

Comparative Evaluation of Supercritical CO₂ and Methanol Extraction of *Ruta graveolens* Polyphenolic Compounds: *In-vitro* Characterization of Antioxidant and Antimicrobial Potentials

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Plant-derived extracts remain a vital source of bioactive molecules with potential medicinal applications. *Ruta graveolens*, a phenolic-rich medicinal herb, is recognized for its diverse antioxidant, and antimicrobial activities. Supercritical fluid extraction (SFE) was carried out using carbon dioxide (CO₂) as fluid. This was compared to Soxhlet extraction (SE) with methanol to obtain *Ruta graveolens* extracts rich in bioactive compounds. High-performance liquid chromatography revealed notable differences in the phenolic profiles of *Ruta graveolens* extracts depending on the extraction method. SFE yielded higher concentrations of gallic acid (1380 µg/g), chlorogenic acid (522 µg/g), catechin (595 µg/g), and rosmarinic acid (218 µg/g), while SE contained more kaempferol (242 µg/g) and catechin (921 µg/g). The IC₅₀ assessments were 6.59 µg/g for SFE and 1.63 µg/g for methanol, indicating potent anti-inflammatory potentials for both extracts. Based on DPPH radical scavenging assay, SFE and SE of *R. graveolens* extracts showed concentration-dependent activity. The IC₅₀ values were 5.81 µg/mL (SFE) and 7.86 µg/mL (SE). SFE showed larger inhibition zones than SE (24 ± 0.2 vs 20 ± 0.3 mm for *B. subtilis*; 17 ± 0.3 vs 11 ± 0.6 mm for *P. aeruginosa*) and stronger effects on *K. pneumoniae* and *C. albicans*.

DOI: 10.15376/biores.21.1.208-220

Keywords: *Ruta graveolens*; Supercritical fluid; Methanol extraction; Antimicrobial; Biomedical; Infected; Diseases; Antioxidant

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INTRODUCTION

Medicinal plants are considered valuable sources of bioactive ingredients, particularly polyphenols, which are well known for their broad spectrum of biological activities (Al-Rajhi *et al.* 2024; Almehayawi *et al.* 2024). These natural metabolites play a pivotal role in health promotion; they act as potent antioxidants capable of delaying aging processes and as antimicrobial agents that inhibit the growth of pathogenic fungi and bacteria (Alawlaqi *et al.* 2023; Al-Rajhi *et al.* 2023; Alsolami *et al.* 2023). Currently, one of the most significant challenges facing global public health is the rise of antibiotic resistance. The inappropriate use and excessive consumption of traditional antibiotics have exacerbated this issue, resulting in persistent microbial infections that are increasingly challenging to treat. As a result, there is an urgent necessity to identify alternative

therapeutic approaches, which has sparked scientific interest in investigating plant-derived compounds that may possess antibacterial properties (Ventola 2015; Abdelghany *et al.* 2021; Swidan *et al.* 2025). Among the notable medicinal plants, *Ruta graveolens* L. (family Rutaceae) has garnered significant attention. This evergreen shrub, indigenous to Southern Europe, has been utilized in both traditional and alternative medicine for an extended period. Its leaves and stems, whether utilized fresh or dried, are incorporated into culinary practices and various preparations, including decoctions and teas. Furthermore, essential oils derived from its aerial parts have extensive pharmaceutical applications (Szopa *et al.* 2012; Elansary *et al.* 2020). Historically, *R. graveolens* has been used to treat inflammation, infections, ulcers, hypotension, reproductive and menstrual disorders, parasitic diseases, wounds, and even as an antidote for scorpion and snake venoms (Sidwa-Gorycka *et al.* 2009).

Phytochemical and pharmacological studies have confirmed the diverse biological potential of *R. graveolens* and other *Ruta* species. Reported activities include antioxidant (Mokhtar *et al.* 2022), anti-inflammatory (Coimbra *et al.* 2020), antibacterial, neuroprotective, anticancer, and antihyperlipidemic effects (Althaher *et al.* 2024a,b). In particular, methanolic extracts of *R. graveolens* have demonstrated strong antioxidant properties (Diwan *et al.* 2012) and promising antibacterial activity against oral pathogens such as *Streptococcus mutans* and *Streptococcus sobrinus* (Salman *et al.* 2018). Moreover, these extracts exhibit significant inhibition of protein denaturation as well as a dose-dependent suppression of collagenase and elastase activities (Althaher *et al.* 2024b). A recent comprehensive review further highlighted the pharmacological spectrum of *R. graveolens*, reporting antibacterial, anthelmintic, anti-inflammatory, antiproliferative, fertility-regulating, antioxidant, and antiviral properties (Luo *et al.* 2024). These findings reinforce the plant's importance as an auspicious candidate for the progress of natural medicinal agents, particularly in the fight against antibiotic-resistant pathogens.

