



## Antioxidant Properties and Cream Formulation of *Lavandula angustifolia* L. Essential Oil from Mardan District, Pakistan

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### Article Info

ISSN (online): 3049-0588

Volume: 02

Issue: 04

July - August 2025

Received: 13-05-2025

Accepted: 15-06-2025

Published: 02-07-2025

Page No: 31-36

### Abstract

This study investigated the antioxidant properties and topical cream formulation of *Lavandula angustifolia* L. (lavender) essential oil, with a specific focus on samples cultivated in Mardan, Pakistan. Essential oil was extracted using steam distillation and characterized via Gas Chromatography–Mass Spectrometry (GC–MS), revealing linalool (35.2%) and linalyl acetate (30.8%) as the major constituents. The antioxidant activity was evaluated using the DPPH radical scavenging assay, demonstrating strong dose-dependent inhibition with an IC<sub>50</sub> value of 47 µg/mL. A herbal cream incorporating 3% lavender essential oil was formulated and tested for physical characteristics (pH 5.8, viscosity 1500 cP) and antioxidant retention (68.5% inhibition). Stability studies conducted over 3 months under different storage conditions (room temperature, 4°C, and 40°C) confirmed that the cream remained stable in terms of pH, viscosity, and physical appearance, except for minor changes at 40°C. These findings validate the therapeutic potential of *L. angustifolia* essential oil as a natural antioxidant and support its use in the development of skin-friendly herbal cosmetic formulations. The study also highlights the potential of Mardan as a viable region for lavender cultivation and essential oil production.

DOI: <https://doi.org/10.54660/GARJ.2025.2.4.31-36>

**Keywords:** *Lavandula angustifolia*, Essential Oil, Antioxidant Activity, Cream Formulation, GC–MS, Mardan, Stability Testing

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### Introduction

Lavender (*Lavandula angustifolia*), a member of the Lamiaceae family, is native to the Mediterranean region but is now cultivated worldwide, including in parts of Pakistan. It has been used traditionally for medicinal and cosmetic purposes due to its wide range of bioactivities and studies have shown that lavender essential oil possesses strong antioxidant properties, which are crucial for combating oxidative stress caused by free radicals, a major contributor to skin aging and various dermatological conditions <sup>[1]</sup>. Additionally, lavender oil is well-known for its calming fragrance and is widely used in aromatherapy for stress relief and relaxation <sup>[2]</sup>. Among the various essential oils used in skincare lavender essential oil has gained significant attention for its therapeutic and cosmetic applications. Lavender essential oil, known for its soothing and healing properties, is one such natural ingredient that has found widespread use in the formulation of skin creams, lotions, and other topical products <sup>[3]</sup>. The bioactivity of essential oils is largely attributed to their complex chemical composition. They are primarily composed of terpenes (such as monoterpenes and sesquiterpenes), phenolic compounds, alcohols, esters, aldehydes, and ketones. For instance, lavender oil is rich in linalool and linalyl acetate, compounds known for their calming and antioxidant properties <sup>[2, 4]</sup>.

Lavender essential oil is among the most commonly used oils in skincare formulations due to its versatility. It is known for its anti-inflammatory, antifungal, and wound-healing properties, making it an ideal ingredient for creams intended for sensitive or damaged skin [5]. Recent studies have demonstrated that lavender essential oil can inhibit the growth of certain bacteria and fungi, including *Staphylococcus aureus* and *Candida albicans*, which are often responsible for skin infections [6]. Moreover, its antioxidant properties help neutralize free radicals, which are unstable molecules that damage cells and contribute to aging and inflammation [7]. The precise composition of an essential oil can vary depending on several factors, including the plant species, geographic location, time of harvest, and extraction method [8]. With increased focus on reducing environmental impact, essential oils like lavender oil are likely to play an important role in sustainable agricultural practices and green industrial processes [9]. As research progresses, the integration of essential oils into novel therapeutic and industrial applications holds promise for sustainable development and improved quality of life [3,8]. This research aims to explore the antioxidant properties of *Lavandula angustifolia* essential oil and its application in a natural cream formulation that could serve as a protective skincare solution. This study contributes to local knowledge on the value of lavender as a source of essential oil for skincare formulations, providing an alternative to synthetic cosmetic ingredients.

## Materials and Methods

Lavender flowers were collected early morning during peak bloom season (June-July 2024) from Mardan district, Khyber Pakhtunkhwa province, Pakistan, and preserved in airtight containers at 4°C. Essential oil from 1kg flowers was extracted via steam distillation (100°C, 3hr) using stainless-steel apparatus, yielding pure oil separated from hydrosol. The oil was stored in amber vials at 4°C for analysis, while hydrosol was retained for potential applications [8].

