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MEDICINAL CHEMISTRY | RESEARCH ARTICLE

Chemical composition, antibacterial and antifungal activities of *Ruta graveolens* L. volatile oils

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Abstract: The *Ruta graveolens* L. (Rutaceae) plant is used medicinally as a homeopathic remedy in various areas of the world. In this study, volatile oils were extracted from the areal parts of the plant which was collected from southern India, then investigated for its chemical constituents and antimicrobial activities. The volatile oils were extracted by hydrodistillation using a Clevenger apparatus and samples were simultaneously analyzed with GC and GC-MS. As a result, a total of 13 chemical constituents were characterized. Representing 100% of the total oil with 2-ketones are the major groups. The principal components were identified, undecanone-2 (43.66%), 2-nonanone (16.09%), 2-acetoxy tetradecanone (14.49%), and nonyl cyclopropanecarboxylate (9.22%), respectively. The extracted volatile oil showed antibacterial activity against gram-positive and gram-negative bacteria, resulting in a number of common human pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* and the yeast *Candida albicans*. The zone of inhibition is from 12.57 ± 0.03 to 27.10 ± 0.02 mm. The minimum inhibitory concentration (MIC) values were within the antimicrobial activity range and varied between 0.70 ± 0.04 and 1.58 ± 0.05 $\mu\text{g}/\text{mL}$. The essential oil showed maximum antifungal activity (35.10 ± 0.02 mm) against *Candida albicans*. This study indicates that *R. graveolens* L. essential oils could be used as a natural medical application in antimicrobial and antifungal treatments.

ABOUT THE AUTHORS

Desam Nagarjuna Reddy (assistant professor, corresponding author), PhD, has research experience in the area for more than five years. His thrust area of research is Natural products. His work in the field can be evidenced by more than 15 papers published in National and international journals of repute. His current research interest focuses mainly on the analysis of bioactive natural compounds and their biological activity determination.

Abdul Jabbar Al-Rajab earned his PhD degree in Agronomy from the prestigious University of Lorraine, France in 2007. Then, he worked at the research center of Agriculture and Agri-Food Canada in London, Ontario, as NSERC postdoctoral fellow. He joined Jazan University (Saudi Arabia) in 2013 as a scientist at Center for Environmental Research and Studies. His research interest includes understanding the behavior of pollutants in the environment and plants, also the natural products as potential anti-agents or bio-pesticides.

PUBLIC INTEREST STATEMENT

The plants of family Rutaceae are big repository of secondary metabolites. A number of plants from this family are used in traditional system of medicine for cosmetics, ornamental plants, and home gardens to repel snakes in south India. Due to its antibacterial and antifungal properties, *Ruta graveolens* L. is an important medicinal plant in the countries of Indian subcontinent having morphological resemblance. The synthetic drug possesses toxic effects including carcinogenic effect. There is intensive search program throughout the world to look for new natural antioxidants. In the present study, we are reporting antibacterial and in vitro antifungal potential with essential oil composition of *Ruta graveolens* L. The extracts and essential oil revealed good antibacterial and antifungal activity. The essential oil possesses 2-aliphatic ketones which are major compounds. Besides its medicinal properties, the herb possesses antioxidant potential, thus it may be a good source as nutraceutical. It will also be helpful to upgrade the scientific knowledge of traditional people.

Subjects: Bioscience; Environment & Agriculture; Food Science & Technology; Physical Sciences

Keywords: *Ruta graveolens* L; essential oils; GC; GC-MS; antibacterial activity; antifungal activity; medicinal plant; Chemical Constituents; aliphatic ketones; 2-Undecanone; 2-Nonanone