Supercritical fluid extraction (SFE), a green and sustainable extraction technique, has become more popular in recent decades for the extraction of bioactive compounds from natural sources (Bazaid *et al.* 2025). The extraction efficiency of bioactive plant components strongly depends on the polarity and solvation capacity of the extraction medium. Supercritical carbon dioxide (CO₂) possesses unique tunable properties that allow it to behave as a non-polar solvent with high diffusivity and low viscosity, enabling the efficient solubilization of oleophilic and moderately polar compounds. Moreover, by adjusting pressure and temperature (Almehayawi *et al.* 2024), the density and solvating power of CO₂ can be optimized for selective extraction of target molecules such as essential oils, flavonoids, and phenolic derivatives. Therefore, it was hypothesized that supercritical CO₂ extraction would yield extracts with higher concentrations of bioactive constituents and superior antioxidant and antibacterial activities compared to conventional solvent-based methods. Higher yields of thermolabile or sensitive compounds with fewer solvent residues are frequently the result of this tunability, which enhances mass transfer and selectivity. Furthermore, the most widely used SFE solvent, carbon dioxide, is non-flammable, non-toxic, and readily extracted from the finished product, making it a more environmentally benign method that can be used in food, medicine, and cosmetics (Qanash *et al.* 2025). In contrast, the conventional method of solvent extraction that employs methanol remains widely used due to its simplicity, low equipment costs, and strong ability to solvate a variety of polar compounds. However, the extraction process involving

methanol has significant disadvantages, including the requirement for large amounts of toxic and flammable solvents, extended extraction times, and the risk of co-extracting undesirable substances that complicate later purification steps. Moreover, the high temperatures sometimes necessary in traditional methods can deactivate sensitive bioactive compounds. Therefore, while methanol extraction is both economical and straightforward, supercritical fluid extraction (SFE) has the potential to be a more efficient, selective, and environmentally sustainable alternative for different natural product matrices.

EXPERIMENTAL

Methanolic Extraction *Ruta graveolens* Using Soxhlet

Four grams of the *R. graveolens* powder were placed in a thimble and subjected to solvent extraction using a Soxhlet apparatus with a processing capacity of 500 mL. The metabolites in the pulverized powder were extracted successively using methanol as solvent *via* Soxhlet extraction (SE). Briefly, Soxhlet was operated at 50 °C and ran for 10 h using methanol. Under reduced pressure, the solvent extract was concentrated using a rotary evaporator (Hahn Vapor, HS-2005S, 200–240 V, Korea). For methanol extraction the water bath temperature and vacuum pressure were maintained at 50 °C and 80 mmHg, respectively, with the condenser temperature set to 4 °C. The residual solvent was then removed by spreading the extract as a thin layer on a glass plate and placing it in a vacuum oven (Thermo Scientific, Model Lab Line 3618-1CE) at −90 kPa and 40 ± 2 °C for 24 h. The dried extract was subsequently reconstituted in dimethyl sulfoxide (DMSO) at a concentration of 400 mg/mL for further studies.

Supercritical Fluid Extraction (SFE) of *R. graveolens* Powder

The powdered *R. graveolens*, which had been dried and finely ground, underwent SFE, utilizing carbon dioxide as the main solvent. In this method, CO₂ was pressurized beyond its critical point (at 30 °C and 50 bar) to create supercritical conditions. In this state, CO₂ demonstrates both liquid-like solvating capabilities and gas-like diffusivity. The system's temperature and pressure were modified to improve the extraction of specific bioactive compounds. A co-solvent, methanol, was added in small amounts to increase the solubility of more polar phytochemicals. Following the extraction process, depressurization enables the CO₂ to transition back to a gaseous form, allowing it to separate from the plant extract, resulting in a concentrate that is rich in phytochemicals and free from solvents (Bazaid *et al.* 2025).

