Academia Open
Vol 10 No 1 (2025): June (In Progress)
DOI: 10.2025.11256 . Article type: (Medicine)

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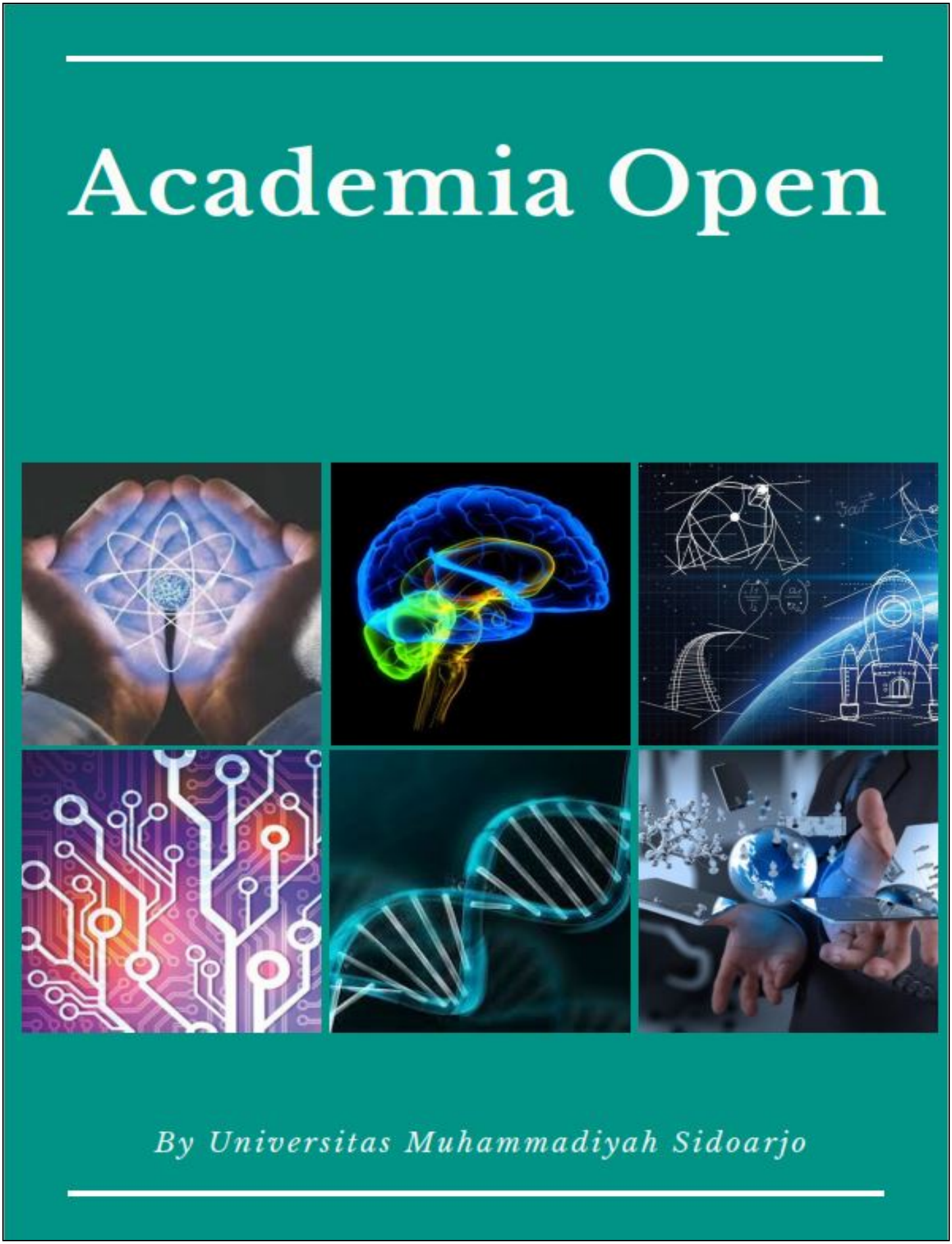
Vol 10 No 1 (2025): June (In Progress)
DOI: 10.2025.11256 . Article type: (Medicine)



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Vol 10 No 1 (2025): June (In Progress)
DOI: 10.2025.11256 . Article type: (Medicine)

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Academia Open
Vol 10 No 1 (2025): June (In Progress)
DOI: 10.2025.11256 . Article type: (Medicine)

Evaluation Study of the Active Compounds and Antioxidant Activity of Lavender Extract

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Abstract

General background: Medicinal plants have long been recognized for their therapeutic properties due to their bioactive compounds. **Specific background**: Lavender (Lavandula spp.) is widely used for its aromatic, medicinal, and preservative properties, but comprehensive analyses of its leaf extract's phytochemical profile and antioxidant potential remain limited. **Knowledge gap**: While numerous studies have explored lavender essential oils, fewer have examined the secondary metabolites and antioxidant capacity specifically from leaf-based alcoholic extracts. **Aims**: This study aimed to evaluate the active compounds and antioxidant activity of the alcoholic extract of lavender leaves by identifying its chemical composition and quantifying key bioactive metabolites. **Results**: Phytochemical screening revealed the presence of phenolic compounds, flavonoids, tannins, glycosides, alkaloids, terpenes, and sterols. The extract showed the highest concentration of total phenolics (25.32 mg/100 g) and the lowest of glycosides (0.076 mg/100 g). Antioxidant assays confirmed its capacity to scavenge free radicals effectively. **Novelty**: This study provides detailed evidence of the potent antioxidant activity of lavender leaf extracts, contributing new insights distinct from flower-based evaluations. **Implications**: These findings support the potential development of lavender leaf-based natural antioxidants for pharmaceutical and nutraceutical applications.