1. Introduction

The use of medicinal plants in natural remedies has increased dramatically in recent years. As such, determination of their chemical constituents and potential medicinal applications has become a priority for pharmacologists and the scientific community. Aromatic and medicinal plants contain volatile oils with antibacterial, antifungal, and antioxidant activities (Guttridge & Halliwell, 1994; Halliwell & Guttridge, 1990, 1998; Hossain, Shah, Sang, & Sakari, 2012; Lee, Shin, Hwang, & Kim, 2003; Mantle, Eddeb, & Pickering, 2000; Maxwell, 1995; Milos, Mastelic, & Jerkovic, 2000; Ruberto & Baratta, 2000). Many medicinal plants are adopted from the Rutaceae family which contains about 150 genders and over 1,600 species of medicinal importance around the world (Albarici, Vieira, Fernandes, Silva, & Pirani, 2010; Januário et al., 2009). Essential oils are known for representing wide variety of secondary metabolites, due to the presence of aromatic compounds presence, which has attracted the attention of several research groups, regarding the oils' chemical constituents and biological importance (Bizzo, Hovell, & Rezende, 2009; Costa et al., 2010; De La Cruz, 2008; Elaissi et al., 2011). *Ruta graveolens* L., commonly known as rue or herb of grace (Anonymous, 2003; Lorenzi & Matos, 2002), has been used in Brazil and other countries in traditional medicine (Al-Qurainy et al., 2011; Souza, Stamford, Lima, & Trajano, 2007). Essential oils of *R. graveolens* L. were reported to have several therapeutic properties which has increased medicinal and pharmacological interest in this plant (Ahmad, Faisal, Anis, & Aref, 2010; Mejri, Abderrabba, & Mejri, 2010; Ratheesh, Shyni, Sindhu, & Helen, 2011; Yamashita, Fernandes Neto, Campos, & Guimarães, 2009). Because, synthetic chemical drugs may cause harmful side effects, many researchers have been examining and looking for naturally available chemical constituents from plants with antibacterial and antifungal activities. Historically, essential oils and their derivatives are sources of therapeutic agent, and antibacterial effects vary among the different plants materials and synthetic drugs (Freiesleben & Jäger, 2014; Koehn & Carter, 2005; Silver & Bostian, 1990).

The mechanisms of antibacterial effects are different between the plant materials and the synthetic drugs. Essential oils are reported to have significant efficiency in the treatment of resistant bacterial strains (Gardete & Tomasz, 2014). Moreover, the use of essential oils naturally extends the half-life of relevant food products, mainly accountable to antibacterial and antioxidant activity. Essential oils are more commonly known to possess mixtures of complex chemical compounds that have good smell and that contribute to therapeutic olfactory use, which is synthesized in the plant organs. Herein, according to the previous literature, and to the best of our knowledge, this paper (or work) presents a novel study on the *R. graveolens* L. species and its use in potential applicability in pharmacology. Therefore, this research reports on the chemical constituents, antibacterial, and antifungal activities of the essential oil extracted from the aerial parts of *R. graveolens* L.

2. Materials and methods

2.1. Reagents and chemicals

All analytical grade standards, chemicals, solvents, and reagents were supplied by Sigma-Aldrich (New Delhi, India). High-purity culture media were supplied by Merck (India).

2.2. Sampling and extraction procedure

Aerial parts of the plant materials were sampled during the flowering stage of the plant in November 2015 from Rayachoty, Rayalaseema region, in southern India. The taxonomic identification of plant materials was identified by plant taxonomist and its herbarium sheet was deposited at the

postgraduate laboratory of Botany, Sri Krishnadevaraya University, India. According to the method described by Viuda-Martos et al. (2011), air-dried areal parts of the plant materials were hydrodistilled for 4 h using a Clevenger apparatus. The oil was separated and dehydrated with anhydrous Na_2SO_4 . Until further analysis, the essential oil was stored at 4°C.

2.3. Qualitative and quantitative analysis

The compositions of volatile oils extracted from aerial parts of *R. graveolens* L. were analyzed with GC and GC-MS. The Agilent GC 6890 N (Agilent, New Delhi, India) was equipped with a flame ionization detector FID and a capillary column (HP-5MS 5% 30 m \times 0.25 mm \times 0.25 μm ; Agilent, New Delhi, India). Gradient temperature program was maintained for the column at 5°C min^{-1} from 50 to 260°C, with the carrier gas (Helium, purity 99.99%) at a flow rate of 1.0 mL min^{-1} . The injector temperature was 300°C, split ratio used was 10:1, and 0.5 μL of each sample was injected manually. For gas chromatography-mass spectrometry analysis, a GC-MS Agilent 6890 N/5975 (Agilent, India) was used. The mass detector was operated in the mode of electron impact ionization EI, the mass spectra were obtained with 70 eV in a mass range of 50–500 m z^{-1} and the GC conditions and column were as described above. The chemical constituents were identified by their retention time and compared to the standards and mass spectra referred in the library Wiley 5, Mass-Finder, and Adams GC/MS libraries (Adams, 2007).