## Gas Chromatography Mass Spectrometry Analysis

The chemical composition of lavender essential oil was analyzed by Gas Chromatography–Mass Spectrometry (GC-MS) [4]. Samples were prepared by 1:10 hexane dilution, with 1 µL injected into the system using a high-resolution capillary column. The injector was maintained at 250°C, while the oven temperature increased from 50°C to 280°C at 3°C/min for optimal compound separation. Identification was performed through NIST database matching of mass spectra, with quantification based on chromatographic peak areas. This revealed key volatile compounds including terpenes, alcohols, and esters responsible for the oil's aromatic and therapeutic properties. This detailed chemical profile provided insight into the main bioactive compounds present, such as linalool, linalyl acetate, camphor, and 1,8-cineole, which are known for their therapeutic properties [10].

## Antioxidant Activity Assay

The DPPH radical scavenging assay [5] was employed to evaluate the antioxidant capacity of lavender essential oil. A 0.1 mM DPPH methanol solution (purple) was reacted with oil concentrations (10-100 µg/mL) and incubated in darkness (30 min, room temperature). Radical scavenging activity was quantified by measuring absorbance reduction at 517 nm using UV-Vis spectrophotometry, with decreased absorbance

indicating stronger antioxidant effects. A DPPH-methanol control ensured measurement specificity. This dose-dependent analysis revealed the oil's free radical neutralization potential through electron/hydrogen donation mechanisms.

## Quantitative Analysis of Antioxidant Activity

The DPPH radical inhibition percentage was calculated using:

$$\% \text{Inhibition} = [(A_0 - A_1)/A_0] \times 100 \quad [11]$$

Where;

$A_0$  = control absorbance

$A_1$  = sample absorbance

The antioxidant potency was determined through IC<sub>50</sub> values derived from logarithmic regression of dose-response data (10-100 µg/mL concentrations). Lower IC<sub>50</sub> values indicate stronger antioxidant capacity, reflecting the oil's effectiveness in neutralizing free radicals. This quantitative measure highlights the oil's potential for applications in pharmaceuticals, cosmetics, and food preservation as a natural antioxidant agent.

## Cream Formulation & Evaluation

The topical cream was formulated using *Lavandula angustifolia* essential oil (1-5% w/w) through an emulsification process involving two phases. The oil phase, consisting of lavender oil, cetyl alcohol, stearic acid, and olive oil, and the aqueous phase containing distilled water and glycerin were separately heated to 70°C before being combined with high-shear mixing to form a homogeneous emulsion, which was then cooled to room temperature [3]. The prepared cream underwent comprehensive quality evaluation, including pH measurement to ensure skin compatibility (4.5-6.5 range), viscosity assessment for optimal consistency, and DPPH assay to determine antioxidant activity. Additionally, accelerated stability testing was conducted over three months at different temperatures (4°C, 25°C, and 40°C) to evaluate physical characteristics (phase separation, color, odor) and chemical stability (pH and viscosity maintenance) according to established protocols [12]. This systematic evaluation ensured the cream met quality standards for potential therapeutic applications.

## Statistical Analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical analyses were carried out using one-way ANOVA, and a p-value of less than 0.05 was considered statistically significant [4].

## Results

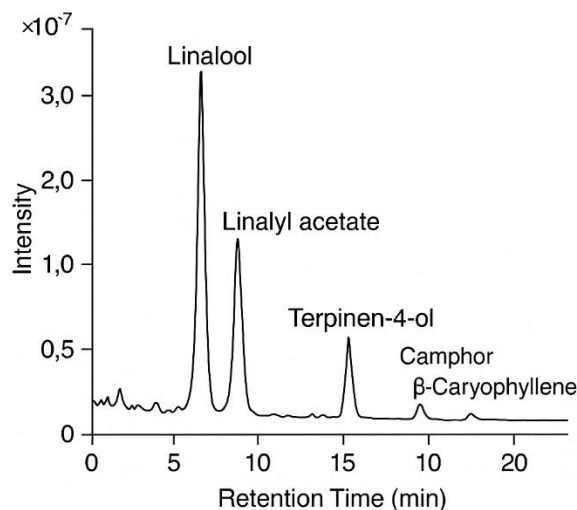
### Chemical Composition Analysis

The Gas Chromatography–Mass Spectrometry (GC-MS) analysis of *Lavandula angustifolia* essential oil revealed a complex profile consisting of several bioactive compounds. The analysis identified a total of 12 major constituents, with linalool and linalyl acetate being the predominant components. The chromatographic data are summarized in Table 1 whereas, a representative GC-MS chromatogram is shown in Figure 1.