Highlights:

- Identified seven major classes of secondary metabolites in lavender leaf extract.
- Highest antioxidant activity linked to phenolic and flavonoid content.

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- Demonstrated potential for pharmaceutical and nutraceutical applications.

Keywords: Lavender Plant, Active Compounds, Antioxidant Activity

Published date: 2025-06-05 00:00:00

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

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Vol 10 No 1 (2025): June (In Progress)
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Introduction

Plants contain biologically active substances and secondary compounds with a variety of functions, including antibacterial, antioxidant, anti-inflammatory, and anticancer activities. Medicinal plants have been used in traditional folk medicine for a long time, as ancient civilizations relied on them for their medicinal properties and therapeutic effects [1]. These plants have played a fundamental role in treating various ailments and have been passed down through the centuries, preserving knowledge of their medicinal benefits [2].

Secondary compounds, also referred to as bioactive compounds, are chemical entities in medicinal plants responsible for their therapeutic properties [3]. These compounds possess many properties that make them valuable in medicine. They exhibit various antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [4]. Identifying the bioactive compounds in medicinal plants is essential to determining their potential therapeutic applications, as they contain substances for the treatment of chronic and infectious diseases [5] [6]. In our quest to isolate these substances, we focus on extraction with acids and bases, which is a proven and efficient method for obtaining these compounds from plants [7].

Lavender (Lavandula angustifolia Mill) is one of the most widely used plants in cosmetics and aromatherapy because of its deodorizing, soothing, antiseptic, and anti-inflammatory effects. In addition, it can be used in creative dish design as an additive that enhances the taste of meat, ice cream, and drinks in Mediterranean countries It is also used as a food preservative. In addition, this plant has the ability to purify the soil of pollutants, especially heavy metals [8].

The most valuable part of the lavender plant is the flowers, which contain up to 4.5% of the essential oil, the main components of which are esterified linalool, free linalool, camphor, and cineole [9]. Lavender leaves typically contain more camphor and cineole than the flowers, making the leaf oil more pungent. According to (Tăbăraşu et al.) [10]. Essential oils can contain up to 300 different compounds, the qualitative and quantitative composition of which depends on the species, morphological part of the plant, and environmental factors affecting the crop. Lavender essential oils, obtained through various extraction processes and methods, contain more than 100 components [11]

Lavender flowers play an important role because they contain 2–4.5% essential oils [12] [13] but also compounds such as flavones, anthocyanidins, phenolic carboxylic acids, zinc, calcium, magnesium, manganese, etc. [14]. In the dried state, lavender flowers have a complex benefit in the cosmetics industry (for personal care products and perfumes), but they can also be used as a pest and insect repellent [15]

The ideal environment for healthy lavender growth should have a pH between 6-8, preferably sandy loam soil, and water should be present in moderate amounts, as lavender is not a water-loving plant but is drought-resistant. In addition, fertilizer supplements or compost are needed, with light being another essential factor that the plant depends on [16] [17].

Lavender planting can begin in spring and end in autumn, but according to various studies, the preferred season for increasing productivity for the following year appears to be autumn [18]. Lavender harvest varies depending on the plant variety, as it can bloom early, mid-season, or late. As a general rule, young crops can be harvested when the flowers are 75-80% open. As for long-term crops, which are harvested within 10-15 days, the picking process can begin when the flowers are 50-60% open and end when they reach a flowering threshold of 95-100% [19].

It should also be noted that the quality of the essential oil depends on the method of obtaining it [20]. The lavender extraction method, using supercritical carbon dioxide, produces more oxygenated compounds, while the simpler and easier steam distillation method produces more terpene hydrocarbons [21]. Lavender vapors are powerful inhibitors of the fungi Trichophyton mentagrophytes and Trichophyton spp., so they can be used as an air freshener in rooms [22].

Furthermore, Kavanagh's research demonstrated that lavender essential oil can be used prophylactically in cases of localized bacterial infections [23]. The quality of plant raw materials, including their antimicrobial and antioxidant properties, largely depends on the level of constituents present in the bioactive compounds, which in turn depends on climatic and soil conditions [24].

In addition, some research has shown the protective role of raw lavender, due to its health and culinary value, it is increasingly cultivated in different regions of the world, because the quality of plant materials depends largely on climatic and soil conditions [25] [26]. In this study, extracts from lavender leaves and flowers, obtained from cultivation in a temperate climate on complex wheat soil, were found to exhibit good antibacterial and antifungal properties. The effect on microorganism viability depends on the extract dosage However, in this study, it was shown that small concentrations of the extract, about 0.32%, were sufficient to significantly reduce the viability of bacteria and fungi of the genus Candida [27] [28].

Methods

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A. Preparation of the Plant

Lavender was purchased from local markets in Baqubah and dried at 40°C for 40 minutes in a drying oven. The plant was then ground using a grinder and the resulting powder was placed in airtight containers until use.

B. Preparation of Extracts

The alcoholic extract of lavender leaves was prepared by soaking it in 70% ethanol solvent. 10g of the plant was weighed and dissolved in 70 cm^3 of the solvent. The mixture was left for a whole day (24 hours) at normal laboratory temperature, then filtered to obtain the solution. It was stored in sealed volumetric bottles, after which the study was conducted.