2.4. Antibacterial activity

2.4.1. Sources of microbial strains

The antimicrobial activity of *Ruta* species essential oils was evaluated using laboratory reference strains (American Type Culture Collection) “ATCC,” American Research Service (ARC) culture collection or (NRRL) for *Candida albicans* and gram-negative and positive; National Museum of Natural History “NMHN” for filamentous fungi, accessed from Laboratory of microbiology, Centre for Cellular and Molecular Biology, India. Gram-positive bacteria are: *Bacillus cereus* (ATCC 10876), *Enterobacter cloacae* (ATCC 13047), *Enterococcus Faecalis* (ATCC 49452), *Listeria monocytogenes* (ATCC15313), *Staphylococcus aureus* (ATCC 25923), *Micrococcus flavus* (ATCC 25923), *Micrococcus luteus* (ATCC 9341). Gram-negative bacteria are: *Acinetobacter Baumannii* (ATCC 19606), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 35659), *Salmonella typhimurium* (ATCC 13311), *Citrobacter freundii* (ATCC 8090), *Enterobacter aerogenes* (ATCC 13048). Fungal micro-organisms are: *Candida albicans* (ATCC 26790), *Aspergillus fumigatus* (MNHN 566), *Aspergillus flavus* (MNHN 994294), *Cladosporium herbarum* (MNHN 3369), *Fusarium oxysporum* (MNHN 963917), *Alternaria alternata* (MNHN 843390). Strain numbers and micro-organisms are given in Tables 3 and 4. Strains were stored in fridge at 4°C, and then 24 h prior to each assay, strains were grown on Luria-Bertani agar. The cultures were diluted with Mueller–Hinton Broth (MHB) for bacteria which might be used for the antibiotic susceptibility tests.

2.4.2. Antimicrobial activity by the disc diffusion method

The antimicrobial activity of the extracted volatile oil was determined using the disc diffusion method according to the NCCLS (NCCLS, 2001; Pfaller, Messer, Karlsson, & Bolmstrom, 1998) using 100.0 μL of tested micro-organisms suspension, which contains 2×10^8 and 1.5×10^6 CFU/mL for bacteria and *C. albicans*, respectively, and 2×10^5 spores/mL of the tested fungal strains. Sabouraud Dextrose Agar (SDA) was used to inoculate *C. albicans*, and bacteria were inoculated overnight at 37°C in Mueller–Hinton agar (MHA). In order to test the antimicrobial and antifungal activities of the extracted essential oil, 10.0 μL of the extract was added individually to a 6-mm Whatman filter paper (Whatman, New Delhi, India), then placed onto the Petri dishes inoculated with the tested micro-organisms. Treated Petri dishes were kept at 4°C for 2 h. The plates containing bacteria were incubated at 37°C for 24 h, while plates containing *C. albicans* and fungal strains were incubated at 30°C for 24 and 48 h, respectively.

2.4.3. Determination of MIC values

The values of minimum inhibition concentration (MIC) were used to calculate the method of broth microdilution as described in the guidelines of National Committee for Clinical Laboratory Standards (NCCLS) for tested bacteria and *C. albicans* (NCCLS, 2001).

The volatile oils were dissolved in 1% DMSO and all the tests on bacteria and yeast were performed with Mueller–Hinton Broth and Sabouraud Dextrose Broth, respectively. Fresh cultures of tested strain were prepared and adjusted to 5×10^5 CFU and 2.5×10^6 CFU/mL, for bacteria and *C. albicans*, respectively. For bacterial and yeast analysis, all test tubes were inoculated at 37°C and at 30°C, respectively, for 48 h. The essential oils for which micro-organisms did not show visible growth were determined (MIC). The growth of micro-organism was determined by turbidity, whereas the MIC values of filamentous fungi were evaluated in agar medium via the dilution method (Soliman & Badea, 2002). Each treatment was used in triplicate. Gentamicin and amphotericin B were used as the reference compounds to compare antimicrobial, antibacterial, and antifungal activity of the oil.

