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Microscopic Evaluation and Qualitative Phytochemical Screening of Leaves and Fruits of *Lycium ferocissimum* Miers.

Beste Atli [1,*], Sayeh Ghahramani[1], Ezgi Ak-Sakalli[1], Nesrin Oztinen[1], Muberra Kosar[1]

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus via Mersin 10, Türkiye.

Abstract

Lycium ferocissimum Miers. is a plant species belonging to the Solanaceae family. This study aimed to investigate the microscopic structure and phytochemical composition of the leaf, green fruit, and red fruit of *L. ferrocissimum*. Plant materials were oven-dried at 50 °C, powdered, and prepared for microscopic analysis using chloral hydrate. Microscopy revealed stomata, epidermis, crystals, and secretory hairs in leaves; and parenchyma, pigment cells, and stone cells in fruits. Phytochemical screening was conducted using standard biochemical reactions for primary and secondary metabolites. Alkaloids were confirmed by Dragendorff's test, producing a reddish-brown precipitate. Carbohydrates were detected using Fehling, Molisch, and Seliwanoff tests, all giving positive results. Flavonoid glycosides were identified via Cyanidin test; red fruit gave a strong orange color and zinc confirmed the presence of flavonoid. Lipid detection using Sudan III resulted in orange spots in both red and green fruits. Carotenoids were identified by pH-dependent color changes. No saponins were detected.

Keywords

Lycium ferocissimum, phytochemical analysis, microscopy, flavonoids, medicinal plant

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***Corresponding author:** Beste Atli **email:** beste.atli@emu.edu.tr

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INTRODUCTION

The genus *Lycium*, belonging to the Solanaceae family, grows in temperate and subtropical climatic regions. Approximately 80 species of the genus have been identified globally. *Lycium* species are distributed across South America, South Africa, Europe, and Asia (Yao et al., 2011). The genus holds significant importance in Traditional Chinese Medicine. In particular, two species, *Lycium barbarum* and *Lycium chinense*, have been widely used for centuries as traditional medicinal plants in China, with *L. barbarum* being commonly cultivated there. The parts of *Lycium* species that are used both as food and medicine are primarily the fruits. In addition, the leaves and root bark are also utilized, and in some cases, the whole plant is used. The fruits are typically consumed fresh or dried, while the fresh leaves are either cooked as food or brewed as tea. Among the most frequently reported species in the literature are *L. barbarum*, *L. chinense*, and *Lycium ruthenicum* from China; *Lycium europaeum*, *Lycium intricatum*, and *Lycium shawii* from the Mediterranean and the Middle East; *Lycium pallidum* from North America; and *Lycium afrum* from Africa (Yao et al., 2018). The traditional uses of *Lycium* species among the public include the treatment of eye diseases, cough (Lev and Amar, 2006), digestive and gastric inflammations (Trillo et al., 2010), headache, rheumatism, kidney disorders (Dhar et al., 2011), toothache, and chickenpox (Yao, 2018).

The traditional uses of *Lycium* species have attracted the attention of many researchers. Numerous phytochemical studies have been conducted on these species. These studies have revealed that *Lycium* species contain polysaccharides, lipids, terpenes, and phenolic compounds, with a particular focus on the types and effects of polysaccharides (Jiang et al., 2021). The chemical constituents reported in *Lycium* species include glycerogalactolipids, phenylpropanoids, coumarins, lignans, flavonoids, amides, alkaloids, anthraquinones, organic acids, terpenoids, sterols, steroids, and their derivatives (Qian et al., 2017). Phytochemical studies on *Lycium* species have identified various chemical constituents, including alkaloids, cyclopeptides, lignans, anthraquinones, coumarins, flavonoids, terpenoids, sterols, and others (Yang et al., 2018). In *L. barbarum* fruits, derivatives of luteolin such as luteolin-7-*O*-glucuronide, luteolin-7-*O*-glucoside, apigenin including apigenin-7-*O*-glucoside and acacetin have been detected (Zhang et al., 2019). The fruits also contain quercetin diglycoside, rutin, kaempferol-*O*-rutinoside, and

phenolic acids such as chlorogenic, caffeoylquinic, and caffeic acids (Wu et al., 2012). In a study carried out on the root bark and leaves of *L. barbarum*, N-trans-caffeoylphenethylamine, N-trans-caffeoyltryptamine, and N-trans-feruloylphenethylamine were identified in the root bark and it was reported that the leaves contained N-trans-caffeoyltyramine and N-trans-feruloylphenethylamine (Wang et al., 2018). Compounds isolated from *L. chinense* include acacetin, apigenin, kaempferide, caffeic acid, luteolin, and vanillic acid, along with flavonoids such as kaempferol and isoscopoletin (Chen et al., 2016). An 80% ethanol extract from the leaves and stems of *L. chinense* also revealed several phenolic compounds, including gallic acid, catechin, chlorogenic acid, and rutin (Liu et al., 2017).