1. Preliminary Tests

Preliminary chemical investigations were conducted on ground lavender extracts to identify the secondary metabolites in them, as follows:

2. Tests for Flavonoids

a. Shinoda's test

A few drops of concentrated hydrochloric acid were added to 1 cm^3 of the extracts, then a small piece of magnesium ribbon or magnesium powder was added. If a red color was formed, this was an indication of the presence of flavonoids.

b. Alkaline's test

Mix 1 cm^3 of ammonium hydroxide reagent with 1 cm3 of the extracts and mix well. Then, add a few drops of the mixture to a filter paper. If a bright color is formed when exposed to ultraviolet (UV) light, this indicates the presence of flavonoids.

c. Ferric chloride test

Mix 1 cm^3 of the extract with a few drops of a 0.046 M neutral ferric chloride solution (pH=7). If a reddish-black or black color is formed, this indicates the presence of flavonoids.

2. Test for phenolic compound and tannin

a. Dichromate test

Add 2 cm^3 of 20% aqueous potassium dichromate solution to 1 cm3 of each extract. If a yellow color is formed, this indicates the presence of tannin and phenols.

b. Ferric chloride test

Add a few drops of 0.1% ferric chloride to 1 cm3 of the extracts. If a dark blue or blackish-green color is formed, this indicates the presence of tannins or phenolic compounds. If a brown color is formed, this indicates a false tannin detection.

3. Test for Glycosides :

a. Killer Killen test

Dissolve 1 cm^3 of each of the extracts in glacial acetic acid and cool in an ice-water bath. Then add two drops of ferric chloride solution and transfer the contents of the test tube to a second test tube containing 2 cm3 of concentrated sulfuric acid. If a reddish-brown color is formed in the form of a ring, this indicates the presence of glycosides.

b. Sodium hydroxide test

1 cm^3 of the extract was mixed with equal amounts of a 5% sodium hydroxide solution. If a yellow color was formed, this indicated the presence of glycosides.

4. Test for Alkaloids

a. Dragendroff's test

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The reagent consists of the following:

1. Solution (A) 0.1 M bismuth subsalicylate (Bi(NO3)): Prepared by dissolving 0.6 g of bismuth subsalicylate in 5 cm3 of distilled water and then making up the volume to 10 cm^3.

2. Solution (B) 3.6 M potassium iodide (KI): Prepared by dissolving 6 g of potassium iodide in 5 cm3 of distilled water and then making up the volume to 10 cm^3.

3. Prepare the reagent by mixing solutions (A and B) in a 400 cm3 volumetric flask with 7 cm3 of concentrated hydrochloric acid, then make up to 400 cm3 with distilled water.

4. Add 1 cm3 of the reagent to 1 cm3 of each extract. If an orange color or an orange color with a red precipitate forms, this indicates the presence of alkaloids.

b. Mayer's test

The reagent consists of the following:

1. A 0.085 molar solution of mercuric chloride: Prepared by dissolving 1.4 g of mercuric chloride in 25 cm3 of distilled water and making up the volume to 60 cm3.

2. A 3.01 molar solution of potassium iodide: Prepared by dissolving 5 g of potassium iodide in 10 cm3 of distilled water.

3. Mix solutions (A and B) in a 100 cm3 volumetric flask and fill to the flask's mark with distilled water.

4. Add drops of Mayer's reagent to 1 cm3 of each of the aqueous, alcoholic, petroleum, and cyclohexane extracts. If a white or pinkish-white precipitate forms, this indicates the presence of an alkaloid.

5. Test for Saponins

a. F oam test

Mix 1 cm^3 of distilled water with 1 cm3 of the extracts in a test tube, then mix using a mixer. Empty the contents of the tube into a graduated cylinder and leave for 15 minutes. If foam appears, this indicates the presence of soaps.

b. Mercuric chloride test

Add 1 cm^3 of mercuric chloride (1%) to 1 cm3 of the five extracts. If a white precipitate forms, this is an indication of the presence of saponins.

c. Sterols Test for

- **Salkowski's test**

Mix 1 cm^3 of each extract with a few drops of concentrated sulfuric acid on the walls of the test tube. If a bright red color is formed, this indicates the presence of sterols.

- **Lieberman's test**

Add a few drops of acetic anhydride to 1 cm3 of the extracts, then heat the mixture until boiling. Then cool the mixture, and add 1 cm^3 of concentrated sulfuric acid to the walls of the tube. If a blue-green color is formed, this indicates the presence of sterols.

6. Test for Terpenoids [29]

a. Trim-Hill test

1 cm^3 of the reagent was added, where 10 cm^3 of glacial acetic acid was mixed with 1 cm^3 of 0.2% copper sulfate solution. The mixture was acidified by adding 0.5 cm^3 of concentrated hydrochloric acid to 1 cm^3 of each extract. The mixture was placed in a boiling water bath. If a blue color was formed, this indicated the presence of diterpenes, and if a green color was formed, this indicated the presence of monoterpenes.

b. Liebermann's test

A few drops of acetic anhydride solution were added to 1 cm^3 of the extracts, the mixture was heated to boiling, then cooled and 1 cm^3 of concentrated sulfuric acid was added to the walls of the tube. If a pink color was formed, this indicated the presence of triterpenes.

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7. Test for amino acids and protein [29] [30]

a. Biuret's test

Mix 1 cm^3 of each extract with 2-3 cm^3 of biuret reagent, 1 cm3 of 40% sodium hydroxide solution, and 2-3 drops of 1% copper sulfate solution. If a pinkish-purple color appears, this indicates the presence of protein.

b. Ninhydrin test

Mix 1 cm^3 of the extract with a few drops of 0.2% ninhydrin reagent and heat the mixture in a water bath. If a blue color appears, this indicates the presence of amino acids.

