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Antioxidant Profiling and GC-MS Analysis of Active Subfractions from *Jasminum sambac* L. Leaf Extract

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ABSTRACT: Jasmine leaves (*Jasminum sambac* L.) are known to contain phenolic and flavonoid compounds with antioxidant potential. This study evaluated the antioxidant activities of the ethanol extract, solvent fractions, and subfractions of jasmine leaves. It characterized the chemical profile of the most active subfraction using gas chromatography–mass spectrometry (GC–MS). Jasmine leaves were extracted by maceration using 96% ethanol and subsequently fractionated with n-hexane, ethyl acetate, and water. The ethyl acetate fraction, which exhibited the strongest antioxidant activity, was further separated by vacuum liquid chromatography to obtain subfractions. Antioxidant activity was determined using the DPPH method and expressed as IC_{50} values. The most active subfraction was analyzed by GC–MS. The results showed that the ethyl acetate fraction had the lowest IC_{50} among the solvent fractions, while subfraction 1 (SF1) exhibited the strongest antioxidant activity, with an IC_{50} of 49.78 ppm, indicating strong activity. GC–MS analysis of the most active subfraction suggested the presence of antioxidant-related compound classes with tentative identification. These findings demonstrate that subfractionation enhances antioxidant activity and enriches antioxidant-related constituents in jasmine leaves, while highlighting the need for advanced analytical techniques to confirm compound identities.

Keywords: Jasmine leaves; *Jasminum sambac*; antioxidant; DPPH

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INTRODUCTION

Free radicals are highly reactive molecules or atoms with one or more unpaired electrons that can damage important macromolecules in the body, such as proteins, lipids, and DNA. This damage can trigger various diseases, including degenerative diseases, autoimmune disorders, and cancer (Khaira, 2015; Pratama & Busman, 2020). To neutralize free radicals, the body needs antioxidants, which work by delaying, preventing, and inhibiting oxidative damage (Maharani *et al*., 2021). Antioxidants can stabilize free radicals by capturing unpaired electrons, thereby reducing their reactivity and increasing their stability (Pratama & Busman, 2020). A common method for measuring antioxidant activity is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, due to its simple procedure, fast analysis time, and relatively low reagent requirements (Ansyori *et al*., 2024). The IC_{50} value (the concentration required to reduce 50% of DPPH free radicals) is the main parameter, where a lower value indicates a stronger antioxidant activity (Prasetyo *et al*., 2023).

Natural plants containing phenolic compounds and flavonoids, such as jasmine leaves (*Jasminum sambac* L.), have the potential to serve as sources of antioxidants and UV-protective agents (Sari *et al*., 2022). Previous studies show that jasmine leaf extract has strong antioxidant activity, although it is still weaker than vitamin C, a super-strong antioxidant (Selfiani *et al*., 2023; Yulianto *et al*., 2024). However, existing studies on jasmine leaves are largely confined to crude extracts and solvent fractions. At the same time, systematic investigation at the subfraction level, particularly regarding antioxidant activity enrichment and chemical characterization, remains limited. Although the antioxidant activity of Jasminum sambac leaf extracts and solvent fractions has been reported, most studies remain limited to the extract or fraction level, where bioactive compounds remain chemically heterogeneous. Subfraction-level investigation is therefore necessary to enrich antioxidant-active constituents and to better understand the chemical complexity underlying antioxidant activity. Moreover, limited information is available regarding the chemical ambiguity of enriched subfractions when analyzed using GC–MS. Therefore, this study combines subfractionation and GC–MS profiling to address these unmet scientific gaps.

To the best of our knowledge, antioxidant evaluation at the subfraction level of Jasminum sambac leaves, combined with GC–MS characterization of the most active subfraction, has not been previously reported. This study aims to evaluate the antioxidant activity of jasmine leaf subfractions and identify their active compounds using *Gas Chromatography-Mass Spectrometry* (GC-MS). The GC-MS method can separate and detect volatile compounds in a mixture with high sensitivity, even at very low concentrations. Thus, this research is expected to provide further scientific data on the potential of jasmine leaves as a source of natural antioxidant and sunscreen compounds, especially at the fraction and subfraction levels (Ahmed *et al*., 2023).