High-Performance Liquid Chromatographic Analysis of Polyphenolic Compounds

Polyphenolic compounds of the *Ruta graveolens* extract were analyzed using an Agilent 1260 Infinity HPLC system equipped with a quaternary pump, autosampler, and multi-wavelength detector. Separation was carried out on a Zorbax Eclipse Plus C8 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of solvent A (water) and solvent B (acetonitrile containing 0.05% trifluoroacetic acid). Gradient elution was programmed as follows: 0 to 1 min, 82% A; 1 to 11 min, 75% A; 11 to 18 min, 60% A; 18 to 22 min, return to 82% A; and 22 to 24 min, re-equilibration at 82% A. The flow rate was

maintained at 0.9 mL/min, the column oven temperature at 40 °C, and detection at 280 nm for optimal polyphenolic profiling. Prior to injection, each sample was filtered through a 0.45 µm membrane filter, and a 5 µL injection volume was used. Data acquisition and processing were performed with Agilent ChemStation software. Identification of compounds was achieved by comparing the retention times and UV spectra of peaks with those of authentic standards, including rutin, quercetin, and kaempferol. Quantification was performed using external standard calibration curves.

Antioxidant Ability of *R. graveolens* Extract

The antioxidant capacity of *R. graveolens* extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging technique with minor adjustments. A stock solution of the plant extract (10 mg/mL) was prepared in methanol, and serial dilutions were made to get concentrations ranging from 1.95 to 1000 µg/mL. The working DPPH solution was freshly prepared at a concentration of 0.1 mM in methanol and kept protected from light. For the assay, 1.0 mL of DPPH solution was mixed with 1.0 mL of the extract at different concentrations in test tubes. The mixtures were vortexed and incubated in the dark at room temperature for 30 min to allow the reaction between antioxidants in the extract and the free radical. Following incubation, the decrease in AS was measured at 517 nm using a UV–Vis spectrophotometer against methanol as a blank (Abdelghany and Bakri 2019). Ascorbic acid was tested under identical conditions as standard reference antioxidants, while a control solution (DPPH with methanol only) was used to determine the maximum radical activity. The Eq. 1 below was used to calculate the radical scavenging activity,

$$\% \text{ Scavenging Activity} = \frac{\text{AS of control} - \text{AS of sample}}{\text{AS of control}} \times 100 \quad (1)$$

where control represents the absorbance of the DPPH solution without extract or standard, and sample represents the absorbance in the presence of *R. graveolens* extract or standard compounds. All assays were conducted in triplicate, and results were expressed as mean ± SD. Dose–response curves were generated, and IC₅₀ values (the concentration needed to neutralize 50% of DPPH radicals) were calculated using non-linear regression.

Antimicrobial Evaluation of *R. graveolens* Extract

The inhibitory effect of the extracts was evaluated against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 90274), *Klebsiella pneumoniae* (ATCC 13883), and *Candida albicans* (ATCC 10221). Each microorganism was freshly cultured and adjusted to the 0.5 McFarland standard. Sterile Mueller–Hinton agar (bacteria) and Sabouraud dextrose agar (*C. albicans*) were inoculated with the standardized suspensions. Circular cavities (6 mm) were aseptically prepared in the agar and filled with defined volumes of the test extracts. The inoculated plates were incubated at 37 °C for 18 to 24 h for bacteria and at 28 °C for 48 h for the yeast. Antimicrobial activity was expressed as the diameter (mm) of the clear inhibition zone surrounding each well (Al-Rajhi and Abdelghany 2023).

Minimum Inhibitory, Bactericidal and Fungicidal Concentrations

The lowest concentrations of the extracts capable of suppressing visible microbial growth (MIC) and killing the test organisms (MBC for bacteria and MFC for *C. albicans*)

were determined by a broth microdilution approach. Serial two-fold dilutions of each extract were prepared in sterile Mueller–Hinton broth (for bacteria) and broth of Sabouraud dextrose (for *C. albicans*) was dispensed into 96-well microplates. Standardized microbial suspensions (adjusted to 0.5 McFarland and diluted to $\sim 10^5$ CFU/mL) were inoculated into each well. Plates were incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h for *C. albicans*. The MIC was defined as the lowest extract concentration without visible turbidity. To establish MBC or MFC, 10 μ L from wells without visible growth were plated onto drug-free agar and incubated under the same conditions; the minimal concentration yielding no colony development was recorded as the MBC (bacteria) or MFC (*C. albicans*).

Statistical Analysis

All experiments were performed in triplicate and outcomes expressed as mean $\pm$ SD. Data were analysed employing one-way ANOVA followed by Tukey's post hoc test (GraphPad Prism v9). Different superscript letters show significant differences at $P \leq 0.05$.