2.5. Statistical analysis

Experiments in this study were made in triplicates, and data were presented in mean values. The statistical analysis was conducted by variance test ANOVA (SPSS 21).

3. Results and discussion

3.1. Chemical composition of essential oil

Volatile oil of light yellow color was extracted from the aerial parts of *R. graveolens* L by hydrodistillation with a 1.29% (v/w). Results of the chemical constituents in the essential oil were determined and analyzed using GC and GC-MS as shown in Table 1. As a result, a total of 13 chemical constituents were characterized. Representing 100% of the total oil with 2-ketones are the major groups. The principal components were identified. Two major compounds are aliphatic 2-ones. The decreasing order of aliphatic ketones is as follows: 2-undecanone (43.66%), 2-nonanone (16.09%), 2-tridecanone (2.59%), 2-decanone (2.58%), and 2-dodecanone (2.23%).

Table 1. Chemical composition of the essential oil of *Ruta graveolens* L.

RRI	Chemical constituent	M.F	%
1096	2-Nonanone	C ₉ H ₁₈ O	16.09
1194	2-Decanone	C ₁₀ H ₂₀ O	2.58
1294	2-Undecanone	C ₁₁ H ₂₂ O	43.66
1303	2-Undecanol	C ₁₁ H ₂₄ O	2.19
1315	2-Acetoxy tetradecane	C ₁₆ H ₃₂ O ₂	14.49
1331	1-Methyl-5,6-divinyl-1-cyclohexene	C ₁₁ H ₁₆	3.38
1397	2-Dodecanone	C ₁₂ H ₂₄ O	2.23
1498	2-Tridecanone	C ₁₃ H ₂₆ O	2.59
1552	Nonyl cyclopropanecarboxylate	C ₁₃ H ₂₄ O ₂	9.22
1598	2,7-Dimethyl-3,6-dimethylene-1,7-octadiene	C ₁₂ H ₁₈	0.63
1828	Ethyl piperonyl acetate	C ₁₂ H ₁₄ O ₄	0.43
1866	1,5-Isobutyl-1,3-benzodioxole	C ₁₁ H ₁₄ O ₂	0.72
2016	Methyl-3-methylene-1,2,3,3a,4,4a,4b,5,6,10b-decahydrocyclopropa[3,4]cyclohepta[1,2a]naphthalen-8-yl ether	C ₁₈ H ₂₂ O	1.79
Total			100.0

Notes: RRI: Relative retention index calculated against n-alkanes on the HP-5MS column; M.F Molecular formula of the chemical compound; % percentages were calculated from flame ionization detector data.

Other compounds in the essential oil included, 2-acetoxy tetradecane (14.49%), aliphatic ester nonyl cyclopropanecarboxylate (9.22%), 1-methyl-5, 6-divinyl-1-cyclohexene (3.38%), 2-Undecanol (2.19%), methyl-3-methylene-1,2,3,3a,4,4a,4b, 5,6, 10b-decahydrocyclopropano [3,4]cyclohepta [1,2a]naphthalen-8-yl ether (1.79%), and others that were found in minimal concentration. A comparative major chemical constituent of the *R. graveolens L.*, essential oil from various studies, including our results, is presented in Table 2.

Our results were not in accord with some published studies regarding the presence of C₈ to C₁₃ aliphatic 2-ketones along with esters, aldehydes, alcohols, and unsaturated hydrocarbons. From the Table 2, the profile obtained in the present study was similar to the previous results reported as 2-aliphatic ketones. But in the present investigation, the percentage of 2-aliphatic ketones and predominate constituents (2-undecanone and 2-nonanone) were smaller than the previous results reported by França Orlanda and Nascimento (2015), Meccia, Rojas, and Usbillaga (2008), were identified 89.94% similarity as aliphatic 2-ketones, predominately, 2-undecanone and 2-nonanone. But the composition of major constituents are: (França Orlanda & Nascimento, 2015) 2-undecanone (47.21%) and 2-nonanone (39.17%) and (Meccia et al., 2008) 2-undecanone (43.0%) and 2-nonanone (33.5%).

