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# Phytochemical Characterization of the Chloroform Extract of *Foeniculum vulgare* Mill. Leaves with Implications for Antiparasitic Agent Development

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**ABSTRACT**

*The development of antiparasitic agents derived from natural products continues to receive considerable attention due to increasing resistance and limitations of conventional therapies. This study aimed to examine the phytochemical profile of the chloroform extract of fennel leaves (F. vulgare Mill.) as a basis for antiparasitic agent development. The extract was obtained using a maceration method and analyzed using thin layer chromatography. Qualitative analysis was conducted based on the observation of visible spots after spraying with appropriate visualization reagents, while supporting analysis was performed based on the calculation of retention factor values for the detected spots. The results revealed the presence of several major groups of secondary metabolites, including flavonoids, terpenoids, alkaloids, and phenolic compounds, as indicated by specific color changes and retention factor values. The diversity of these metabolites suggests the possible involvement of multiple biochemical pathways related to antiparasitic mechanisms. Overall, the phytochemical profile of the chloroform extract of fennel leaves provides important preliminary data to support further research on natural product–based antiparasitic agents.*

## 1. INTRODUCTION

Parasitic diseases remain a significant global health and economic burden due to their high prevalence, morbidity, and mortality rates, particularly in tropical and subtropical regions where access to effective treatments is limited and resistance to conventional therapies continues to emerge. Phytochemical characterization represents a critical preliminary step in the rational development of antiparasitic agents derived from natural products, as it enables systematic identification of secondary metabolites that may contribute to antiparasitic activity. Such characterization provides a scientific basis for prioritizing specific extracts, solvent systems, and compound classes for subsequent biological evaluation, thereby increasing the efficiency and reproducibility of natural product–based drug discovery. Moreover, understanding the chemical profile of plant extracts is essential for interpreting future bioactivity data and for guiding bioassay-guided fractionation strategies. Natural products have historically contributed to successful antiparasitic therapies, exemplified by artemisinin and its derivatives, underscoring their continued relevance in contemporary antiparasitic drug discovery efforts (Shang *et al.*, 2025).

*Foeniculum vulgare* Mill., commonly known as fennel, is an aromatic herb belonging to the Apiaceae family and is widely recognized for its diverse phytochemical composition and extensive ethnomedicinal applications. Traditionally, this plant has been used across various cultures for gastrointestinal disorders, as a culinary spice, and for general health maintenance, reflecting the presence of a broad spectrum of bioactive secondary metabolites. Phytochemical

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studies have reported that *F. vulgare* contains flavonoids, phenolic compounds, volatile oils such as anethole and fenchone, phytosterols, and other metabolite classes that contribute to its documented pharmacological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Khan et al., 2025; Zahi et al., 2025). Notably, the leaves of *F. vulgare* are known to accumulate semi-polar secondary metabolites, and chloroform extraction has been widely applied for the efficient isolation of bioactive compounds such as alkaloids, terpenoids, and certain phenolic constituents. These metabolite groups have been frequently associated with antiparasitic potential in medicinal plants, thereby providing a rational scientific basis for focusing on leaf-derived chloroform extracts in preliminary phytochemical investigations aimed at supporting antiparasitic agent development.

Despite the growing body of literature on the phytochemistry and biological activities of *F. vulgare*, most existing studies have focused on general phytochemical screening, essential oil composition, and broad pharmacological properties, with limited attention to solvent-specific extracts in the context of parasitology. In particular, investigations on chloroform extracts, which are known to preferentially solubilize semipolar to nonpolar secondary metabolites often associated with bioactivity, remain scarce. Moreover, while phenolic compounds, flavonoids, terpenoids, and other metabolites have been reported in *F. vulgare*, their systematic characterization in chloroform extracts derived specifically from fennel leaves has not been adequately addressed. This represents a critical knowledge gap, especially given that leaves are a metabolically active plant organ and a renewable source of secondary metabolites with potential relevance for antiparasitic research (Khan *et al*., 2025; Moumen *et al*., 2025).