C. Method of Scavenging Free Radicals using 2,2-Diphenyl -1-picrylhydrazyl (DPPH) [31]

1. Dissolve 0.04 g of DPPH in 100 ml of methanol, noting that the concentration of DPPH is 400 µg/cm^3.

2. Vitamin C standard solution and sample solution were prepared at a concentration of 5000 ppm by dissolving 0.5 g of vitamin C and sample in 100 ml of methanol solvent and distilled water. Then, the dilution law was used to prepare the diluted concentrations (30, 60, 120, 250, 500 (µg/cm3) for both the standard solution and the sample.

3. 200 µL of DPPH solution was added with 500 µL of each of the standard solution and the sample (for each of the prepared concentrations), then the mixture was shaken and left at room temperature for 30 minutes in a dark place, after which the absorbance was measured at a wavelength of 517 nm against the equivalent solution consisting of DPPH in its solvent only.

4. The IC50 value of the sample was calculated using the inhibition curve, which is the concentration of the sample required to inhibit 50% of the DPPH free radicals. The lower absorbance of the reaction mixture indicates a higher activity to scavenge the free radicals.

5. Calculations:

The percentage of DPPH free radical scavenging was calculated using the following equation:

$$\% \text{ Inhibition of DPPH} = \frac{Ac - As}{Ac} x100$$

Figure 1.

% Inhibition of DPPH=(Ac-As)/Ac x100 D. Quantitative Estimation of Some Secondary Metabolites

The phenolic content of lavender was estimated using the Modified Folin method [32], as shown in Figure (1). Flavonoids were also quantitatively estimated using Method, as shown in Figure (2). In addition, the concentration of tannins was quantitatively estimated for lavender plants using method as in Fig. (3). The glycoside content was quantitatively estimated using method [33] as in Fig. (4). The alkaloid content was estimated for lavender plants using method [34]. The saponin content in the samples was also determined using method [35]

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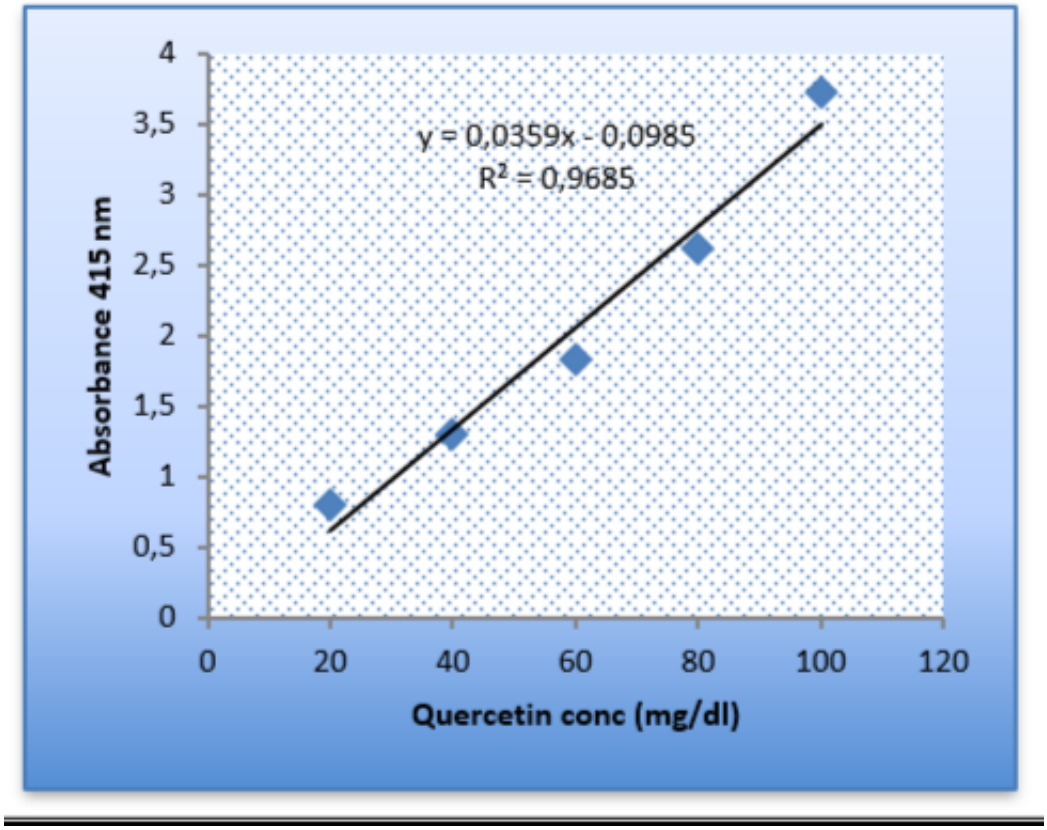