METHODS

Tools and Materials

Various essential tools and chemical materials had been utilized throughout the study. The tools had included an analytical balance for accurate sample weighing, a rotary evaporator for solvent evaporation, an oven for drying, and standard laboratory glassware. For the primary analysis, a UV-Vis Spectrophotometer had been employed to measure absorbance in the DPPH assay, and Gas Chromatography-Mass Spectrometry (GC-MS) had been used to identify specific compounds.

The materials had consisted of jasmine leaves (Jasminum sambac L.) as the sample, alongside solvents such as 96% ethanol, n-hexane, ethyl acetate, and water, which had been prepared for the extraction and fractionation processes. Furthermore, the reagent 1,1-diphenyl-2-picrylhydrazyl (DPPH) had been used as a free radical, and quercetin had been selected as the positive control standard.

Research Procedures

Sample Preparation

The initial stage of the research consisted of preparing the jasmine leaf samples. The collected leaves were taxonomically determined to ensure species authenticity. Next, the leaves were washed thoroughly and dried either under sunlight or in an oven at a low temperature to prevent compound degradation. Once the leaves had been dried, they were ground into a fine powder. This powder was then extracted using the maceration method with 96% ethanol for 72 hours at room temperature. After the mixture had been filtered, the resulting filtrate was concentrated using a rotary evaporator until the solvent had evaporated, leaving a viscous extract (Kholifah et al., 2023).

Compound Identification

Compound identification involved qualitative antioxidant tests, as well as TLC tests for flavonoids, alkaloids, saponins, and tannins. Silica gel GF254 was used as the stationary phase. The mobile phase for the flavonoid test consisted of n-butanol, acetic acid, and water (4:1:5), with ammonia vapor as the reagent. For alkaloids, a methanol:chloroform (0.5:9.5) mixture was used with Dragendorff's reagent. The resulting chromatograms were detected at 254 and 366 nm, after which the values were calculated (Harborne, 1987).

Extract Fractionation

The viscous jasmine leaf extract was fractionated using liquid-liquid extraction with solvents of varying polarity: n-hexane, ethyl acetate, and water (Mustarichie et al., 2017). This process aimed to separate compounds based on their polarity. After the fractionation had been completed, each resulting fraction was concentrated again. Each fraction was subsequently tested for antioxidant activity to identify the fraction with the highest potential.

Subfractionation

The ethyl acetate fraction, which had shown the strongest antioxidant activity, was further separated using Vacuum Liquid Chromatography (VLC). This process yielded several subfractions. Each subfraction was retested to find the one with the highest activity, which was then analyzed via GC-MS.

Antioxidant Activity Assay

a. DPPH Solution: After 50 mg of DPPH powder had been dissolved in 50.0 mL of methanol p.a., a 1000 ppm concentration was obtained. The sample was then diluted to 100 ppm (Gülçin, 2023).
b. Quercetin Standard: A 100 µg/mL stock solution was prepared and diluted to 1–6 µg/mL. These solutions were mixed with DPPH, incubated at 37°C for 30 minutes, and the absorbance was measured at 514 nm.
c. Sample Solutions: Extract, fraction, and subfraction solutions were prepared at concentrations of 60–140 ppm. These concentrations were selected because preliminary observations had indicated a linear inhibition response. Each sample was mixed with DPPH, vortexed, incubated in the dark, and measured at 514 nm. All treatments were performed in triplicate.

UV-Vis Spectrophotometry

To determine the maximum wavelength, the absorbance of the DPPH stock solution was scanned from 400–800 nm. To determine the operating time, the mixture was measured every minute until the absorbance had stabilized.

The final antioxidant activity assay was conducted using the DPPH method. After the mixtures had been incubated in a dark room for 30 minutes, the absorbance was measured at 514 nm. The values were determined via linear regression. The fraction that exhibited the smallest value was considered to have the strongest antioxidant activity (Azizah et al., 2024). The absorbance values were then used to calculate the percentage of free radical scavenging activity using the following formula:

$$\frac{\text{(Abs Control - Abs Sample)}}{\text{Abs Control}} \text{ x } 100\%$$

After the inhibition percentage for each replication had been obtained, a linear regression was performed between the sample concentration (ppm) (X) and the DPPH inhibition percentage (%) (Y). This resulted in the equation:

$$Y = bx + a$$

The IC_{50} value was obtained from the x value after substituting y with 50 by inserting the values of B and A. The same calculation was also performed on the Quercetin standard (Rizikiyan, 2019).