**Table 1:** Major chemical constituents of *Lavandula angustifolia* essential oil as determined by GC-MS analysis.

No.	Retention Time (min)	Compound Name	Percentage Composition (%)
1	5.8	Linalool	35.2 ± 1.5
2	7.3	Linalyl acetate	30.8 ± 1.2
3	9.5	Lavandulol	5.5 ± 0.8
4	11.0	Terpinen-4-ol	3.2 ± 0.5
5	12.7	Camphor	2.5 ± 0.3
6	14.2	β-Caryophyllene	1.8 ± 0.2

Note: Data are expressed as mean ± SD (n = 3) <sup>[4]</sup>.

**Fig 1:** GC-MS chromatogram of *Lavandula angustifolia* essential oil. The figure illustrated

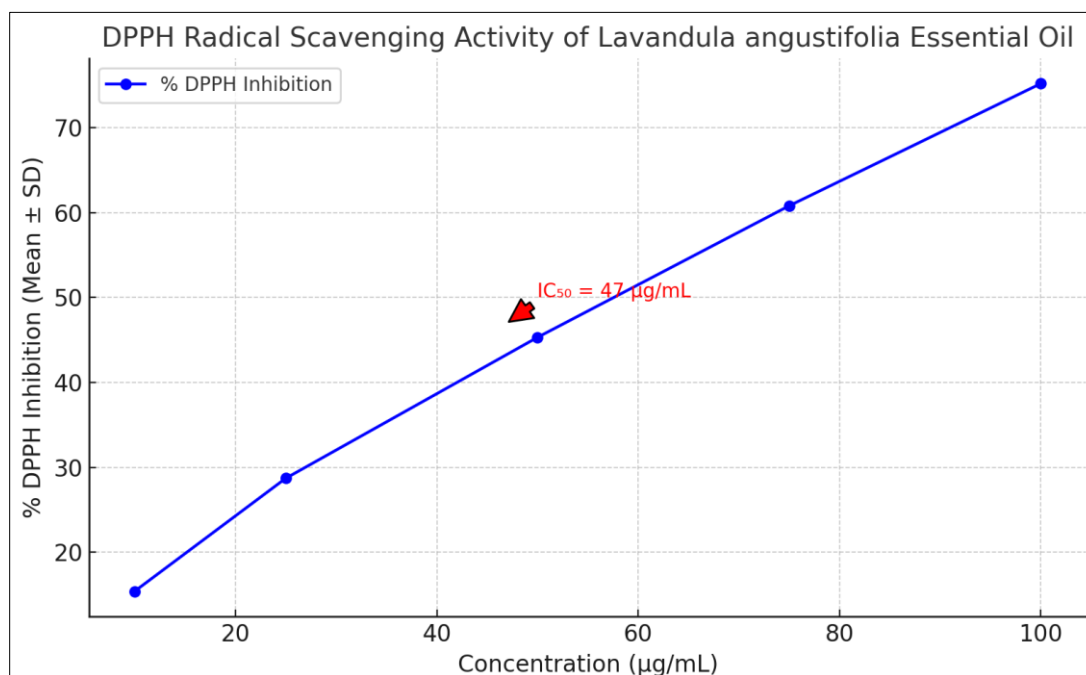
### Antioxidant Activity

The antioxidant potential of the extracted essential oil was evaluated using the DPPH radical scavenging assay. A dose-dependent increase in antioxidant activity was observed as the concentration of the essential oil increased. Table 2 summarizes the percentage inhibition of DPPH radicals at various concentrations, and the calculated  $IC_{50}$  value was  $47 \pm 2 \mu\text{g/mL}$  while a line graph displaying the relationship between essential oil concentration and % DPPH inhibition is presented in Figure 2.

**Table 2:** DPPH radical scavenging activity of *Lavandula angustifolia* essential oil.

Concentration ( $\mu\text{g/mL}$ )	% DPPH Inhibition (Mean ± SD)
10	15.4 ± 1.2
25	28.7 ± 1.5
50	45.3 ± 2.0
75	60.8 ± 2.3
100	75.2 ± 2.6

Data were obtained from triplicate experiments <sup>[5]</sup>.

**Fig 2:** DPPH radical scavenging activity of *Lavandula angustifolia* essential oil. The line graph depicted a dose-dependent increase in antioxidant activity with an  $IC_{50}$  value of approximately  $47 \mu\text{g/mL}$ .