Figure 2. *Concentration of phenolic content in lavender plant*

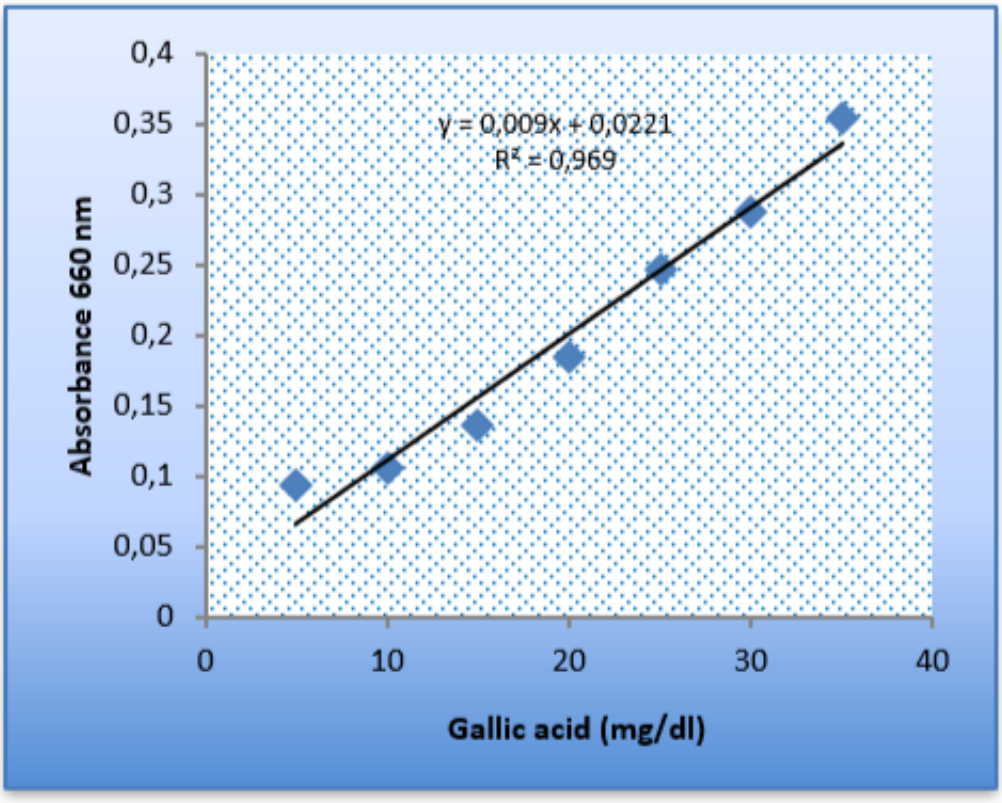

Figure 3. *Concentration of flavonoid content in lavender plant*

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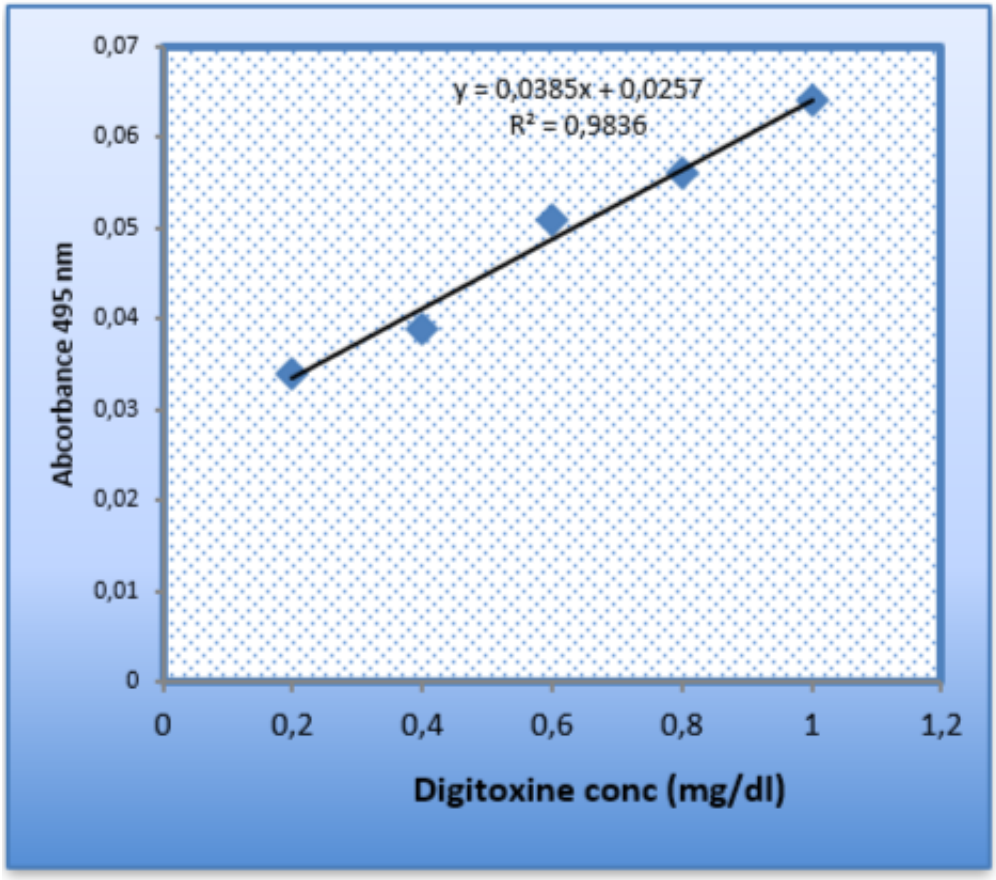