GC-MS Analysis

The process of identifying active compounds was performed using Gas Chromatography-Mass Spectrometry (GC-MS). The subfraction sample that had exhibited the best antioxidant activity was injected into the GC-MS instrument. Within the gas chromatograph (GC), the components were separated based on their boiling points and polarity. Once they had been separated, each compound entered the mass spectrometer (MS), where it was ionized and fragmented. The resulting mass spectra were then compared against a standard spectral database (e.g., NIST) to identify the chemical structures present in the sample.

RESULT AND DISCUSSION

Extraction and Phytochemical Screening

The extraction of *Jasminum sambac* L. leaves using 96% ethanol via maceration yielded a concentrated extract with a satisfactory yield of 16.22%. Ethanol had been selected for its polarity, which had enabled the efficient dissolution of flavonoids and phenolic compounds. TLC analysis of the ethanol extract confirmed the presence of flavonoids, alkaloids, tannins, and saponins (Sofowora, 1993).

The dominance of semi-polar spots observed in the ethyl acetate fraction and SF1 suggested an enrichment of phenolic-related compounds, which corresponded to the lower values observed in these samples. This qualitative confirmation was essential, as it had highlighted the presence of active constituents responsible for the antioxidant activity prior to further separation.

Fractionation Yields

Fractionation of the crude extract was performed by liquid–liquid partitioning to separate compounds based on polarity. The highest yield resulted from the ethyl acetate fraction, followed by the water and n-hexane fractions (Inayah & Sari, 2023). By the time the fractionation had concluded, it was evident that the semi-polar solvents had captured the largest portion of the extract's mass.

Table 1. Yield of Jasmine Leaf Fraction

Ethanol Extract (gram)	Solvent Fraction	Total Thick Fraction (gram)	Yield (%)
50,2394	n-Hexane	5,1632	10,28
	Ethyl Acetate	17,6502	35,13
	Water	10,2254	20,35

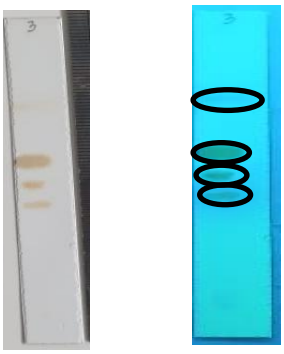
Antioxidant Activity and VLC Separation

Antioxidant testing using the DPPH method showed that the ethyl acetate fraction had the strongest activity, with an of 55.53 ppm, which was lower than those of the n-hexane and aqueous fractions. This result was consistent with the semipolar nature of ethyl acetate, which had effectively dissolved the phenolic and flavonoid compounds (Nursamsiar, 2023). These findings indicated that the key antioxidant compounds in jasmine leaves were primarily semipolar.

The ethyl acetate fraction, which had shown the strongest antioxidant activity, was further separated by Vacuum Liquid Chromatography (VLC). This method was chosen because the vacuum conditions allowed the sample to migrate more efficiently through the stationary and mobile phases, thereby facilitating a more effective isolation of active compounds (Mutmainnah et al., 2017). In this procedure, 5 grams of the ethyl acetate fraction were accurately weighed, mixed with approximately 5 grams of silica gel, and eluted using a gradient of n-hexane, ethyl acetate, and methanol (Rahimah et al., 2013).

TLC Optimization and Mobile Phase Selection

The purpose of this analysis was to determine the optimal solvent system for separating compounds based on the number of spots observed (Wardhani et al., 2023). Among the various mobile phases tested, as shown in Figure 1, the fifth mobile phase—n-hexane:ethyl acetate (6:4 v/v)—provided the most distinct separation pattern. It was determined to be the optimal mobile phase for the TLC analysis of the VLC subfractions because the spots had provided a superior separation pattern compared to the crude ethanol extract (Kusuma, 2023). The resulting chromatogram was observed under UV light at a wavelength of 254 nm and under visible light, as illustrated in Figure 1.