Several studies have reported antiparasitic or antimicrobial effects of *F. vulgare* extracts against non-*Plasmodium* organisms; however, direct evidence linking specific phytochemical constituents of fennel leaf chloroform extracts to antiparasitic potential remains unavailable. The lack of detailed phytochemical characterization limits the ability to rationally interpret biological findings and hampers the identification of metabolite groups that may contribute to antiparasitic activity. Importantly, the identification of major classes of secondary metabolites through systematic phytochemical profiling constitutes a fundamental preliminary step in natural product-based drug discovery, as it provides essential chemical insight prior to bioassay-driven investigations.

Therefore, the objective of this study was to characterize the phytochemical profile of the chloroform extract of *F. vulgare* Mill. leaves using thin-layer chromatography (TLC), with the aim of identifying the major groups of secondary metabolites present based on qualitative spot detection and retention factor (Rf) values. This study is designed as a preliminary investigation and does not include biological or antiparasitic activity assays. The resulting phytochemical profile is expected to provide foundational chemical data that enhance the scientific understanding of fennel leaf chloroform extracts and their potential relevance in parasitology-oriented natural product research. Ultimately, these findings are anticipated to serve as a scientific basis for subsequent studies, including bioassay-guided fractionation and in vitro antiparasitic screening using relevant protozoan models.

## 2. METHOD

*Plant Material and Extraction*

Simplicia of *F. vulgare* Mill. leaves were collected, authenticated, and processed. The dried leaves were pulverized into a fine powder prior to extraction. Chloroform extraction was carried out using the maceration method, in which 500 g of powdered leaf material was immersed in 1 L of chloroform for 24 hours with periodic stirring to enhance solvent penetration. The mixture was filtered, and the residual plant material was remacerated twice under the same conditions to ensure optimal extraction. All filtrates were combined and concentrated under reduced pressure using a rotary evaporator to obtain a semisolid chloroform extract, which was stored at 4 °C until further analysis.

*Thin-Layer Chromatography Analysis*

Phytochemical profiling of the chloroform extract of *F. vulgare* Mill. leaves was conducted using thin-layer chromatography. The extract was applied as discrete spots onto silica gel $GF_{254}$ plates, which served as the stationary phase. Suitable mobile phase systems were selected to achieve effective separation of secondary metabolites. After chromatographic development, the plates were air-dried and initially observed under ultraviolet light at wavelengths of 254 nm and 366 nm to detect fluorescent or quenching spots.

Subsequently, the plates were sprayed with specific detection reagents to facilitate the identification of major classes of secondary metabolites based on characteristic color reactions. Liebermann–Burchard reagent was used to detect terpenoids and sterol compounds, indicated by the appearance of blue, green, or violet coloration. Ferric chloride ($FeCl_3$) reagent was applied for the detection of phenolic compounds, producing dark blue, green, or black spots. Alkaloids were identified using Dragendorff reagent, which yielded orange to brown precipitate spots. Flavonoid compounds were detected by exposing the plates to ammonia vapor and observing the development of yellow to yellow-green fluorescence under ultraviolet light at 366 nm. The observed color changes and fluorescence patterns were recorded and interpreted to determine the presence of specific metabolite groups in the extract.

*Data Collection and Analysis*

Qualitative data were obtained through visual observation of the number, color, and intensity of chromatographic spots appearing on the TLC plates after reagent spraying. Each spot was interpreted according to established phytochemical indicators to determine the presence of specific metabolite groups. Quantitative analysis was conducted by calculating the retention factor (Rf) value of each detected spot. The Rf value was determined using the following formula:

$$Rf = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

The calculated Rf values were used to support qualitative identification by comparing them with reference values reported in the literature. All data obtained were analyzed descriptively and presented to illustrate the phytochemical profile of the chloroform extract of *F. vulgare* leaves.