The essential oil extracted from the same plant in Algeria presented 81.08% of aliphatic ketones and major compounds 2-undecanone (55.4%) and 2-nonanone (21.62%) (Farah Haddouchi et al., 2013). Similar results were reported by Tang, Yang, Yang, and Xu, (2011) in the oil extracted from plants collected from China where the major compounds 2-undecanone (46.15%) and 2-nonanone (27.01%) and showed mainly aliphatic ketones (80.04%). Another results reported by De Feo, De Simone, and Senatore (2002) showed mainly the 2-series of aliphatic ketones (83.4%) and the major

Table 2. Comparative study of *R. graveolens L.* essential oils with previous results

Major compounds	Percentage (%)	Place	Reference
Undecanone-2, 2-Nonanone, 2-Acetoxy tetradecanone, Nonyl cyclopropanecarboxylate	43.66, 16.09	India	Present study
	14.49, 9.22		
2-Nonanone, Chalepinsin, 2-Undecanone, Chalepin, Bergapten, Psoralen, Hexatriacontane	8.97, 7.11, 6.81, 6.66, 5.54, 5.32, 5.14	Colombia	Stashenko et al. (2000)
2-Undecanone, 2-Heptanol acetate, 1-Dodecanol, Geyrene, 2-Nonanone	33.9, 17.5, 11.0, 10.4, 8.8,	North of Iran	Soleimani et al. (2009)
2-Undecanone, 2-Nonanone, Pregeijerene	50.93, 16.85, 8.72	Mérida	Janne Rojas et al. (2011)
2-Undecanone, 2-Nonanone, Pregeijerene	37.80, 28.28, 6.8	Miranda states	
2-Undecanone 2-Nonanone	46.8, 18.8,	Italy	De Feo et al., 2002
2-Undecanone 2-Nonanone	55.4, 21.62	Algeria	Farah Haddouchi et al. (2013)
2-Undecanone 2-Nonanone, 2-Acetyl-tridecane	46.15, 27.01	China	Tang et al. (2011)
	12.73		
2-Undecanone 2-Nonanone, 2-Nonylacetate, Xanthotoxin	30.73, 18.06	Malaysia	Yaacob et al. (1989)
	11.03, 7.24		
2-Undecanone 2-Nonanone	49.20, 24.70	Egypt	Aboutabl et al. (1988)
2-Undecanone 2-Nonanone	43.0, 33.5	Mérida state	Meccia et al. (2008)
2-Undecanone 2-Nonanone	50.50, 17.47	Egypt	El-Sherbeny et al. (2007)
2-Nonanone, 2-Undecanone followed by 2-Nonanol, 2-Octyl acetate	38.66, 27.34, 12.25, 7.71	Tunisia	Fredj et al. (2007)
2-Undecanone 2-Nonanone	47.21, 39.17	Brazil	França Orlanda and Nascimento (2015)

components like 2-undecanone (46.8%) and 2-nonanone (18.8%) in the essential oil from plants collected from Italy. In addition, the oil extracted from plants collected in Egypt by El-Sherbeny, Khalil, and Hussein, (2007) presented 2-undecanone (50.50%) and 2-nonanone (17.47%), aliphatic compounds (74.86%), and mainly 2-ketones. Aboutabl, Elazzouny, and Hammerschmidt (1988) reported that essential oil extracted from plants growing in Egypt contained mainly aliphatic (76.89%) ketones, but major compounds were 2-undecanone (49.20%) and 2-nonanone (24.70%). Moreover, the major constituents of essential oil from two samples collected from Merida and Meridia state in Venezuela by (Rojas et al., 2011) reported 2-undecanone, 2-nonanone, and pregeijerene, but mainly the aliphatic 2-ketones from Merida and Meridia state were 74.4 and 72.03%, respectively. The essential oil in samples collected from Tunisia by Fredj, Marzouk, Chraief, Boukef, and Marzouk (2007) reported 69.77% of aliphatic ketones and major compounds 2-undecanone (38.66%) and 2-nonanone (27.34%).