[the stationary phase: silica gel GF 254;mobile phase: n-hexane:ethyl acetate (v/v) as (6:4)]

Figure 1. Chromatogram profile of ethanol extract optimization

Subsequently, 11 eluates were analyzed via TLC using the previously determined optimal mobile phase of n-hexane:ethyl acetate (6:4 v/v). Eluates that exhibited identical spot patterns under UV light were combined to simplify the resulting fractions. The consolidated chromatogram patterns of these fractions were then recorded, as shown in Table 2.

Table 2. Result of chromatogram pattern of Jasmine fraction

Eluate	Eluents	Total	Total of spots	Rf	Subfraction Code
1st	*n*-hexane	100% (v/v) 100 mL	2	0,65 0,88	SF1
2nd	*n*-hexane : ethyl acetate	8 : 2 (v/v) 100 mL	2	0,65 0,88	SF1
3rd	*n*-hexane : ethyl acetate	6 : 4 (v/v) 100 mL	2	0,65 0,88	SF1
4th	*n*-hexane : ethyl acetate	4 : 6 (v/v) 100 mL	2	0,65 0,88	SF1
5th	*n*-hexane : ethyl acetate	2 : 8 (v/v) 100 mL	4	0,13 0,35 0,48 0,65	SF2
6th	ethyl acetate	100% (v/v) 100 mL	4	0,13 0,35 0,48 0,65	SF2
7th	ethyl acetate : methanol	8 : 2 (v/v) 100 mL	4	0,13 0,35 0,48 0,65	SF2
8th	ethyl acetate : methanol	6 : 4 (v/v) 100 mL	3	0,13 0,35 0,65	SF3
9th	ethyl acetate : methanol	4 : 6 (v/v) 100 mL	3	0,13 0,35 0,65	SF3
10th	ethyl acetate : methanol	2 : 8 (v/v) 100 mL	3	0,13 0,35 0,65	SF3
11th	methanol	100% (v/v) 100 mL	3	0,13 0,35 0,65	SF3

Furthermore, the four combined fractions were subjected to quantitative antioxidant activity testing using the same methods and procedures that had been applied to the jasmine leaf extract. The maximum wavelength was 514 nm, with an absorbance of 0.732, which fell within the acceptable range (0.2–0.8). These results are presented in Table 3. Based on the statistical analysis, the Quercetin group showed a significant difference from the SF1 group, as indicated by the distinct superscript letters in Table 3. This demonstrated that SF1 did not exhibit antioxidant activity comparable to that of the positive control; instead, SF1 showed strong activity that was significantly lower than that of Quercetin.

As shown in Table 3, Subfraction 1 (SF1) exhibited the lowest IC_{50} value (49.78 ppm) among all tested samples. Based on IC_{50} classification, this value indicated strong antioxidant activity, although it was not comparable to the positive control (IC_{50} = 9.37

ppm). The lower IC_{50} of SF1 compared to the parent ethyl acetate fraction indicated that further fractionation had successfully enriched the antioxidant bioactive compounds. It should be noted that the antioxidant activity reported in this study was based on the DPPH assay, which measures radical scavenging activity via a single-electron transfer mechanism. Therefore, the obtained IC_{50} values reflected in vitro radical scavenging potential rather than comprehensive biological antioxidant effects.

In contrast, as shown in Table 3, SF2 and SF3 exhibited higher IC_{50} values, indicating moderate antioxidant activity (94.52 ppm and 103.47 ppm, respectively). These findings suggested that polarity-based fractionation had effectively concentrated the phenolic and flavonoid constituents responsible for the antioxidant effects. The enrichment observed in SF1 highlighted the role of subfractionation in isolating potent antioxidant components from complex plant extracts.

Compared with previous studies that had reported IC_{50} values of approximately 60–80 ppm for Jasminum sambac leaf extracts, the IC_{50} value of SF1 (49.78 ppm) obtained in this study indicated greater antioxidant potency. This improvement was attributed to deeper fractionation and the enrichment of semi-polar bioactive constituents. Statistical analysis of the IC_{50} values was performed using one-way analysis of variance (ANOVA), followed by post hoc multiple-comparison tests. Different superscript letters in Table 3 denote statistically significant differences among samples at $p<0.05$..