RESULTS AND DISCUSSION

HPLC

HPLC analysis revealed the incidence of diverse phytochemical compounds in *Ruta graveolens* extracts obtained from both SFE and methanol SE (Fig. 1), with variations in their abundance and concentration (Table 1). In the SFE extract, gallic acid was the predominant compound (38.5% area, 1380 μ g/g), followed by cinnamic acid (12.9%, 137 μ g/g), and chlorogenic acid (7.54%, 522 μ g/g), whereas catechin, methyl gallate, vanillin, ferulic acid, and rosmarinic acid were also detected in moderate amounts. Conversely, the SE showed gallic acid as the major compound as well (37.6%, 1100 μ g/g), but catechin (9.84%, 921 μ g/g), vanillin (7.8%, 106 μ g/g), and kaempferol (5.9%, 242 μ g/g) were also highly abundant. Some compounds such as chlorogenic acid and rutin were present in the SFE extract but absent or negligible in SE, while kaempferol was notably higher in the methanol fraction depending on the solvent. Interestingly, daidzein and hesperetin were not detected in either extract. The results from this study demonstrate that both extraction methods yielded profiles abundant in polyphenols; nonetheless, SFE proved to be more efficient in obtaining greater quantities of gallic acid, ellagic acid, methyl gallate, ferulic acid, naringenin, rosmarinic acid, quercetin, and chlorogenic acid. On the other hand, SE enhanced the recovery of catechin, vanillin, and kaempferol. This suggests solvent selectivity in extracting different phenolic and flavonoid constituents, which may contribute differently to the biological activities of the extracts. Recent investigations have highlighted the richness of *R. graveolens* in phenolic constituents. Mokhtar *et al.* (2022) reported that the extract contains a wide array of phenolic compounds, identifying nine in total—three phenolic acids and six flavonoids—with rutin (465 μ g/g) being the most abundant, followed by syringic acid (180 μ g/g) and naringenin (110 μ g/g). Noori *et al.* (2019) analyzed the aerial parts of *R. graveolens* and documented a diverse flavonoid profile including kaempferol, quercetin, apigenin, rutin, isorhamnetin, myricetin vitexin, and chrysin. Similarly, Elansary *et al.* (2020) characterized the phenolic composition of *R. graveolens* as well as reported multi-biological activities. Melnyk *et al.* (2018) presented a comprehensive analysis of both the quantitative and qualitative phenolic content,

identifying six flavonoids—quercetin, apigenin, luteolin, rutin, isoquercetin, and hyperoside—and four hydroxycinnamic acids—rosmarinic, caffeic, *p*-coumaric, and chlorogenic. In contrast, Asgharian *et al.* (2020) identified caffeic acid as the only phenolic acid detected, in addition to five flavonoids: rutin, apigenin, quercetin, naringenin, and luteolin in the extract of *R. graveolens*.