In the present study, percentage of aliphatic 2-ketone and major constituents (2-undecanone and 2-nonane) was greater than the values reported from Malaysia (Yaacob, Abdullah, & Joulain, 1989), North of Iran (Soleimani, Aberoomand-Azar, Saber-Tehrani, & Rustaiyan, 2009), and Colombia (Stashenko, Acosta, & Martínez, 2000) which reported mainly 2-aliphatic ketones (53.24%), (47.13%), and (18.61%), respectively. Our results were in accord with previous reported results which showed that aliphatic ketones, which present the major components, were as following: 2-undecanone 43.66% and 2-nonanone 16.09% and others are esters followed by 2-acetoxy tetradecane (14.49%) and nonyl cyclopropanecarboxylate (9.22%). Abundance of aliphatic ketones in the essential oils was also in accord with results reported in previous studies. However, the percentage of aliphatic ketones was comparable with values presented in previous studies. It may be due to the climatic effect, seasonal genotype, growth location, rainfall, geographic conditions, soil salinity, harvest period (Corduan & Reinhard, 1972; Pala-Paul et al., 2005), and the light effect on the composition of the essential oil.

3.2. Antimicrobial activity

The extracted essential oil was employed *in vitro* under controlled conditions against microorganisms to analyze its antimicrobial activity. For this assessment, the parameters examined were the zone of inhibition, zone diameter (mm), and MIC values.

The antibacterial activities of the essential oil against bacteria are shown in Table 3. The present method (Weerakkody, Caffin, Turner, & Dykes, 2010) measured the zone of inhibition present on the disk that contained the antibacterial agent. The results in Table 3 display the inhibition zones including size of the filter paper (6 mm). A significant variation was observed in the antibacterial properties of the volatile oil against different bacteria. The volatile oil showed maximum, intermediate, minimal (or no) antibacterial activity in inhibition zone >20, <15–20, and >15 mm, respectively. Based on the zone of inhibition diameter, the volatile oil showed maximum antibacterial activity against *E. Faecalis*, *B. cereus*, *S. aureus*, *P. mirabilis*, and *M. flavus*, with respect to inhibition zones of 27.10 ± 0.02 , 26.60 ± 0.03 , 23.40 ± 0.06 , 22.00 ± 0.02 , and 21.20 ± 0.02 mm ($p \leq 0.01$). Intermediate activity was observed against *M. luteus*, *A. baumannii*, *L. monocytogenes*, *K. pneumonia*, *E. cloacae*, *E. coli*, and *C. freundii* with 19.06 ± 0.05 , 18.60 ± 0.02 , 18.30 ± 0.04 , 18.10 ± 0.04 , 18.00 ± 0.05 , 17.70 ± 0.02 , and 16.02 ± 0.03 mm inhibition zones ($p \leq 0.01$), respectively. Minimal or no activity was observed against *P. aeruginosa* and *E. aerogenes* with 15.30 ± 0.06 and 12.57 ± 0 mm inhibition zones ($p \leq 0.01$), respectively.

By observing Table 3, it can be suggested that this essential oil is a potential antibacterial agent, because the organisms corresponding to each value of MIC were low and ranged from 0.70 ± 0.04 to 1.58 ± 0.05 $\mu\text{g/mL}$, with the exception of the standard strain *E. aerogenes* which required 72.0 ± 0.09 $\mu\text{g/mL}$ to become sensitive to the oil.

The essential oil has a maximum value of MIC at 500 $\mu\text{g/mL}$, intermediate at MIC 600.0–1,500.0 $\mu\text{g/mL}$, and minimum above 1,500 $\mu\text{g/mL}$ (Aligiannis, Kalpoutzakis, Mitaku, & Chinou, 2001). While, the

Table 3. Zones of growth inhibition (mm) and MIC, showing antibacterial activity of *R. graveolens* L. essential oil