Figure 4. *Total tannin concentration in the lavender plant*

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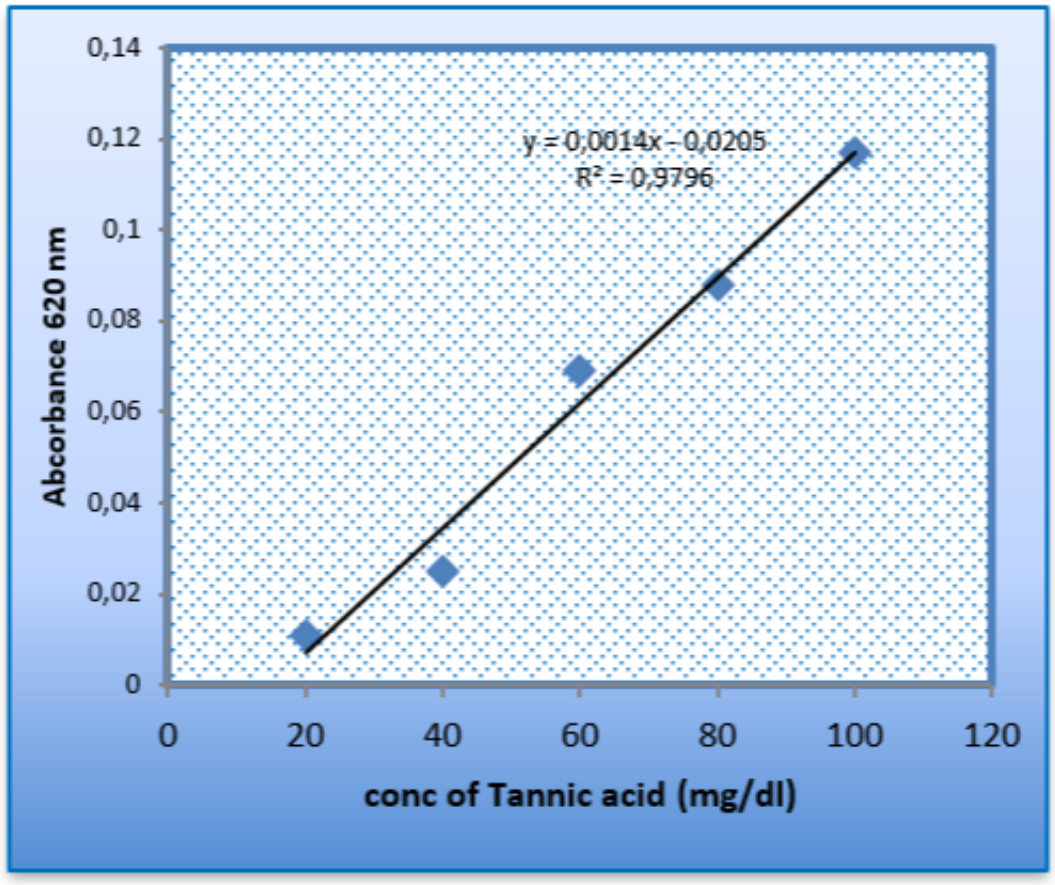

Figure 5. *Glycoside content concentration in the lavender plant*

Results and Discussion

A. Qualitative Determination

Qualitative inferential detection of the primary and secondary metabolites of lavender in the alcoholic extract was conducted. The results showed a clear variation in the plant components, as shown in Table (1).

Metabolic Compound	Type of Reagent	Alcohol Extract
Phenols and tannin	Dichromate	++
	Ferric chloride	+
Flavonoid	Shinoda	+
	Basic solution	++
	Ferric chloride	+
	Mercuric chloride	+
Trephines	Trim Hill	+
	Lieberman	-
Alkaloids	Drakendov	+
	Mayer	++
Sterols	Salkowski	++
	Lieberman	+
Saponin	Foma	+
	Mercuric chloride	+
Glycoside	Kilkerkillen	+

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	NaOH	+

Table 1. *Results of qualitative detection of secondary metabolites in lavender.*

Preliminary test results indicated that lavender contains phenolic compounds, flavonoids, tannins, glycosides, alkaloids, terpenes, and sterols. The alcoholic extract of lavender leaves also contained concentrations of secondary metabolites. All active compounds yielded positive results with all reagents for the alcoholic extract of lavender leaves.

B. Qualitative Content of Secondary Metabolites

The secondary metabolites present in lavender were quantitatively estimated, and the results indicated the presence of various metabolites in the plant, including phenolic compounds, flavonoids, tannins, glycosides, alkaloids, and saponins, as shown in Table (2).

Total Concentration of Estimated Metabolites	Concentration
Total Phenolic Content	25.32± 4.021
Total Tanini Content	2.43 ± 0.215
Total Flavonoid Content	18.45 ± 5.000
Total Alkaloids Content	1.79 ± 0.067
Total saponin Content	3.54± 0.321
Total Glycoside Content	0.076 ± 0.006

Table 2. *Total concentration of estimated metabolites in the plant.*

The results of the current research showed that the alcoholic extract of lavender leaves contained secondary metabolites, as it contained the total phenolic content with the highest concentration of 25.32 mg/100 g and the lowest concentration of the total glycoside content, as the concentration reached 0.076 mg/100 g.

A study found that lavender contains nine phenolic compounds in samples of leaves and flowers grown in Al-Ula (Muğla) during the flowering period. The highest levels of phenolic compounds detected in samples of leaves and flowers of lavender (Lavandula angustifolia Mill.) were protocatechuic acid (189.38 µg/kg), 3,4-dihydroxybenzaldehyde (168.97 µg/kg), and 4-vanillic acid (77.54 µg/kg) [36]

The study of (Gergana et al) [37] also showed the determination of the chemical composition and antioxidant capacity of the lavender plant. Nine phenolic acids were discovered (gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, paracoumaric acid, ferulic acid, salicylic acid, and rosmarinic acid) and five flavonoids, including [(+)-catechin, [(-)-Epicatechin, rutin, hesperidin, and quercetin] in the 70% ethanolic extract of lavender. The highest amount of phenolic compounds was found at 0.38±16.08 mg gallic acid equivalent/g dry weight in the 70% ethanolic extract of lavender, and the ethanolic extract was characterized by the highest amount of flavonoids (quercetin) at 0.44±3.89 mg equivalent/g.