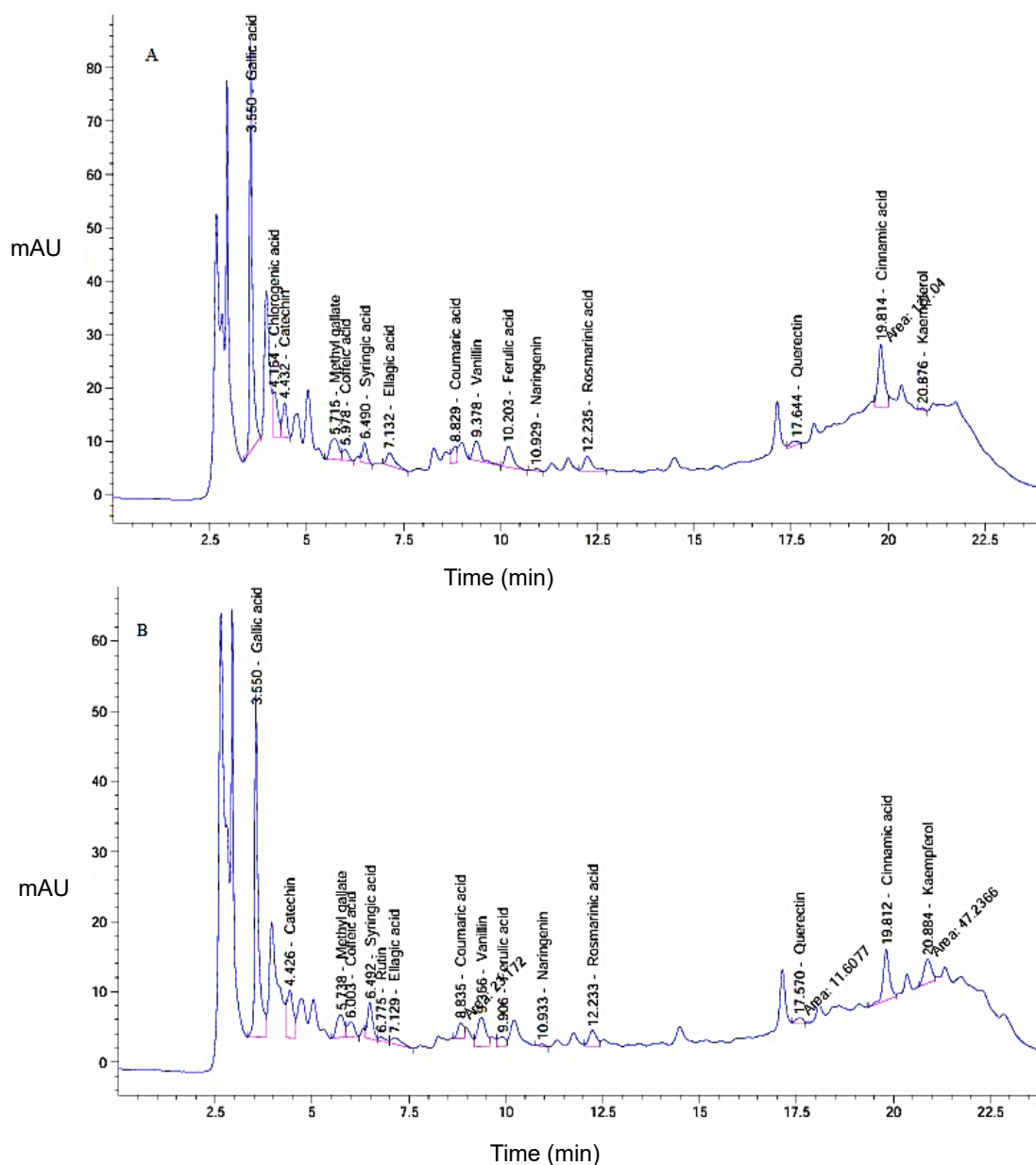


Fig. 1. HPLC chromatograms of phenolic and flavonoid compounds in *Ruta graveolens* extracts obtained by (A) Supercritical fluid extraction and (B) Soxhlet extraction with methanol

Table 1. HPLC Detection of Phytochemical Compounds in *Ruta graveolens* Extracted via SFE and SE

Detected Compound	SFE			SE		
	RT	Area (%)	Concentration (µg/g)	RT	Area (%)	Concentration (µg/g)
Gallic acid	3.550	38.4820	1376.50 ^a	3.550	37.6226	1098.75 ^a
Chlorogenic acid	4.164	7.5419	521.70 ^a	4.210	0.00	0.00 ^b
Catechin	4.432	5.1912	594.97 ^a	4.426	9.8443	921.19 ^a
Methyl gallate	5.715	6.2031	173.59 ^a	5.738	5.4885	125.40 ^a
Caffeic acid	5.978	2.1514	63.58 ^a	6.003	3.2083	77.41 ^a
Syringic acid	6.490	3.2426	102.47 ^a	6.492	5.9806	154.31 ^a
Rutin	6.802	0.00	0.00 ^a	6.775	0.9554	55.99 ^b
Ellagic acid	7.132	3.6407	204.34 ^a	7.129	2.1560	98.80 ^b
Coumaric acid	8.829	3.1931	57.56 ^a	8.835	2.8747	42.31 ^a
Vanillin	9.378	5.0445	84.29 ^a	9.366	7.7832	106.18 ^a
Ferulic acid	10.203	5.4712	156.20 ^a	9.906	2.3210	54.10 ^b
Naringenin	10.929	0.4352	20.77 ^a	10.933	0.3585	13.97 ^a
Rosmarinic acid	12.235	4.9680	217.91 ^a	12.233	4.0265	144.19 ^a
Quercetin	17.644	1.2945	82.75 ^a	17.570	1.4400	75.16 ^a
Cinnamic acid	19.814	12.8675	137.48 ^a	19.812	10.0802	87.93
Kaempferol	20.876	0.2730	13.82 ^a	20.884	5.8601	242.16 ^b

