. In vitro antimicrobial activities of *Jacaranda acutifolia* essential oil using agar well diffusion method

Table 3. Antimicrobial Potency of 1 mg oil sample compared to standard antibiotics against different microorganisms

Microorganisms	Equivalent activity of antibiotics
Gram (+)	
Staphylococcus aureus	1.05 µg penicillin
Gram (-)	
Escherichia coli	13.55 µg gentamicin
Salmonella typhimurium	1.3 µg gentamicin
Shigella flexneri	2.72 µg gentamicin
Yeast	
Candida albicans	2.72 µg nystatin

Table 4. Minimum inhibitory concentration of *Jacaranda acutifolia* essential oil against different pathogens using agar diffusion method

Microorganisms	Minimum inhibitory concentration (MIC)			
	EO (mg/ml)	Penicillin (µg/ml)	Gentamicin (µg/ml)	Nystatin (µg/ml)
Gram (+)				
Staphylococcus aureus	3.6	0.1	NT	NT
Gram (-)				
Escherichia coli	0.9	NT	28.7	NT
Salmonella typhimurium	>20	NT	0.1	NT
Shigella flexneri	>20	NT	73	NT
Yeast				
Candida albicans	10.9	NT	NT	0.02

EO, essential oil; NT, not tested.

An interesting aspect related to the antimicrobial activity of essential oils is that the risk of pathogenic microorganisms developing resistance is very low because these products contain a blend of different antimicrobial substances that have different modes of action [15]. This is a beneficial characteristic of plant-derived products as compared to synthetic antimicrobial agents, as their application in food products may provide better food safety and longer shelf life [15].

4. CONCLUSION

The essential oil of *Jacaranda acutifolia* flowers contains dodecanoic acid, decanoic acid, tetradecanoic acid, hexahydrofarnesyl acetone, hexadecanoic acid and nonacosane as the main chemical markers. These chemical markers would be a powerful tool for maintaining quality control in the extraction of essential oils for use in medicinal applications, as well as in identification of plant specimens to a taxonomist. The essential oil exhibited potential antimicrobial activity against foodborne bacteria and yeast, and this *in-vitro* activity makes its local traditional uses rational. Therefore, the essential oil is a potential to be used in food conservation to extend the shelf life and increase the safety of the processed food as alternative to chemical preservatives and can be used as a natural antimicrobial agent in new drugs for therapy of infectious diseases. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine.

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COMPETING INTERESTS

The authors declare that no competing interests exist.

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