The results of (TRUZZI E et al) [38] on extracts of by-products resulting from steam distillation of Lavandula angustifolia Mill and Lavandula x intermedia Emeric ex Loisel using several solvents, and they studied their total content of polyphenols and flavonoids.

The total polyphenol content of Lavandula angustifolia Mill. and Lavandula x intermedia Emeric ex Loisel extracts was 19.22 ± 4.16 and 17.06 ± 3.31 mg/g, respectively, slightly higher compared to the total polyphenol content, while the total flavonoid content was 1.56 ± 0.21 and 1.41 ± 0.10 mg/g for Lavandula angustifolia Mill. extracts.

The levels of Lavandula x intermedia Emeric x Loisel extracts were, respectively, about two times lower than the extract of L-SD21 M.

1. **The Effectiveness of Extracts as Antioxidants in Vitro**

The antioxidant activity of the extracts was estimated using two methods:

a. **Reducing Power Assay**

The results shown in Figure (5) indicate the reducing power of the alcoholic extract of lavender leaves compared to ascorbic acid as a standard substance.

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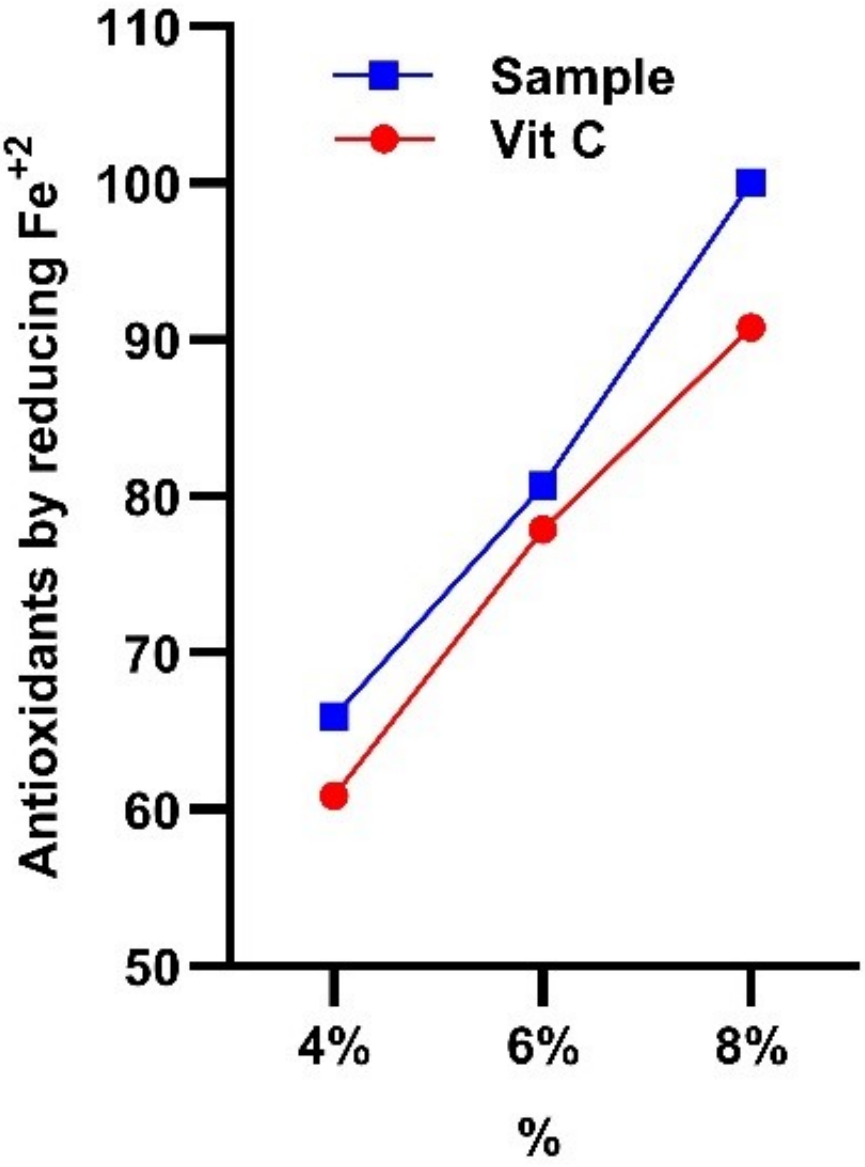

Figure 6. *Reducing power of isolated flavonoids.*

The results showed that the reducing power of the extract is higher than that of ascorbic acid as a standard substance, as shown in Figure (5). The reason may be attributed to the plant containing active compounds, as Sadia et al indicated that flavonoids and phenols have antioxidant activity, so they are considered natural antioxidants [39]. Since the reducing power is related to antioxidant activity and may be an important reflection of its antioxidative activity, compounds with reducing power indicate that they are electron donors and can reduce the oxidative intermediates of lipid peroxidation processes, as they act as primary and secondary antioxidants [40].

The reason may be attributed to the fact that flavonoids donate hydrogen atoms to convert ferric 3 Fe+ to ferrous 2 Fe+, which act as reductants, and that increasing the concentration of flavonoids leads to an increase in the reducing power. They can be reductants in larger quantities if a higher concentration is used [41]. It was found that one of the basic mechanisms of antioxidant activity exhibited by polyphenolic phytochemicals is believed to be the strong scavenging capacity of flavonoid extract due to its ability to remove reactive oxygen and nitrogen species as well as free radicals, which is in agreement with Moreno et al [42]

b. Radical Scavenging Activity DPPH

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The DPPH radical scavenging activity of the alcoholic extract of lavender leaves was estimated, with the lower absorbance of the reaction solution indicating higher free radical scavenging activity.

