

Phytochemical, Anatomical, and Histochemical Investigation of *Felicia abyssinica*: A Chemical Analysis of Tissues in Relation to Their Structural Organization

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ABSTRACT

The genus *Felicia*, family Asteraceae, is used in South African folk medicine. The Zulu and Xhosa healers prescribed the aqueous extract of *Felicia* species to treat headaches, fever, stomach diseases, and catarrh due to its analgesic, anthelmintic, antibacterial, antifungal, and anti-inflammatory properties. This study examines *Felicia abyssinica* aerial parts to disclose the chemical nature of its secondary metabolites as well as their histochemical localization. The freshly prepared extracts were qualitatively examined to detect phytochemical constituents using different chemical reagents. Selected plant sections and powdered parts were investigated using a light microscope. The transverse sections of stems and leaves were stained with chemical reagents specific for detecting various phytoconstituents. We detected high amounts of nitrogenous compounds, flavonoids, phenolic compounds, steroids, and low quantities of carbohydrates, glycosides, mucilage, and saponins. The leaves showed anomocytic and anisocytic stomata, one layer of epidermis, dorsiventral mesophyll, and a collateral vascular bundle. The multicellular, uniseriate, non-glandular trichomes with tapered apical cells are characteristic of the leaves. The stem shows a multicellular uniseriate stalk with multicellular head glandular trichomes. In the leaves, nitrogenous compounds were localized in the mesophyll, phenolics, and lignins in the xylem vessels, while flavonoids in the epidermis and xylem. However, in the stem, nitrogenous compounds, flavonoids, and lignins were found in hypodermis; and the area of vascular bundles, including xylem vessels and fibers. This study for the first time, investigates the anatomy of *F. abyssinica* L aerial parts and also correlates the phytochemical analysis with the structural organization of *F. abyssinica* tissues.

Keywords: *Felicia abyssinica*, Anatomy, Histochemical localization, Phenolic compounds, Nitrogenous compounds.

1. INTRODUCTION

The family Asteraceae (Compositae) comprises 1500–1900 genera and 20,000–35,000 species¹. It is considered one of the largest medicinally important families, with members that have both economic and therapeutic benefits.

Felicia is a genus of small annual or perennial herbaceous plants with 85 known species², which contain different classes of phytoconstituents, such as volatile oils

containing compounds such as limonene, myrcene, α -Pinene, *trans*-ocimene α -terpineol, linalool, methyl eugenol, eugenol, *trans*-farnesene, and farnesol. This genus also has acetylenic compounds for instance matricaria methyl ester and 5-dihydrofuran. Some species as well contain phenolic compounds, iso-coumarin derivatives, and terpenoids especially sesquiterpenes such as germacrene D, and farnesene. Besides cytotoxic activities, *Felicia* spp. exhibit anti-inflammatory, antinociceptive, antipyretic, antioxidant, anthelmintic, antibacterial, and antifungal properties^{3,4,5,6,7,8}.

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The genus *Felicia* was used in traditional medicine to treat pain, fever, headache, inflammation as well as stomach diseases, and constipation. *Felicia erigeroides* DC extracts were administered as enemas for intestinal parasites, and abdominal pains, and also as purgatives.⁹

Felicia abyssinica L. is a perennial herb, which grows 5–50 cm tall and forms a woody rootstock along with a woody stem base. Its leaves are alternate, dense, dark green, and linear to narrowly lanceolate. Its capitulum is solitary, with ray florets \pm 25. The rays are pale pink to pale mauve to white, linear, 5–10 mm long, 1.5 mm wide, with numerous disk florets which are \pm 3 mm long^{7,10}.

Previous studies were concerned with the growth of *F. abyssinica* in arid conditions. Several studies examined *F. abyssinica* growth performance and physiological changes in response to various levels of drought stress. It was found that the plant had various drought-related adaptation mechanisms including the increase in root biomass and drought circumstances, higher root-to-shoot ratio, smaller leaves, and higher antioxidant levels indicating its adaption to dry environments^{11,12,13}.