## 3. RESULT AND DISCUSSION

### Result

The phytochemical profile of the chloroform extract of *F. vulgare* Mill. leaves was determined using thin-layer chromatography. The results are presented in the form of chromatographic spot visualization and retention factor (Rf) values to describe the qualitative and quantitative characteristics of the detected secondary metabolites. Visualization of the developed TLC plates under ultraviolet light at 254 nm and 366 nm, followed by spraying with specific detection reagents, revealed the presence of several distinct chromatographic spots with characteristic color responses. These spots corresponded to major groups of secondary metabolites, including terpenoids, phenolic compounds, alkaloids, and flavonoids, as identified based on their reaction with Liebermann–Burchard, ferric chloride ($FeCl_3$), Dragendorff reagent, and ammonia vapor, respectively.

Representative TLC plate images showing the separation pattern, color development, and relative migration distances of the detected compounds are presented in Figure 1. These images provide visual confirmation of the chromatographic profiles obtained from the chloroform extract of *F. vulgare* leaves.

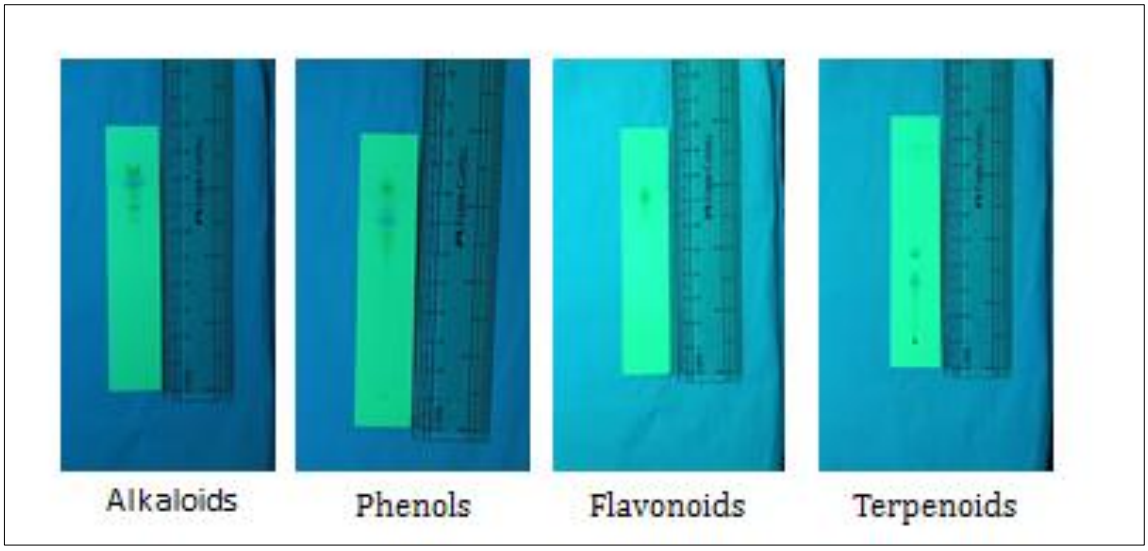


**Figure 1.** TLC profile visualized under UV light (254 nm)

Figure 1 Thin-layer chromatography profile of the chloroform extract of *F. vulgare* Mill. leaves observed under ultraviolet light at 254 nm. Quenching spots indicate the presence of UV-absorbing compounds, and the distances traveled by the spots and the solvent front were recorded for retention factor (Rf) determination.

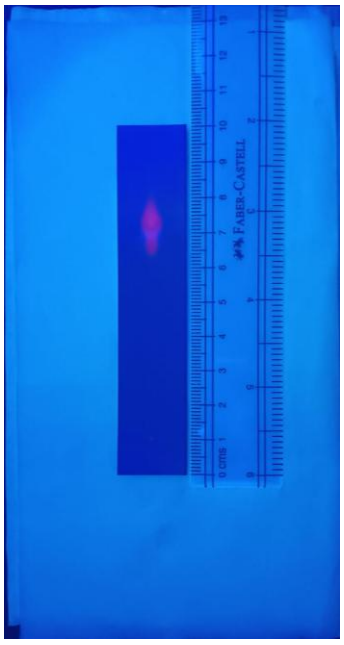

Figure 2. TLC profile of the chloroform extract of *Foeniculum vulgare* Mill. leaves after exposure to ammonia vapor under UV 366 nm