Values bearing different superscript letters differ significantly at $P \leq 0.05$

The results of the DPPH scavenging assay demonstrated a clear concentration-dependent antioxidant activity for all tested samples. At low concentrations, ascorbic acid showed significantly higher scavenging activity compared with both SFE and SE of *R. graveolens* (Fig. 3). As the concentration increased, all treatments exhibited a gradual rise in activity, with ascorbic acid consistently maintaining the highest inhibition across nearly all tested doses. Between the two extracts, the SFE fraction generally showed stronger antioxidant activity than the SE, although both remained significantly lower than the standard. At the highest tested concentrations (500 to 1000 µg/mL), all samples reached high levels of inhibition above 90%, indicating potent free radical scavenging capacity. The IC₅₀ values further confirm these trends, with ascorbic acid exhibiting the lowest IC₅₀ (2.87 µg/mL), followed by the SFE extract (5.81 µg/mL), and the SE (7.86 µg/mL), reflecting the higher potency of the standard antioxidant and the relatively stronger effect of the SFE extract compared to the SE one.

In agreement with previous findings, the present results demonstrate that *R. graveolens* extracts have notable antioxidant potential. Pushpa *et al.* (2015) informed that the ethanolic *R. graveolens* extract exhibited strong antioxidant activity *in vitro*, requiring 9 µg/mL to record 50% scavenging of the DPPH free radical. In this study, the noteworthy radical-scavenging ability indicates the presence of strong phenolic and flavonoid components. The SFE extract obtained demonstrated superior antioxidant effects in comparison to the SE, as evidenced by its lower IC₅₀ value. These findings reinforce the theory that green extraction methods like SFE can enhance the recovery of bioactive phytochemicals that contribute to the antioxidant properties of *R. graveolens*. Furthermore, the phenolic extract of *R. graveolens* displayed notable antioxidant potential, as shown by its results in the DPPH radical scavenging assay (Mokhtar *et al.* 2022).

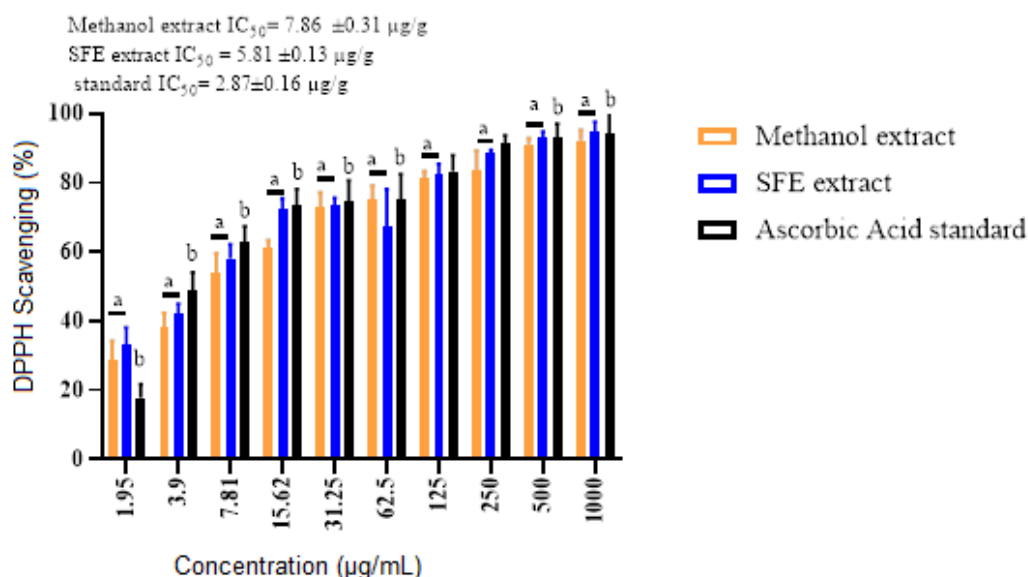
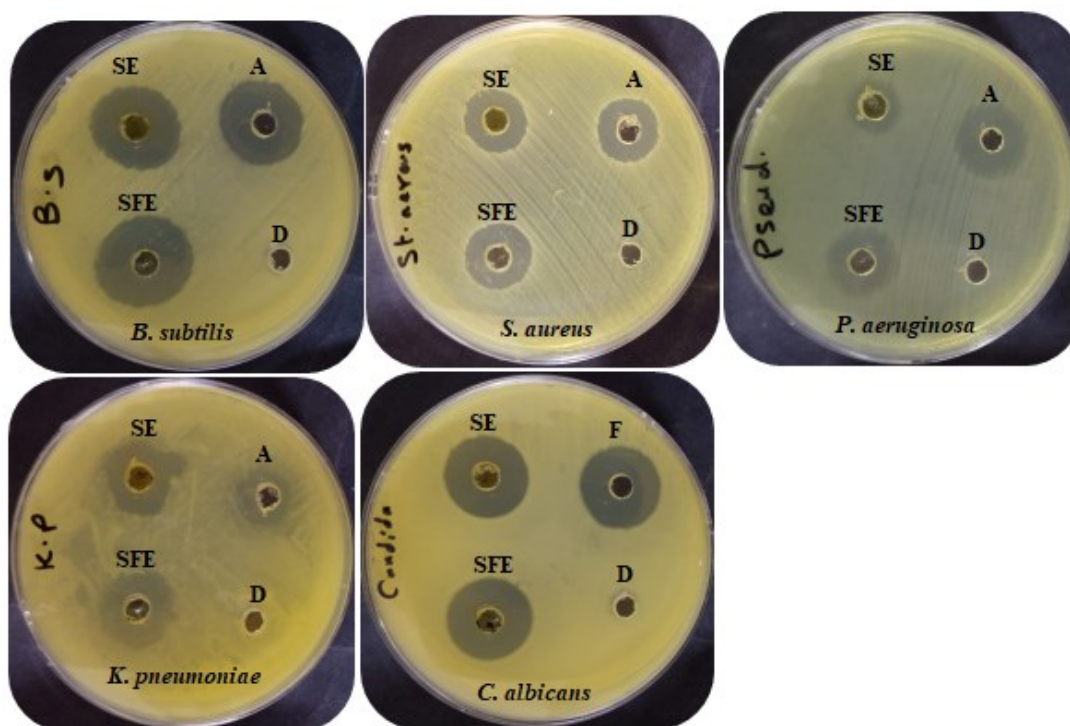