The anatomical characteristics, phytochemical, and histochemical investigation of *F. abyssinica* have not been studied so far. Therefore, the present study aimed to investigate the anatomy of *F. abyssinica* aerial parts. In addition, we have identified the classes of the secondary metabolites present in the different tissues of *F. abyssinica* to explore a new area for new potential therapeutic agents. Moreover, the histochemical investigation was conducted to locate the phytoconstituents in the different tissues. Finally, we present a correlation between the chemical analysis of tissues and their structural organization, besides providing a detailed description of the stem and leaves of *F. abyssinica*.

2. MATERIAL AND METHODS

Plant material:

F. abyssinica L. (F. Asteraceae) aerial parts in the flowering stage were collected from Al Baha, Southwest Saudi Arabia, at a height of 7000 feet in March 2017. The plant material was kindly identified by Prof. Dr. A.A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen is kept as a reference in the Herbarium of the Faculty of Science, Assiut University, Assiut, Egypt which is alphabetically ordered.

Solvents for general purposes:

The solvents used for extraction and phytochemical investigations (Ethanol, dichloromethane, xylene, and glycerol) (El-Nasr Company for Pharmaceutical Chemicals, Egypt)

Chemicals and reagents:

Dragendorff's reagent is prepared according to Ciulci (1994). (Mixing a concentrated solution of potassium iodide with a solution of bismuth subnitrate in diluted tartaric acid). (Piochem Company for laboratory chemicals, Egypt).

Conc. H₂SO₄, Conc. HCl, Lead acetate, Sodium hydrogen phosphate, Picric acid, Sodium hydroxide, Ammonium hydroxide, Ruthenium red, Copper sulfate, Potassium sodium tartrate, Ferric chloride, Paraffin wax, Canada balsam, Clove oil, Toluidine blue, Chloral hydrate, Glycerin

gelatin, Phloroglucinol, Aluminum chloride (Piochem Company for laboratory chemicals, Egypt).

2.1 Phytochemical screening

The air-dried powdered *F. abyssinica* L. aerial parts (2.5 kg) was exhaustively extracted with 70 % ethanol. The total ethanolic extract obtained after evaporation (250 gm) was kept for further investigations. Part of the prepared aerial parts ethanolic extract (5 gm) was qualitatively examined using chemical reagents specific for detecting the phytochemical constituents, such as alkaloids, carbohydrates, flavonoids, glycosides, tannins, steroids, and saponins, by identifying characteristic color changes using standard procedures^{14,15,16}.

Alkaloids: Dragendorff's Test

In a test tube, 2-3 drops of Dragendorff's reagent were added to 1 ml of the extract, orange precipitate indicated the presence of alkaloids¹⁵.

Terpenoids: Salkowski Test

In a test tube, 5 ml of extract was mixed with 2 ml of dichloromethane, and then 3 ml of Conc H₂SO₄ was added to form a layer an aqueous layer. A reddish-brown coloration forms at the interface indicating the presence of terpenoids¹⁶.

Anthraquinone glycosides: Modified Borntrager's test

Combined forms: Add 3 ml of ethanolic extract to 1 mL of Conc. HCl. After filtration, 5 mL of the dichloromethane is added, and the solution is stirred. After decantation, the organic solvent is evaporated to dryness. Diluted Ammonia (2ml) was added to the residue. A yellow coloration, which develops and turns red after heating in the water bath, indicates the presence of the combined forms of Anthraquinone glycosides¹⁶.

Cardiac Glycosides: Baljet's test

The extract (3ml) is added to 3 ml lead acetate and then filtered, Sodium hydrogen phosphate (3ml) is added then filtration, and this step is repeated until the filtrate becomes colorless. Baljet's reagent (2ml picric acid+ 2ml sodium hydroxide) is added¹⁷.

Saponins: Froth Test

The extract (5 ml) was shaken with 2 ml of distilled water. If froth is produced and persists for ten minutes this indicates the presence of saponins¹⁶.

Tannins: FeCl₃ Test

The extract (4ml) was treated with 4 ml FeCl₃, the formation of brownish brownish-greenish or a bluish-black color indicates the presence of tannins¹⁶.