MATERIALS AND METHODS

Plant Material and Extraction

Mature (unripe) and ripe fruits, as well as leaf samples of *Lycium ferocissimum*, were collected from the Salamis region of Northern Cyprus, Famagusta. The collected plant materials were dried, ground into powder, and extracted with 80% ethanol using a shaker at room temperature. Additionally, 5% aqueous decoctions were prepared from each plant part. The resulting extracts were concentrated using a rotary evaporator, water was removed with freeze drying method and stored at −18°C until further analysis. The extracts were labeled as follows: GFE for mature fruit 80% ethanol extract, GFD for mature fruit water extract (decoction), RFE for ripe fruit 80% ethanol extract, RFD for ripe fruit water extract (decoction), LE for leaf 80% ethanol extract, and LD for leaf water extract (decoction).

Microscopic Evaluation of *Lycium ferocissimum* Miers.

For microscopic analysis, leaf, green fruit, and red fruit samples were dried in an oven at 50°C. After one day of drying, each sample was separately ground using a mortar and pestle. The ground samples were then transferred to three microscope slides using needles, and a few drops of chloral hydrate were added as a reagent to enhance clarity for microscopic observation. A cover slip was placed on top of each specimen. The microscope slides were then heated using a Bunsen burner to fix the samples. The slides were then examined under the microscope one by one to observe and document the particulate structures.

Phytochemical Screening of *Lycium ferocissimum* Miers.

Test for Alkaloids

A 60% hydrochloric acid ethanol solution (in 6% HCl, 20 mL) was prepared, and 0.5

g of dried leaves and 1.8 g of fruits were added into the solution. The mixture was then boiled and filtered. For the alkaloid extraction, a liquid-liquid extraction method was employed. First, a basic solution (10% ammonium hydroxide) was added, and the pH was checked using litmus paper. The alkaloids were then extracted from the aqueous layer by adding 10 mL chloroform. The resulting extraction solution was treated with 10% acetic acid, 10 mL of water, and 1.5 mL of acetic acid. To separate the acetic acid from the upper layer, the bottom layer was discarded. Dragendorff's reagent was subsequently added to test for alkaloids. The presence of alkaloids was indicated by the formation of an orange or reddish-brown color precipitation.

Test for Carbohydrates

Seven grams of the plant sample were weighed and finely crushed using a mortar and pestle, after which the powder was transferred into a beaker. Forty milliliters of distilled water were added to the crushed plant material in the beaker and stirred for 5 minutes. The aqueous portion of the suspension was then filtered. To the filtrate, 3.5 mL of 10% lead acetate solution was added dropwise, and the mixture was re-filtered. This step resulted in the precipitation and separation of compounds such as chlorophyll, flavonoids, and tannins from the aqueous extract. Subsequently, 4 mL of 2.5% disodium hydrogen phosphate solution was added dropwise to the filtrate, and the solution was filtered once more. The obtained extract was used for the subsequent determination tests.

Fehling's Test: One milliliter of the extract was placed in a test tube, followed by the addition of 2 mL of Fehling A solution and 2 mL of Fehling B solution. The mixture was heated using a Bunsen burner. The formation of a red-colored precipitate (Cu_2O) indicates the presence of carbohydrates.

Molisch's Test: To 1 mL of the extract, 5 drops of 5% alcoholic α-naphthol solution were added in a test tube. The tube was slightly tilted, and concentrated sulfuric acid was carefully added down the side of the tube. The formation of a violet-purple ring confirms the presence of carbohydrates.

Seliwanoff's Test: To 1 mL of the extract, 2.5 mL of Seliwanoff reagent was added in a test tube. The solution was then heated to boiling. The presence of a ketose is indicated by the formation of a red color, while aldoses produce a delayed reaction and form a light red color. Saccharides such as pentoses result in a blue-green coloration.