Microbial Strains	Zone inhibition (mm) ^a ± standard deviation		MIC (µg/mL) ^b
	Essential oil	RA ^b	Essential oil
Gram-positive			
<i>Bacillus cereus</i> (ATCC 10876)	26.60 ± 0.03	22.20 ± 0.02	1.0 ± 0.06
<i>Enterobacter cloacae</i> (ATCC 13047)	18.00 ± 0.05	20.04 ± 0.03	0.89 ± 0.05
<i>Enterococcus faecalis</i> (ATCC 49452)	27.10 ± 0.02	23.85 ± 0.05	1.0 ± 0.08
<i>Listeria monocytogenes</i> (ATCC15313)	18.30 ± 0.04	23.40 ± 0.02	0.70 ± 0.04
<i>Staphylococcus aureus</i> (ATCC 25923)	23.40 ± 0.06	19.46 ± 0.04	1.0 ± 0.02
<i>Micrococcus flavus</i> (ATCC 25923)	21.20 ± 0.02	17.06 ± 0.06	1.48 ± 0.09
<i>Micrococcus luteus</i> (ATCC 9341)	19.06 ± 0.05	24.02 ± 0.02	1.0 ± 0.03
Gram-negative			
<i>Acinetobacter baumannii</i> (ATCC 19606)	18.60 ± 0.05	19.00 ± 0.08	1.22 ± 0.06
<i>Escherichia coli</i> (ATCC 25922)	17.70 ± 0.02	20.40 ± 0.02	0.65 ± 0.04
<i>Klebsiella pneumonia</i> (ATCC 700603)	18.10 ± 0.04	19.48 ± 0.03	1.58 ± 0.05
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	15.30 ± 0.06	18.24 ± 0.06	1.0 ± 0.07
<i>Proteus mirabilis</i> (ATCC 35659)	22.00 ± 0.02	15.23 ± 0.03	0.76 ± 0.04
<i>Salmonella typhimurium</i> (ATCC 13311)	13.03 ± 0.03	17.04 ± 0.05	1.34 ± 0.05
<i>Citrobacter freundii</i> (ATCC 8090)	16.02 ± 0.03	18.80 ± 0.06	1.0 ± 0.03
<i>Enterobacter aerogenes</i> (ATCC 13048)	12.57 ± 0.03	16.88 ± 0.02	72.0 ± 0.09

^aValues represent means ± standard deviations for triplicate experiments ($p < 0.05$).

^bRA = reference of antibiotics gentamicin for gram-positive bacteria and mikacin for gram-negative bacteria used was 30 µg/dick.

essential oil showed a higher value of MIC for *E. aerogenes* at 72.0 µg/mL, it generally showed strong activity for all analyzed bacteria. Further, it showed the lowest antibacterial activity against *E. aerogenes* which may be accountable to the high resistance level of gram-negative organisms to the tested essential oil. Our findings were in accordance with previous studies (Angienda, Onyango, & Hill, 2010; Gilles, Zhao, An, & Agboola, 2010; Kivrak et al., 2009; Oyedeji, Lawal, Shode, & Oyedeji, 2009) which reported that the volatile oils showed higher efficiency inhibiting the growth of gram-positive organisms rather than gram-negative organisms.

3.3. Antifungal activity

The essential oil was spiked at a rate of 10 µL per filter paper and demonstrated significant antifungal effects for all tested strains (Table 4). Activity against fungi was compared to that of amphotericin B, a synthetic antifungal drug.

The volatile oil showed maximum, intermediate, and no antifungal activity in zones > 30, <20–30, and > 15 mm, respectively. Based on the diameter of the inhibition zones, the essential oil proved to exhibit the greatest antifungal activity against *C. albicans* with an inhibition zone of 35.10 ± 0.02 mm ($p \leq 0.01$).

In addition, *C. herbarum*, *A. alternaria*, *A. flavus*, and *F. oxysporum* (26.06 ± 0.05, 25.60 ± 0.02, 22.30 ± 0.04, and 20.02 ± 0.03 mm) showed intermediate inhibition growth ($p \leq 0.01$). Against the oil, while minimal activity was reported against *A. fumigates* (14.30 ± 0.06 mm, $p \leq 0.01$). On the other hand, the most significant antifungal activity of the *R. graveolens* L. extracted oil was reported against *C. albicans* with inhibition zones of 35.10 ± 0.02 mm. *R. graveolens* essential oil activity of the