Table 3. IC_{50} Value Results of Jasmine Leaf Extract, Fractions and Subfractions

No	Sample	IC_{50} (ppm) ± SD
1	Quercetin (a)	9,3686 ± 0,7477 ^{b,c,d,e,f,g,h}
2	Ethanol 96% extract (b)	68,3267 ± 1,3765 ^{a,c,d,e,f,g,h}
3	n-Hexane fraction (c)	79,3471 ± 3,3841 ^{a,b,d,e,f,g,h}
4	Ethyl Acetate fraction (d)	55,5349 ± 3,5486 ^{a,b,c,e,g,h}
5	Water fraction (e)	133,5438 ± 2,5902 ^{a,b,c,d,f,g,h}
6	SF1 (f)	49.7826 ± 1,3547 ^{a,b,c,d,e,g,h}
7	SF2 (g)	94,5206 ± 1,9746 ^{a,b,c,d,e,f,h}
8	SF3 (h)	103,472 ± 1,9037 ^{a,b,c,d,e,f,g}

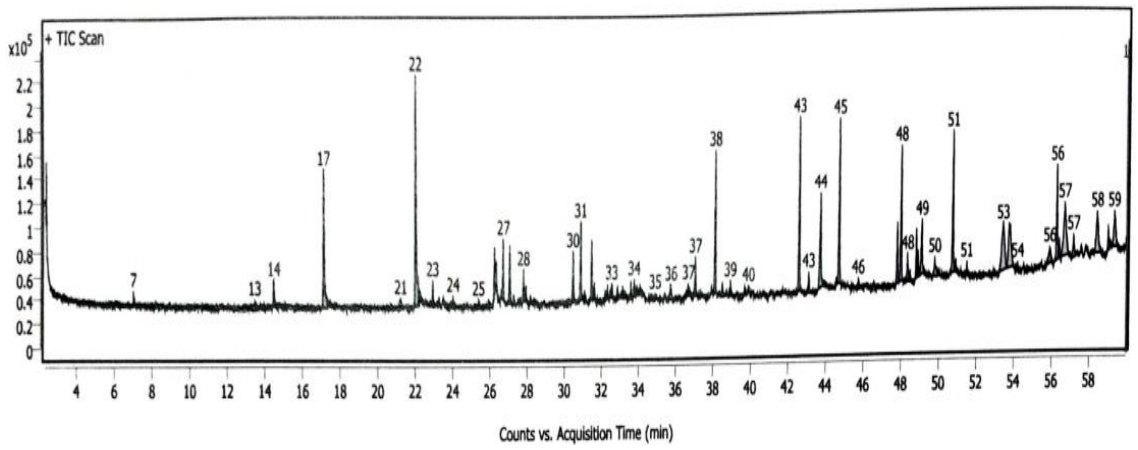
Note:
a = Quercetin
b = Ethanol 96% extract
c = n-Hexane fraction
d = Ethyl Acetate fraction
e = Water fraction
f = Subfraction 1 (SF1)
g = Subfraction 2 (SF2)
h = Subfraction 3(SF3)

GC–MS Analysis and Compound Identification

The subfraction was analyzed using GC–MS to determine its chemical profile. GC–MS is a sensitive and selective analytical technique that identifies volatile and semi-volatile compounds based on retention time and mass-to-charge ratio () (Gross, 2011). Four key parameters were used to interpret the chromatogram: retention time, peak area, mass fragmentation pattern, and similarity index.

Retention time assisted in compound identification through comparison with spectral libraries. Peak area represented the relative proportion of each compound within the chromatogram, calculated as the percentage of each peak's area relative to the total ion chromatogram (TIC) area. Mass fragmentation patterns provided structural information, while the similarity index compared the spectra to reference libraries such as NIST or Wiley, where values typically indicate reliable identification (Sparkman et al., 2011). These combined parameters supported the tentative identification and relative estimation of the compounds present in the sample. The GC–MS chromatogram of subfraction 1 (SF1) is shown in Figure 2.