The results in Figure (6) indicate the strength of the isolated lavender plant in its free radical scavenging activity. The results show that the alcoholic extract of lavender has a higher free radical scavenging activity than the standard compound, ascorbic acid.

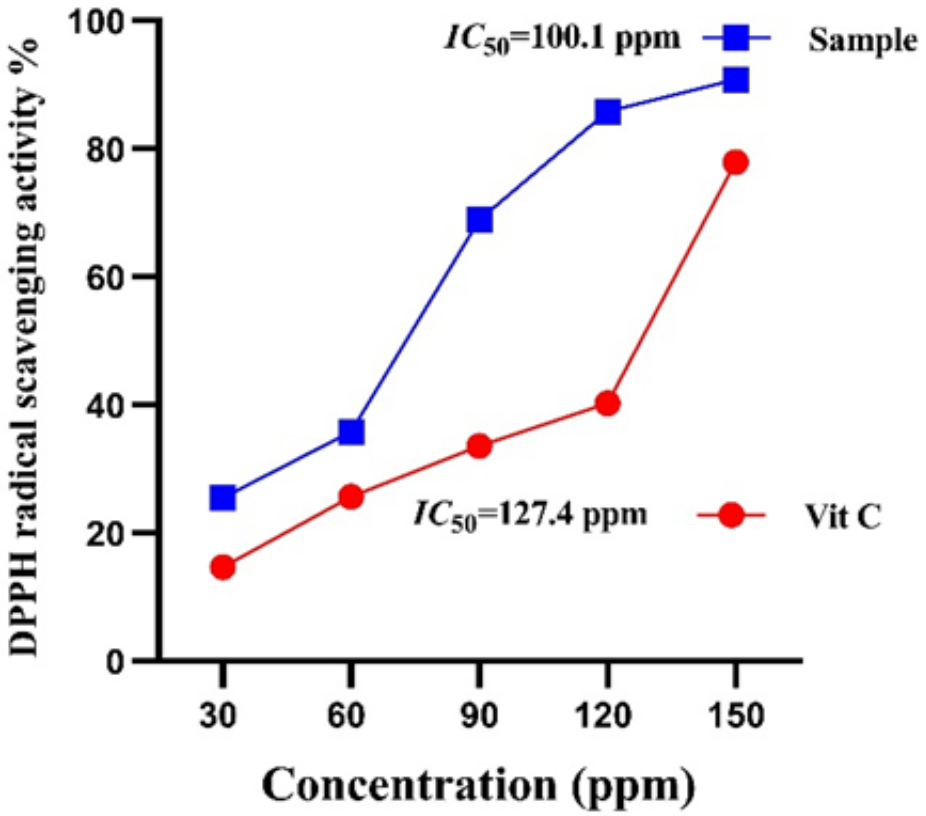

Figure 7. *Efficiency of DPPH free radical scavenging.*

The DPPH method is one of the methods used to determine the antioxidant activity and scavenge of free radicals. This method is easy, fast and sensitive to scan the antioxidant activity of certain compounds or plant extracts to know their antioxidant effects on the DPPH radical. Its activity was compared with the standard substance, corsagin, which is an antioxidant [43].

A study by Wendy et al [44] showed that lavender possesses antioxidant properties through the DPPH method. The extraction method also plays an important role in the content of biologically active substances and their antioxidant properties. The diverse chemical composition of lavender makes it highly active against antioxidants.

This is particularly evident in the presence of phenolic compounds. Rosmarinic acid and midorisinol are among the main compounds attributed to these antioxidant properties [45].

They have antioxidant properties due to their ability to neutralize DPPH radicals.15 While Briones et al [46] reported a 73.85% inhibition of the aqueous extract of Rosmarinus officinalis researchers evaluated the antioxidant capacity of lavender (Lavandula angustifolia) essential oil using FRAP and ABTS techniques, obtaining results of 88.24 µmol Fe2+/g and 101.23 µmol/g, respectively [47].

Medicinal plants are mainly used for the prevention and treatment of human diseases. Among the bioactive compounds in medicinal plants, phenols are of great interest due to their antioxidant properties and potential positive health effects. The antioxidant properties of phenols are mainly related to the number and positions of free hydroxyl groups on the aromatic ring, and their ability to chelate redox metals (Cu, Fe) [48]. Given the essential role of phenolic compounds in immunity, many agricultural and food scientists, as well as farmers, have focused on technical agricultural practices that stimulate the synthesis of phenolics and other antioxidants in plants. One such practice is undoubtedly biostimulant therapy [49].

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Conclusion

This study has successfully demonstrated that the alcoholic extract of lavender (Lavandula angustifolia Mill.) leaves contains a rich diversity of bioactive secondary metabolites, notably phenolic compounds, flavonoids, tannins, glycosides, alkaloids, terpenes, and sterols. The extract exhibited strong antioxidant properties, particularly evident through DPPH radical scavenging and reducing power assays, suggesting that these compounds contribute significantly to its biological activity. The high total phenolic content (25.32 mg/100 g) and substantial flavonoid levels underscore the plant's potential as a natural antioxidant source. These findings validate the traditional medicinal uses of lavender and highlight its promise in pharmaceutical and nutraceutical applications. The implications of this study suggest the potential for developing lavender-based formulations for managing oxidative stress-related conditions. However, further research is recommended to isolate and characterize individual active constituents, explore their specific mechanisms of action, and assess their efficacy through in vivo studies and clinical trials to establish comprehensive therapeutic profiles.

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