Table 4. In vitro antifungal activity of essential oils

Essential oil strains	Inhibitory diametersa(mm)	
	<i>R. graveolens</i> . L. (10 µL/disc)	Amphotericin B. (20 µg/disc)
<i>Alternaria alternata</i> (MNHN 843390)	22.30 ± 0.04	25.01 ± 0.04
<i>Aspergillus flavus</i> (MNHN 994294)	25.60 ± 0.02	19.04 ± 0.06
<i>Aspergillus fumigates</i> (MNHN 566)	14.30 ± 0.06	26.02 ± 0.07
<i>Candida albicans</i> (ATCC 26790)	35.10 ± 0.02	24.00 ± 0.04
<i>Cladosporium herbarum</i> (MNHN 3369)	26.06 ± 0.05	34.03 ± 0.09
<i>Fusarium oxysporum</i> (MNHN 963917)	20.02 ± 0.06	11.01 ± 0.05

^aDisc diameter (mm) included.

fungi *F. oxysporum*, *A. alternaria*, *A. flavus*, and *C. albicans* was similar to that of amphotericin B. Table 5 shows MIC values of the essential oil and amphotericin B that correspond to their inhibitory effects. Against *C. albicans*, *R. graveolens* essential oil proved the most inhibition (22.00 ± 0.09 µg/mL) compared to the other strains, yet amphotericin B had showed greater activity than the oil. Amphotericin B ranged between 1.00 ± 0.01 and 6.00 ± 0.05 µg/mL, and then greatly increased for *F. oxysporum* (25.00 ± 0.09 µg/mL). It is significant to note that compared to the other strains, *A. fumigates* proved to be the most sensitive strain to the essential oil.

The correlation between the percentage of the chemical constituents, the most naturally abundant compound and the interactions between them and the antibacterial activity was reported in previous studies (Delaquis, Stanich, Girard, & Mazza, 2002; Dorman & Deans, 2000). Greater presence of free heteroatoms such as oxygen-containing components has exhibited contribution to maximum antibacterial activity (Dorman & Deans, 2000; Lambert, Skandamis, Coote, & Nychas, 2001). According to (Griffin, Wyllie, Markham, & Leach, 1999; Mailhebiau, 1994), oxygenated terpenes also show greater antibacterial activity compared normal terpene hydrocarbons. The present study has further extended knowledge on the contribution of the chemical constituents of volatile oils to anti-agent properties.

Regarding antibacterial properties (Ben-Bnina, Hammami, Daamii-remadi, Ben-Jannet, & Mighri, 2010; Proestos, Boziaris, Nychas, & Komaitis, 2006), many studies have expressed that essential oils from *Ruta* species display minimal potency. The present study shows more antibacterial activity of the oil toward gram-positive than gram-negative bacteria, due to the natural abundance of ketones and alcohols in the essential oils. According to Belghazi et al. (2002), *Mentha pulegium* volatile oil shows highest antifungal activity against *Penicillium* and *Mucor*, because of a major component, ketone.

Table 5. In vitro MIC values (µg/mL) of essential oils against filamentous fungi and yeast

Essential oil strain	<i>R. graveolens</i> L.	Amphotericin B
<i>Alternaria alternata</i> (MNHN 843390)	9.80 ± 0.05	1.00 ± 0.01
<i>Aspergillus flavus</i> (MNHN 994294)	9.05 ± 0.03	6.00 ± 0.05
<i>Aspergillus fumigates</i> (MNHN 566)	4.50 ± 0.07	3.51 ± 0.02
<i>Candida albicans</i> (ATCC 26790)	22.00 ± 0.09	3.00 ± 0.02
<i>Cladosporium herbarum</i> (MNHN 3369)	8.50 ± 0.03	3.03 ± 0.06
<i>Fusarium oxysporum</i> (MNHN 963917)	9.06 ± 0.04	25.01 ± 0.09

4. Conclusion

This study showed that *R. graveolens* L. chemical compounds have significant antibacterial and antifungal activity properties. Our findings suggest that *Ruta graveolens* L. essential oils could be used as a natural source of medicinal application for antimicrobial and antifungal activities. However, further investigations on other species of the family Rutaceae are encouraged to determine their potential anti-agent activities.

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