Test for Carotenoids

The soluble components of the plant sample were extracted by dissolving the material in ethanol or methanol. If the sample was in

solid form, it was ground using a mortar and pestle or subjected to ultrasonic treatment to accelerate the extraction process. The resulting extract was divided into three equal portions and transferred into separate test tubes. To the first and second test tube, 1% hydrochloric acid (HCl) and 1% sodium hydroxide (NaOH) was added, respectively. The third test tube was left untreated and served as a control. Solutions containing anthocyanins exhibited a red or pink coloration under acidic conditions (HCl) and a green or blue coloration under basic conditions (NaOH). In instances where carotenoids were present and oxidized into anthocyanin-like compounds, similar color changes were also observed.

Test for Flavonoids

Two grams each of red and green fruits were accurately weighed and subsequently soaked to facilitate the extraction of soluble compounds. The samples were then homogenized with 5 mL of ethanol to ensure the efficient dissolution of target metabolites. The resulting mixture was filtered to separate the solid residues from the liquid extract. To isolate the flavonoid components, ethyl acetate was added to the filtrate using a separation funnel, allowing for phase separation. The upper organic layer, enriched with flavonoids, was collected and evaporated under controlled conditions to obtain a concentrated residue. The residue was subsequently re-dissolved in 2–3 drops of distilled water for further analysis. A solvent mixture of methanol and water (1:1:1) was prepared and added to the solution, followed by the introduction of a small quantity of magnesium or zinc powder. Color change observed.

Test for Lipids

Sudan III solution was used as a reagent to visualize lipids and plant fats. To prepare the solution, 0.25 g of Sudan III powder was weighed, and 35 mL of ethanol along with 15 mL of water were added to a beaker. The mixture was stirred thoroughly and heated until a homogeneous solution was obtained. Subsequently, 1 g of dried powder from red and green leaves was weighed and transferred into separate test tubes. To each tube, 10 mL of hexane was added, and the mixtures were stirred using a magnetic stirrer for 5–10 minutes. Following stirring, the mixtures were filtered, and the resulting filtrates were subjected to gentle heating. A portion of each filtered solution was then applied onto individual filter papers, which were left to dry completely. Once dried, the filter papers were sprayed with the prepared Sudan III solution for lipid detection.

Test for Saponins

Foam Test: Approximately 0.5 g of the powdered plant material was placed into a test tube containing 10 mL of hot water. After allowing the mixture to cool to room temperature, it was shaken vigorously for about 10 seconds. The formation of a stable

foam layer measuring between 1 to 10 cm in height, which persists for at least 10 minutes and remains unaffected by the addition of 1–2 drops of 2N HCl, indicates the presence of saponins.

RESULTS

Microscopic Evaluation of *Lycium ferocissimum* Miers

The powdered leaf sample was prepared using chloral hydrate as a mounting reagent. Microscopic examination revealed the presence of various anatomical structures, including stoma, epidermis, calcium oxalate crystals, covering hair and secretory hair (Figure 1).