Flavonoids: Alkaline (NaOH)

The extract was mixed with NaOH. yellow-orange color indicates the presence of flavonoids compared to the blank¹⁷.

Phenols: FeCl₃ Test

The extract was mixed with 2 ml of 2% solution of FeCl₃. A blue-green or black coloration indicates the presence of phenols¹⁶.

Coumarins: UV Fluorescence with Ammonia

Evaporate ethanolic solution (5 ml), dissolve the residue in 1-2 ml of hot distilled water, and divide the volume into two parts. Take the first volume as a negative control. The second volume was added to 0.5 ml 10% NH₄OH. Put two

spots on filter paper and examine under UV light. Intense blue fluorescence indicates the presence of coumarins¹⁷.

Reducing sugars: Fehling

The ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (A and B) Fehling A (copper sulfate in water), Fehling B (potassium sodium tartrate in sodium hydroxide solution) in a test tube was added too. The formation of a precipitate brick-red in the bottom of the tube indicates the presence of reducing sugars¹⁶.

Test for mucilage: Ruthenium red

Ethanol extract (2ml) was mixed with 2ml of 0.1% ruthenium red, the red color indicates the presence of mucilage¹⁷.

2.2 Anatomical studies

2.2.1 Preparation of transverse sections of *F. abyssinica* stems and leaves

To soften the dry plant material, it was soaked in a solution of glycerol, 96% ethanol, and distilled water (1:1:1v/v) for 24 hrs. till it was steadily softened¹⁸.

In conjunction with the anatomical investigation (plant at the flowering stage), 0.5 cm of leaf specimens were taken from the midrib of the middle part of the leaf. Additionally, 0.5 cm of stem specimens were also taken from the middle part of the internodes of the main stem, washed gently with sterile water, and dehydrated in a series of increasing concentrations of ethanol (50%, 70%, 80%, 90%, and 100%), cleared in ethanol: xylene (3:1, 1:1, 1:3, and 100% xylene), and embedded in paraffin wax (52°C–54°C melting points).

Sections 10–15 µm thick were prepared using a rotary microtome, stained with toluidine blue to detect lignified cell walls to be able to study the plant tissues¹⁸, cleared in clove oil, and mounted in Canada balsam. The selected sections were investigated under a light microscope (Olympus CX41, Philippines) equipped with a digital camera (TUCSEN, USB2, H Series).

2.2.2 Preparation of powdered leaves stems, and inflorescences

The dried aerial parts were crushed in a grinder, and placed at the center of the slide, and 1–2 drops of chloral hydrate solution were added. Phloroglucinol/concentrated hydrochloric acid was added to some slides to detect lignified elements. The sample on the slide was mixed well, covered with a cover glass, and examined under a light microscope at magnification powers of 40×, 100×, and 400×.

2.3 Histochemical studies

2.3.1 Preparation of transverse sections of *F. abyssinica* stems and leaves

Transverse sections of stems and leaves were sliced with a rotary microtome, placed on a slide, and stained with different reagents corresponding to various secondary metabolites¹⁹.

2.3.2 Staining procedures

The prepared sections, 10–15 µm thick, were subjected to the following procedures¹⁹:

A. Alkaloids: Sections were treated with Dragendorff's reagent for 20 min, rinsed with 5% sodium nitrite, and mounted in distilled water.

B. Phenolic compounds: Sections were treated with 10% ferric chloride for 30 min, washed twice with distilled water to remove surplus reagent, and mounted in glycerin gelatin.

C. Lignin: Sections were treated with 10% phloroglucinol for 15 min and mounted carefully with 25% hydrochloric acid for 2 min.

D. Flavonoids: Sections were treated with 5% aluminum chloride for 20 min, washed twice in distilled water to remove the surplus stain, and mounted in glycerin gelatin.

3. RESULTS

3.1 Phytochemical screening

To detect different active constituents in the plant, we used different reagents in ethanolic extracts. Tests and results of the phytochemical investigation are shown in Table 1.

Table 1: Phytochemical screening of the powdered aerial parts of *Felicia abyssinica* L.