Figure 2 Thin-layer chromatography profile of the chloroform extract of *Foeniculum vulgare* Mill. leaves observed under ultraviolet light at 366 nm after exposure to ammonia vapor. Yellow to yellow-green fluorescent spots indicate the presence of flavonoid compounds. The distances traveled by the spots and the solvent front were measured for the determination of retention factor (Rf) values.

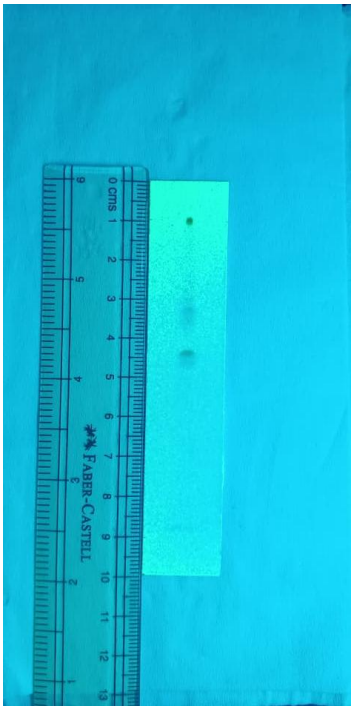


Figure 3. TLC profile after spraying with Liebermann–Burchard reagent

Figure 3 Thin-layer chromatography profile of the chloroform extract of *F. vulgare* Mill. leaves after spraying with Liebermann–Burchard reagent. Visualization with Liebermann–Burchard reagent produced red to reddish-brown spots commonly associated with terpenoid compounds, and spot migration distances were used for retention factor (Rf) calculation.


Figure 4. TLC profile after spraying with ferric chloride ($FeCl_3$) reagent

Figure 4 Thin-layer chromatography profile of the chloroform extract of *F. vulgare* Mill. leaves after spraying with ferric chloride ($FeCl_3$) reagent. Dark-colored spots indicate the presence of phenolic compounds, and retention factor (Rf) values were determined based on measured migration distances.

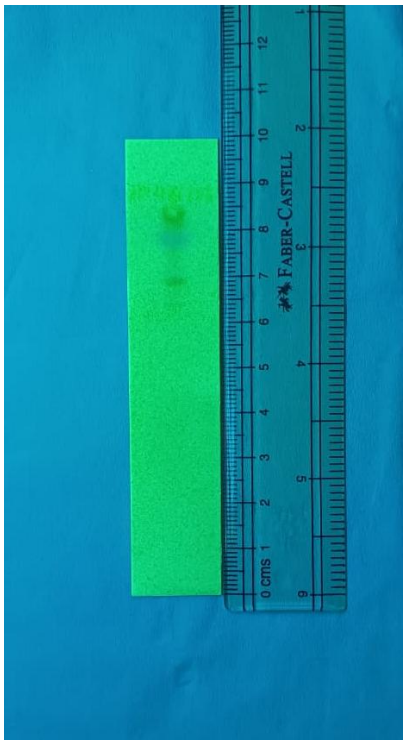


Figure 5. TLC profile after spraying with Dragendorff reagent

Figure 5 Thin-layer chromatography profile of the chloroform extract of *F. vulgare* Mill. leaves after spraying with Dragendorff reagent. Orange to brown spots indicate the presence of alkaloid compounds, and the distances traveled by the spots and the solvent front were used to calculate retention factor (Rf) values.

Quantitative assessment was conducted by measuring the distances traveled by detected spots and the solvent front, from which the retention factor values were calculated. The resulting Rf values varied among the observed spots, indicating differences in chromatographic behavior of compounds present in extract. Complete set of Rf values, along with corresponding detection reagents and visual characteristics of each spot, is summarized in Table 1.