Figure 2. GC-MS chromatogram profile of subfraction 1 (SF1) of jasmine leaves



Interpretation of SF1 Chromatographic Data

GC–MS analysis of jasmine leaf subfraction 1 (SF1) revealed several chromatographic peaks that were tentatively assigned to compounds based on spectral similarity. The three major peaks showed spectral similarity to compounds tentatively assigned as phenolic derivatives, long-chain alcohols, and ester-type compounds, with similarity indices of 67.12%, 71.94%, and 65.29%, respectively.

Compounds with phenolic or long-chain alcohol structural features have been reported in antioxidant-active fractions of various plant extracts, suggesting that similar compound classes may have been associated with the antioxidant activity observed in SF1. However, definitive identification could not be established in this study. Because these similarity scores fell well below the commonly accepted threshold for reliable identification (), these results were interpreted cautiously and were not considered definitive identifications.

The compound with the largest chromatographic peak exhibited the highest relative abundance in the TIC. However, its 100% spectral base-peak abundance did not indicate absolute concentration nor confirm the exact structure. Instead, the mass fragmentation pattern suggested that the compound might have shared structural features with substituted phenolic compounds, which are often associated with antioxidant activity. However, given the limited confidence level, it was not possible to conclude that this specific phenolic compound was the primary contributor to the antioxidant activity of SF1. Therefore, the GC–MS findings were described as indicating the presence of compounds with structural similarity to phenolic or long-chain alcohol derivatives, rather than as a

definite identification. The tentative fragmentation pattern of the peak showing similarity to Phenol, 2-(1,1-dimethylethyl)-6-methyl- is presented in Figure 3.