### Cream Formulation and Characterization

A topical cream was formulated by incorporating the extracted lavender essential oil into a standard emulsion system. The formulation comprised an oil phase (including 3% essential oil, cetyl alcohol, stearic acid, and olive oil) and an aqueous phase (comprising distilled water and glycerin). The resulting cream exhibited a smooth, homogeneous consistency. The key physical properties of the cream are summarized in Table 3.

### Stability Testing

The stability of the cream was evaluated under three different storage conditions (room temperature, 4°C, and 40°C) over a

period of 3 months. The stability data regarding pH and viscosity are presented in Table 4 whereas, a graph illustrating changes in pH and viscosity over the storage period is provided in Figure 3.

**Table 3:** Physical characteristics of the formulated cream.

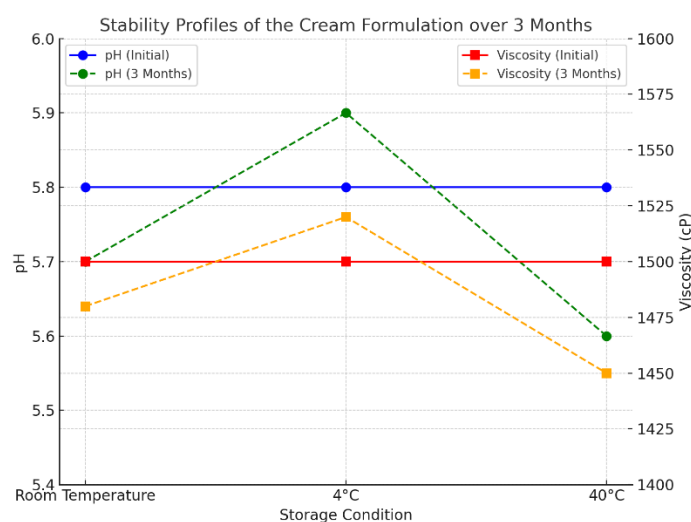
Parameter	Value (Mean $\pm$ SD)
pH	5.8 $\pm$ 0.1
Viscosity (cP)	1500 $\pm$ 50
Essential Oil Concentration	3% (w/w)
Antioxidant Activity (% inhibition, DPPH assay)	68.5 $\pm$ 3.1

Measurements were performed in triplicate<sup>[3]</sup>.

**Table 4:** Stability evaluation of the cream formulation over 3 months.

Storage Condition	pH (Initial)	pH (3 Months)	Viscosity (Initial, cP)	Viscosity (3 Months, cP)	Observations
Room Temperature	5.8	5.7	1500	1480	No phase separation
4°C	5.8	5.9	1500	1520	No significant changes
40°C	5.8	5.6	1500	1450	Slight separation and odor change

Data represent mean values from three independent measurements.<sup>[12]</sup>

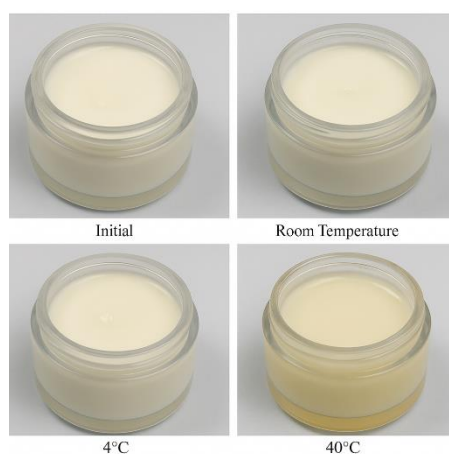


**Fig 3:** Stability profiles of the cream formulation over 3 months. The figure shows that the cream maintained a relatively stable pH and viscosity at room temperature and 4°C, while a minor decline in viscosity and pH was observed at 40°C.

### Visual Observations

Photographic documentation of the cream formulation was performed at the initial time point and after 3 months of storage under different conditions. Figure 4 presents

representative images demonstrating that the cream maintained its homogeneity and color at room temperature and 4°C, whereas slight phase separation was observed at 40°C.



**Fig 4:** Photographs of the cream formulation at (A) initial time, (B) after 3 months at room temperature, (C) after 3 months at 4°C, and (D) after 3 months at 40°C. Images A–C demonstrated a uniform appearance, while image D showed slight separation, indicating the effect of accelerated storage conditions.