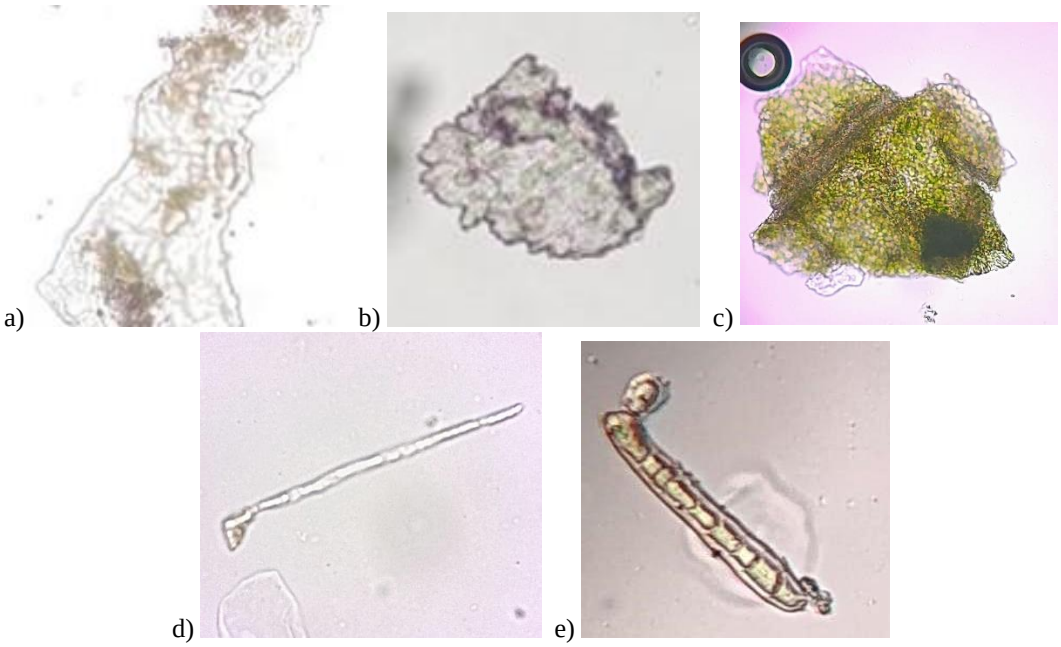

Figure 1: Microscopic images of *L. ferocissimum* leaves (a: stoma, b: epidermis, c: calcium oxalate crystals, d: covering hair, e: secretory hair).

Microscopic examination of the green fruit revealed the presence of various anatomical structures, including parenchyma, stone cells, pigment cells and exocarp (Figure 2).

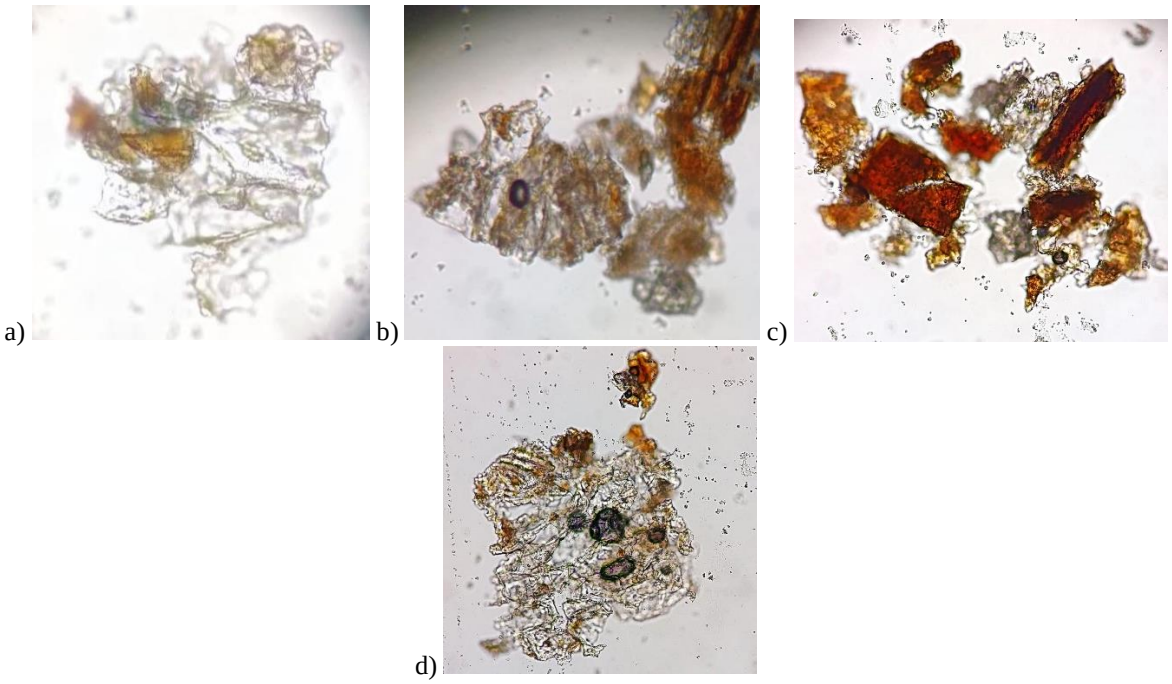

Figure 2. Microscopic images of *L. ferocissimum* green fruits (a: parenchyma, b: stone cells, c: pigment cells, d: exocarp).

Various anatomical structures, including endocarp, stone cells and exocarp were observed as a result of microsvopic examination of red fruit samples (Figure 3).