Fig. 2. Antioxidant influence of *R. graveolens* extract via SFE and SE by DPPH scavenging. Values represent mean $\pm$ SD ($n = 3$). Different superscript letters within the same row denote significant differences ($P < 0.05$).

In the study, different inhibition levels were recorded against tested microbes depending on extraction method and microbe species (Table 2 and Fig. 3). The SFE of *Ruta graveolens* extract reflected higher inhibition zones 24 ± 0.2 and 17 ± 0.3 mm than SE 20 ± 0.3 and 11 ± 0.6 mm particularly against *B. subtilis* and *P. aeruginosa*, respectively. *K. pneumoniae* was highly sensitive to *R. graveolens* extract via SFE (18 ± 0.4 mm) and SE (17 ± 0.5 mm) compared to effect of standard antibiotic (14 ± 0.5 mm). Also, *C. albicans* was inhibited by *R. graveolens* extract with inhibition zones 21 ± 0.4 and 19 ± 0.8 mm via SFE and SE, respectively. MIC of the extract via SFE was lower than the MIC via SE against *B. subtilis* and *P. aeruginosa*, while no difference in case *S. aureus* and *K. pneumoniae*. On the other hand, MBC was low in the utilizing SFE compared to SE against *B. subtilis*, *S. aureus*, and *P. aeruginosa*, as well as MFC in case *C. albicans*. The differences in microbial sensitivity to the current extracts detected extensive alteration in the nature of chemical ingredients. These findings are in line with earlier reports that described strong antibacterial and antifungal properties of *R. graveolens*. For example, Samir *et al.* (2015) found that 70% ethanol extracts of *R. graveolens* leaves and flowers yielded inhibition zones ≥ 22 mm against *Helicobacter pylori*. Similarly, Reddy and Al-Rajab (2016) reported that volatile oil from *R. graveolens* inhibited a broad range of bacteria, comprising methicillin-resistant *Staphylococcus aureus*, and yeast (*C. albicans*) with inhibition zones up to 27.10 ± 0.02 mm and MIC values from 0.70 ± 0.04 to $1.58 \pm 0.05 \mu\text{g/mL}$. Attia *et al.* (2018) further demonstrated that the essential oil disrupted *C. albicans* morphology and impeded germ tube formation, confirming its antifungal mechanism. By contrast, several studies using conventional solvent extraction reported weaker or selective activity. Pushpa *et al.* (2015) noted that SE of the aerial parts mainly inhibited Gram-negative *K. pneumoniae* but showed limited effects on typhoid bacilli and *E. coli*.