Table 1. Results of secondary metabolite analysis of *Foeniculum vulgare* Mill. leaves using TLC

| No. | Spray Reagent Used | Nilai Rf | Warna yang dihasilkan | Metabolite Group |
|---|---|---|---|---|
| 1. | Lieberman-Burchard reagent | 0.91 | Red to reddish brown | Terpenoids |
| 2. | $FeCl_3$ | 0.44 | Dark blue to black | Phenols |
| 3. | Dragendorff reagent | - | Orange to brownish orange | Alkaloids |
| 4. | Ammonia vapor (UV 366 nm) | 0.79 | Yellow fluorescence | Flavonoids |

Based on the thin-layer chromatography analysis, the chloroform extract of *F. vulgare* Mill. leaves exhibited distinct chromatographic spots with characteristic color reactions after visualization using different spray reagents. Visualization with Liebermann–Burchard reagent produced red to reddish-brown spots with a high Rf value, indicating the presence of terpenoid compounds. Ferric chloride reagent generated dark blue to black coloration at a moderate Rf value, suggesting phenolic constituents. Alkaloid compounds were indicated by the appearance of orange to brownish-orange spots following Dragendorff reagent application, although the spots were less sharply defined. Additionally, exposure to ammonia vapor and observation under ultraviolet light at 366 nm revealed yellow fluorescent spots characteristic of flavonoid compounds. Overall, the observed chromatographic profiles and Rf values confirm the presence of multiple groups of secondary metabolites in the chloroform extract, providing a phytochemical basis for further investigation of its biological potential.

## Discussion

In this study, the qualitative and semi-quantitative thin-layer chromatography analysis of the chloroform extract of *Foeniculum vulgare* Mill. leaves revealed the presence of several major secondary metabolite groups, including terpenoids, phenolic compounds, alkaloids, and flavonoids. The identification of these groups is consistent with reports that such metabolites often contribute to biological effects relevant to parasitic infections. Secondary metabolites, particularly flavonoids and terpenoids, have been reported to exhibit antiplasmodial properties in *in vivo* models by reducing parasitemia in experimental animals infected with *Plasmodium berghei* and other rodent malaria parasites. For example, various plant fractions rich in flavonoids and alkaloids significantly reduced parasitemia and increased mean survival time in *P. berghei*-infected mice, indicating a dose-dependent antiplasmodial effect of these compounds (Pimenta, *et al*., 2024).

Mechanistically, flavonoids are known to exert antiplasmodial effects through multiple pathways, including interference with fatty acid biosynthesis within the parasite apicoplast and modulation of host immune responses, which can contribute to suppression of parasite growth and reduction of parasitemia levels (Babylon, *et al*., 2025). Terpenoids, particularly sesquiterpenoids such as artemisinin, represent another class of plant metabolites that have demonstrated potent antimalarial activity both *in vitro* and *in vivo* by generating free radicals that damage parasitic biomolecules and inhibit vital metabolic processes (Sankhuan, *et al*., 2022). Although compounds such as artemisinin are specific examples, the general presence of terpenoid structures in the chloroform extract suggests a plausible contribution to antiparasitic activity via similar biochemical interactions.

Furthermore, phenolic compounds and alkaloids detected in the extract may synergistically support antiplasmodial outcomes observed in other studies by acting as antioxidant agents and affecting parasite survival through oxidative stress modulation, which can indirectly influence parasitemia progression (Ahmed, *et al*., 2021). The combined presence of these metabolite classes in the chloroform extract of *F. vulgare* leaves provides a phytochemical basis for future research to evaluate their influence on parasitemia in *in vivo* malaria models, potentially through suppression of parasite replication, enhancement of host defense mechanisms, or both.