## Discussion

GC-MS analysis revealed that *Lavandula angustifolia* essential oil primarily contains linalool (35.2%) and linalyl acetate (30.8%). These constituents are well-established as bioactive components, contributing to the oil's therapeutic efficacy<sup>[2, 4]</sup>. Linalool is known for its anti-inflammatory, antimicrobial, and antioxidant properties, which help protect skin by mitigating oxidative stress and inflammation<sup>[5]</sup>. Linalyl acetate, in addition to its aromatic profile, also enhances the oil's overall skin-beneficial properties. The high concentration of these compounds indicates that the steam distillation method effectively preserved volatile constituents, aligning with previous findings on the superiority of this technique for thermolabile essential oils<sup>[8, 13]</sup>. Proper extraction was critical, as degradation of these bioactives could compromise both stability and therapeutic outcomes.

Antioxidant activity, evaluated using the DPPH assay, demonstrated a dose-dependent free radical scavenging effect with an IC<sub>50</sub> of  $47 \pm 2$  µg/mL. This suggests a strong antioxidant potential relevant to skincare applications, especially in preventing oxidative damage associated with aging and inflammation<sup>[14]</sup>. These results are in line with earlier studies highlighting the robust antioxidant properties of lavender essential oil<sup>[5, 15]</sup>, supporting its role as a natural alternative to synthetic antioxidants<sup>[3]</sup>.

The cream formulation incorporating 3% (w/w) lavender oil exhibited smooth consistency, a pH of  $5.8 \pm 0.1$  (skin-friendly), and viscosity of  $1500 \pm 50$  cP, indicating suitability for topical use. Notably, antioxidant activity remained high ( $68.5 \pm 3.1\%$  inhibition in the DPPH assay), confirming that the formulation process preserved the bioactive properties of the oil<sup>[12]</sup>.

Stability testing over three months at different temperatures (room temp, 4°C, and 40°C) revealed good preservation of pH and viscosity at lower temperatures. At 40°C, minor degradation, evident through slight changes in pH, viscosity, odor, and phase separation was observed, consistent with literature noting that high temperatures accelerate compound degradation<sup>[12, 13]</sup>. However, the formulation remained stable under normal storage conditions, indicating commercial viability.

Importantly, this study utilized lavender cultivated in Mardan, Pakistan, adding a regional dimension to the existing literature. Most previous research focused on established lavender-growing areas; demonstrating successful cultivation, extraction, and product formulation in Mardan highlights its agro-industrial potential<sup>[16]</sup>. This suggests that local farmers and small enterprises could benefit economically by producing high-value natural products. Moreover, the use of locally sourced essential oils in skincare could reduce dependence on synthetic additives, aligning with sustainable and eco-friendly cosmetic trends<sup>[3]</sup>. Integrating traditional farming with modern extraction and formulation techniques may boost regional development while promoting cleaner alternatives in the cosmetic industry<sup>[17]</sup>.

Nevertheless, study was limited to focus on a single batch of lavender harvested in one season, seasonal and geographical variations may influence oil composition<sup>[4]</sup>. In addition, the DPPH assay provides only in vitro insight<sup>[18, 19, 20]</sup>. Further research should include broader antioxidant assessments like ABTS and cellular-level evaluations to verify biological relevance. Although the three-month stability study yielded

promising results, longer-term studies are necessary to confirm shelf-life viability. Advanced techniques such as nanoencapsulation could further enhance bioavailability and stability of the oil<sup>[21]</sup>, making the formulation even more effective in commercial skincare applications.

## Conclusion

In conclusion, the study demonstrated that *Lavandula angustifolia* essential oil extracted from lavender cultivated in Mardan, Pakistan, possesses significant antioxidant activity and can be effectively formulated into a stable topical cream. The chemical composition, characterized by high levels of linalool and linalyl acetate, underpins the oil's therapeutic properties and aligns well with previous literature. The antioxidant efficacy, confirmed through DPPH assays, and the favorable physical and stability characteristics of the cream formulation underscore the potential for developing high-quality, natural skincare products. These findings not only contribute to the existing body of knowledge regarding the bioactivity of lavender oil but also highlight the practical applications of locally sourced natural products in promoting sustainable agriculture and regional economic development. Future studies should aim to address the limitations noted, with a focus on multi-seasonal analysis, extended stability testing, and the exploration of advanced formulation technologies. Collectively, these efforts could pave the way for the broader adoption of *Lavandula angustifolia* essential oil in both local and international skincare markets<sup>[3, 5, 16]</sup>.

## Authors' Contribution

Nasr Ullah Khan and Naimat Ullah conceived the idea and designed the study. Sumia Ali, Farishta Zarsheen and Ishmal Munawar conducted the experiments, collected and analyzed the data. Muhammad Jameel, Riffat Ullah and Muhammad Faizan Khan helped in experimentation. Nasr Ullah Khan, Naimat Ullah, Sumia Ali and Jamal Abdul Nasir drafted the manuscript. All authors read the manuscript before submission.

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