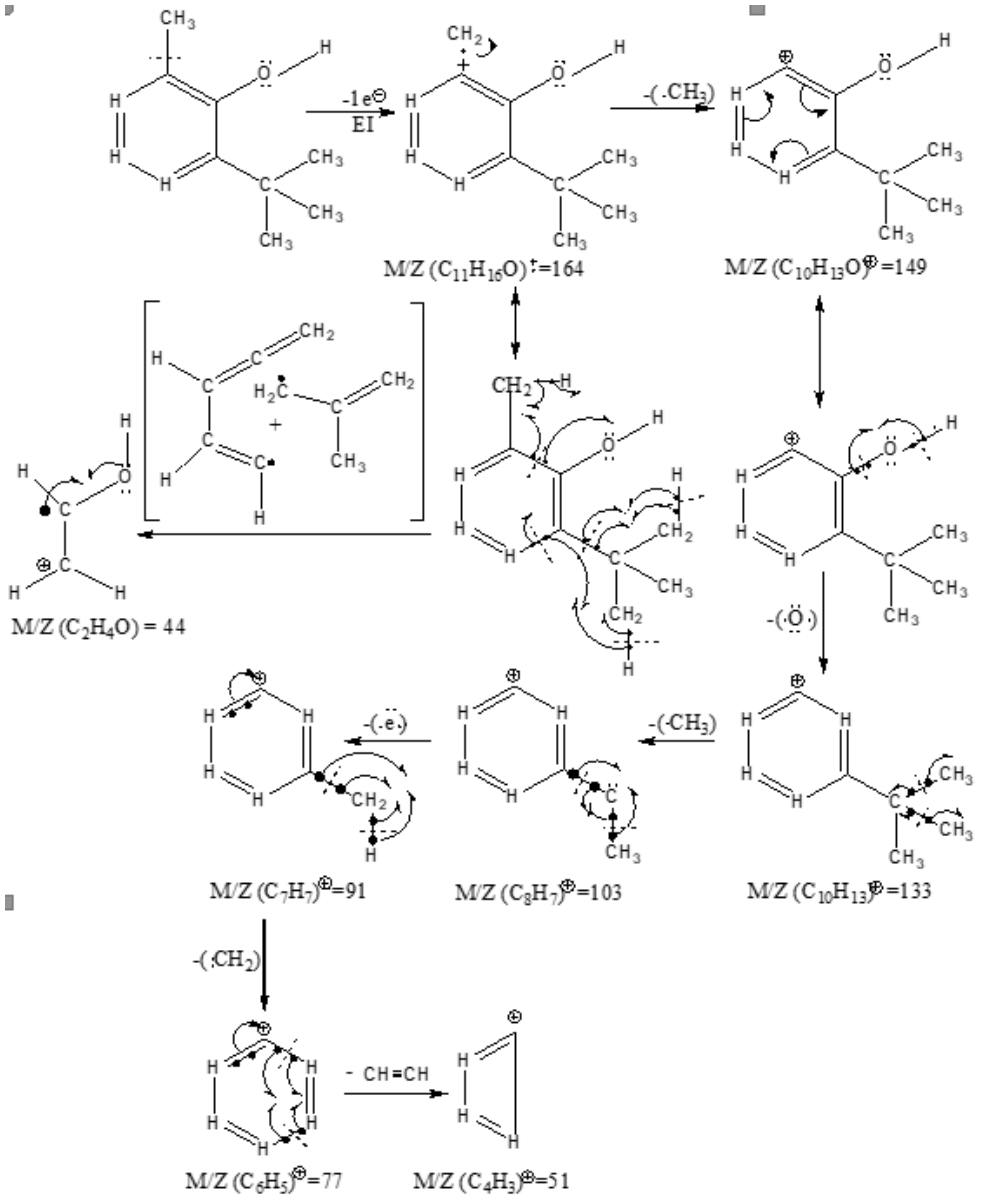


Figure 3. The fragmentation pattern of the Phenol compound, 2-(1,1-dimethylethyl)-6-methyl-

The peak showing similarity to 1-Eicosanol (48.79% peak area; 71.94% similarity) represented a potential long-chain alcohol derivative, although the identification was not definitive due to the low similarity score. Compounds of this structural class are often reported in the literature in relation to membrane-associated properties and antioxidant-related compound classes; however, such functions could not be confirmed in this study based solely on GC–MS data. Likewise, the peak tentatively associated with heptafluorobutyric acid, hexadecyl ester (26.56% peak area; 65.29% similarity) suggested the presence of a fatty-acid-ester–like structure; however, the low confidence level prevented any firm conclusion regarding its biological role. These tentative identifications suggested that the antioxidant activity of SF1 likely resulted from a combination of multiple constituents, although the specific mechanisms and contributions of each compound remained uncertain.

As illustrated in Figure 2, compared with the parent ethyl acetate fraction, SF1 exhibited a chromatographic profile with relatively higher proportions of several major peaks, suggesting that fractionation had led to an increase in certain compound classes, such as phenolic-like structures and long-chain alcohol–like derivatives. However, due to the limited similarity indices, these results were interpreted as indications of possible chemical enrichment rather than confirmation of specific compounds. It is important to emphasize that no direct causal relationship between individual GC–MS peaks and the observed antioxidant activity could be inferred from this analysis.

Overall, the GC–MS data suggested that jasmine leaf extracts might contain constituents with structural features commonly associated with antioxidant activity; however, further studies using higher-resolution spectroscopic techniques (e.g., NMR, LC–MS/MS) are required to conclusively identify the active compounds responsible for the antioxidant effects of SF1.

CONCLUSION

This study demonstrated that Subfraction 1 (SF1), derived from the ethyl acetate fraction of Jasminum sambac L. leaves, possessed the strongest antioxidant activity (IC_{50} =49.78 ppm). GC–MS analysis indicated that subfractionation successfully enriched certain compound classes, such as phenolic-like and long-chain alcohol derivatives, which likely contributed to the enhanced radical-scavenging potential.

However, because similarity indices remained below the 90% threshold, these identifications remained tentative. The study concluded that the observed activity resulted from the synergistic effect of multiple constituents rather than a single compound. While subfractionation effectively boosted potency, further research using LC–MS/MS or NMR is necessary to definitively identify the active metabolites and validate their biological mechanisms.

AUTHOR CONTRIBUTION

MRP: Concepts or ideas; design; definition of intellectual content; literature search; experimental studies; data analysis; manuscript preparation.
I: Provided guidance on study design and methodology, contributed to data interpretation, and approved the final thesis.
NH: Advised on data analysis and manuscript revision, and approved the final thesis.

CONFLICT OF INTEREST

None to declare

ETHICAL CONSIDERATION

This study did not involve human participants or experimental animals; therefore, ethical clearance was not required.

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