Overall, the integration of chromatographic profiling with known antiplasmodial activities of secondary metabolites underscores the relevance of phytochemical characterization as a foundational step in exploring natural product-based antiparasitic agents and guides subsequent biological investigations in parasitology-oriented research. Previous phytochemical studies on *F. vulgare* have consistently reported the presence of flavonoids, phenolic compounds, terpenoids, alkaloids, and other secondary metabolites in various plant parts, including leaves. Similar findings have been described for ethanolic and non-polar extracts of *F. vulgare*, indicating that the phytochemical profile observed in the present study aligns with earlier reports. Several national studies have demonstrated that flavonoids and phenolic compounds are among the dominant metabolites in fennel leaf extracts and are associated with antioxidant and protective biological activities, which may indirectly influence host–parasite interactions during parasitic infections (Amelia, *et al.*, 2024).

The coexistence of multiple metabolite groups within a single extract suggests the possibility of synergistic interactions that enhance biological effects. Previous investigations have highlighted that combinations of flavonoids, terpenoids, and phenolic compounds often produce stronger biological responses than isolated compounds, particularly through modulation of oxidative stress, immune responses, and cellular homeostasis. These mechanisms are relevant to parasitemia control *in vivo*, as oxidative balance and immune regulation play critical roles in limiting parasite proliferation within the host bloodstream (Rajčević, *et al*., 2022).

From a mechanistic perspective, flavonoids have been reported to interfere with essential metabolic pathways of malaria parasites, including inhibition of fatty acid biosynthesis within the apicoplast and modulation of redox signaling. Such actions may suppress parasite growth and contribute to reduced parasitemia levels observed in experimental models.

Terpenoids, on the other hand, are known to exert antiparasitic effects through induction of oxidative stress and disruption of parasite metabolic processes. Although this study did not identify individual compounds, the detection of these metabolite groups by thin-layer chromatography provides a plausible biochemical explanation for the biological relevance of the extract in parasitology-oriented research (Riaz, *et al.,* 2023).

Previous research conducted by Ihtiaringtyas (2025), demonstrated that secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds, present in the alga *Nostoc commune* were capable of reducing *Trypanosoma evansi* parasitemia level in an *in vivo* model. These findings emphasize the important role of specific classes of secondary metabolites in inhibiting the development of blood parasites through diverse biological mechanisms. The similarity in secondary metabolite profiles between *N. commune* and the chloroform extract of fennel leaves (*F. vulgare* Mill.), particularly with respect to alkaloids, flavonoids, terpenoids, and phenolic compounds, suggests a comparable biological potential. Consequently, the presence of similar metabolite groups in fennel leaves provides a scientific rationale for further investigations aimed at exploring their potential as natural antiparasitic resources.

Despite this promising phytochemical similarity, the present study has several limitations. The phytochemical analysis was restricted to qualitative and semi-quantitative characterization using thin-layer chromatography, without further structural elucidation or compound isolation. In addition, direct *in vivo* evaluation of parasitemia reduction was not performed within this study. Variations in metabolite composition due to environmental factors, plant origin, and extraction conditions may also influence the reproducibility of results. Therefore, further studies integrating chromatographic profiling with standardized *in vivo* parasitemia assays and advanced analytical techniques are necessary to clarify the specific roles of individual metabolites and their contribution to antiparasitic activity.

## 4. CONCLUSION

This study demonstrates that the chloroform extract of fennel leaves (*F. vulgare* Mill.) contains several major groups of secondary metabolites, including flavonoids, terpenoids, alkaloids, and phenolic compounds, as identified through thin-layer chromatography based on spot characteristics and retention factor values. The phytochemical profile obtained provides preliminary insight into the complexity of bioactive constituents that may contribute to antiparasitic-related biological effects. The presence of metabolite groups similar to those reported in other natural sources with documented *in vivo* parasitemia-reducing activity highlights the biological relevance of fennel leaf extract in parasitology-oriented research. Although direct antiparasitic evaluation was not conducted in this study, the findings establish a scientific basis for further investigations involving compound isolation, structural characterization, and standardized *in vivo* parasitemia assays to elucidate the specific contributions of individual metabolites.

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