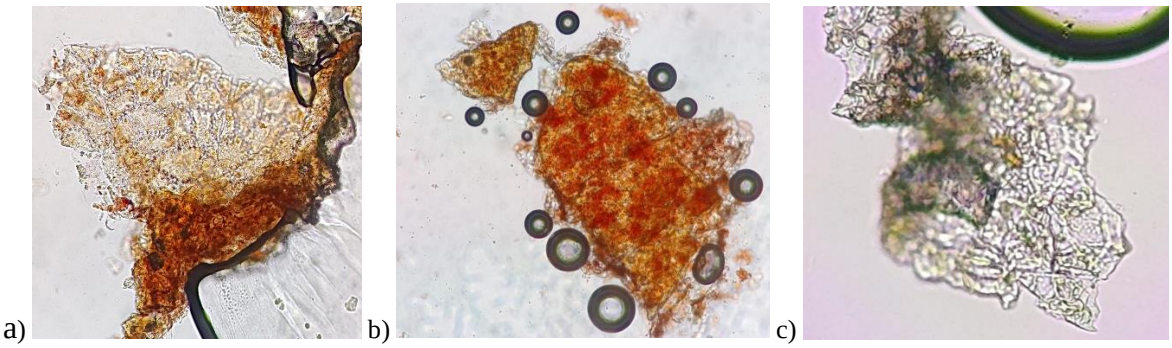

Figure 3. Microscopic images of *L. ferocissimum* red fruits (a: endocarp, b: stone cells, c: exocarp)

Phytochemical Screening of *Lycium ferocissimum* Miers

Phytochemical screening was conducted to identify novel sources of bioactive compounds with potential therapeutic and industrial applications, particularly those derived from medicinal plants. Both primary and secondary metabolites were qualitatively analyzed. The phytochemical analysis demonstrated the presence of several active constituents, including alkaloids, carbohydrates, carotenoids, flavonoids, and lipids (Table 1).

Table 1: Qualitative phytochemical screening of *L. ferocissimum* leaves, green fruits, and red fruits.

Primary/Secondary metabolites	Leaf	Green Fruit	Red Fruit	Name of tests
Alkaloids	+	+	+	Precipitation test (Dragendorff reagent)
Carbohydrates	+	+	+	Fehling test, Molisch test, Seliwanoff test
Carotenoids	ND*	+	+	Anthocyanin test
Flavonoids	ND*	+	+	Cyanidin test
Lipids	ND*	+	+	Sudan III test
Saponins	-	-	-	Foam test

*ND: Not determined

DISCUSSION

In the present study including microscopic examination of the red fruits of *Lycium barbarum*, anatomical structures such as the epidermis, stomata, hypodermis, mesocarp, cuticle, endocarp, and endosperm were investigated (Konarska, 2018). In another study conducted on 11 *Lycium* species, leaf analysis was performed using microscopy. The examined anatomical features included calcium oxalate crystals, glandular and non-glandular trichomes, palisade parenchyma, spongy parenchyma, stomata, and epidermal cells (Joubert et al., 1984). A comparative anatomical investigation of *Lycium europaeum*, *Lycium shawii*, and *Lycium schweinfurthii* var. *aschersonii* focused on several structural features, including the adaxial and abaxial epidermal layers, type of mesophyll, presence of crystals, the number of palisade and spongy parenchyma layers, and the organization of the vascular system (Ragab et al., 2023).

In our previous study with *L. ferocissimum*, phenolic acids and flavonoids were detected from leaves and fruits (Kosar et al., 2024). In another study investigating the leaf and stem extracts of *Lycium ruthenicum*, the presence of 69 compounds, including steroids, terpenes, fatty acids, esters, phenolics, aldehydes, furans, and pyridine derivatives were indicated (Kumar et al., 2022). Studies conducted on *Lycium* species have identified the presence of various phytochemicals, including alkaloids, flavonoids, carotenoids, polysaccharides, terpenes, lignans, coumarins, and anthraquinones (Yao et al., 2011). Because, only a limited number of microscopic and phytochemical investigations have been carried out on this species, such analyses are crucial for accurate identification and characterization of plant taxa. Further comprehensive studies are required to uncover additional properties of *L. ferocissimum*.

CONCLUSION

In conclusion, the present study provides insight into the characteristics and phytochemical profile of *L. ferocissimum* leaves, green fruits, and red fruits. Future research focusing on the pharmacognostic analysis of the leaves may establish specific diagnostic criteria for precise identification of the species.

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