Table 2. Anti-microbial Properties of *Ruta graveolens* Extract with Estimation of MIC and MFC

Investigated Microbes	Zone of inhibition (mm)			MIC ($\mu\text{g/mL}$)		MBC ($\mu\text{g/mL}$)	
	SE Extract	SFE Extract	Control	SE Extract	SFE Extract	SE Extract	SFE Extract
<i>B. subtilis</i>	20 $\pm$ 0.3	24 $\pm$ 0.2	22 $\pm$ 0.3	15.62	7.8	31.25	15.62
<i>S. aureus</i>	15 $\pm$ 0.1	16 $\pm$ 0.5	16 $\pm$ 0.1	31.25	31.25	62.5	31.25
<i>P. aeruginosa</i>	11 $\pm$ 0.6	17 $\pm$ 0.3	19 $\pm$ 0.6	125	31.25	250	62.5
<i>K. pneumoniae</i>	17 $\pm$ 0.5	18 $\pm$ 0.4	14 $\pm$ 0.5	62.5	62.5	125	125
<i>C. albicans</i>	19 $\pm$ 0.8	21 $\pm$ 0.4	20 $\pm$ 0.8	15.62	31.25	62.5	31.25

**Fig. 3.** Microbial Sustainability to *R. graveolens* Extract via SFE and Methanol (SE). Tested microbes including *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 90274), *K. pneumoniae* (ATCC 13883) and *C. albicans* (ATCC 10221). D (DMSO, negative control), A (antibiotic, positive control for bacteria), and A (antifungal, positive control for fungi)

Ivanova *et al.* (2005) described bacteriostatic but largely Gram-positive-specific activity for ethyl acetate, methanolic, and aqueous methanolic, and petroleum ether extracts, with no inhibition of *C. albicans* or *E. coli*. Amabye and Shalkh (2015) observed maximal activity of chloroform extracts against *E. coli* (2.4 cm) but minimal effects on *P. aeruginosa* (0.8 cm) and moderate action on *B. subtilis* and *S. aureus* (1.8 cm and 1.4 cm, respectively). Taken together, these comparisons highlight that the choice of extraction method substantially alters the chemical profile and hence antimicrobial potency of *R. graveolens*. In the present study, SFE not only increased the inhibition zones but also reduced MIC/MBC/MFC values for several test organisms, suggesting that SFE concentrates or preserves active constituents more effectively than SE. The marked activity

against bacteria as well as *C. albicans* underscores the broad-spectrum potential of SFE-derived *R. graveolens* extracts. Mechanistically, supercritical carbon dioxide is known to solubilize and protect thermolabile, volatile, and non-polar bioactive compounds that are often lost or degraded during conventional solvent extraction. This can lead to a higher recovery of alkaloids, coumarins, flavonoids, and essential oils—phytochemicals previously connected to the antimicrobial activity of *R. graveolens*. The lower MIC and MBC/MFC values observed for SFE extracts in our investigation therefore likely reflect both a greater concentration and a more intact chemical profile of these active constituents, providing a plausible explanation for the enhanced antimicrobial efficacy we recorded.

CONCLUSIONS

1. This research revealed that *R. graveolens* is a valuable source of phenolic compounds with substantial pharmacological potential. Supercritical fluid extraction (SFE) using CO₂ produced extracts with higher concentrations of gallic acid (1380 µg/g), chlorogenic acid (522 µg/g), catechin (595 µg/g), and rosmarinic acid (218 µg/g) than solvent extraction (SE), and the extractant exhibited superior bioactivities. SFE extracts achieved stronger antioxidant assays (5.81 vs. 7.86 µg/mL). In addition SFE achieved larger inhibition zones (24 ± 0.2 mm for *Bacillus subtilis*; 17 ± 0.3 mm for *Pseudomonas aeruginosa*) than SE.
2. The present investigation was performed *in vitro* and focused on a single extraction pressure and temperature profile. *In vivo* safety and efficacy, and pharmacokinetics effects with conventional antimicrobials remain unexplored. Future studies should optimize SFE parameters, evaluate mechanism(s) of action, and *in vivo* evaluations to confirm therapeutic applicability.

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Article submitted: September 23, 2025; Peer review completed: November 25, 2025;
Revised version received: October 28, 2025; Accepted: October 30, 2025; Published:
November 14, 2025.

DOI: 10.15376/biores.21.1.208-220