No	Chemical constituent	Test	Results
1	Alkaloids	Dragendorff	++
2	Terpenoids	Salkowski	++
3	Anthraquinone glycosides	Modified Borntrager	-(a)
4	Cardiac glycosides	Baljet	-
5	Saponins	Froth	+(b)
6	Tannins	Ferric Chloride	+
7	Flavonoids	Alkaline (NaOH)	++(c)
8	Phenols	Ferric Chloride	++
9	Coumarins	UV Fluorescence with Ammonia	+
10	Reducing sugars	Fehling	+
11	Mucilage	Ruthenium Red	+

(a) Absent; (b) Present in low concentration; (c) Present in high concentration.

3.2 Anatomy

3.2.1 Leaf anatomy

The transverse sections of leaves showed one layer of polygonal upper epidermal cells. The mesophyll was dorsiventral, represented by elongated rectangular palisade cells with straight walls and polygonal spongy cells with wavy walls. The vascular bundles were open collateral. The midrib contained one vascular bundle and 3–4 bundles were observed around two large vacuoles on both sides (Fig. 1a and b). The lower epidermis was represented by one layer of polygonal cells that appeared to have wavy walls in the surface view.

3.2.2 Stem anatomy

The stem had a circular or oval contour with some ribs (Fig. S1). The epidermis was represented by one layer of rectangular cells covered with a thin smooth cuticle showing a multicellular uniseriate stalk and a multicellular head glandular trichome (Fig. 1). The hypodermis was represented by one layer of collenchyma cells followed by 3–4 layers of parenchyma cells, and many layers of fibers. The open collateral vascular bundles formed a ring in the middle region. Sometimes, vascular bundles appeared in the cortex area of the stem due to secondary growth (Fig. S1 and 1c). Phloem and xylem elements were differentiated, while the cambium cells were not distinguishable. The pith was wide and composed of round or oval parenchymatic cells (Fig. S1 and 1c).

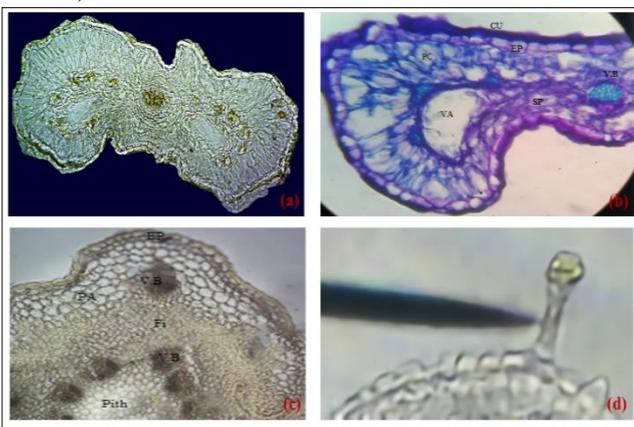


Figure 1: Transverse sections of *Felicia abyssinica* leaf and stem (a, c, e: 100×; B, D: 400×) **a.** Transverse section of a leaf stained with Aluminum chloride. **b.** Part of the transverse section of a leaf stained with toluidine blue (TB) shows one vascular bundle in the midrib and 3–4 vascular bundles around one of the large vacuoles on one side. **c.** A part of the transverse section of the stem. **d.** A part of the stem epidermis showing a multicellular uniseriate stalk and a multicellular head glandular trichome. CU, cuticle; Fi, fiber; EP, epidermis; PC, palisade tissue; PA, parenchyma; SP, spongy tissue; VB, vascular bundle; Pi, pith.

3.2.3 Examination of leaves stems, and inflorescences in powdered form

Leaves: The leaf was amphistomatic. The powdered leaves showed anomocytic stomata with 4–5 subsidiary cells (Fig. 2a and 2b) and anisocytic stomata with 3–4 subsidiary cells (Fig. 2c). The lower epidermal cells showed more stomata than the upper layer. The upper epidermal cells were polygonal with straight thick walls, sometimes beaded, while the lower epidermal cells were polygonal with wavy thick walls. Both were covered by a striated cuticle (Fig. 2a, b, and c). Two types of non-glandular trichomes were observed: unicellular trichomes with warty cuticle (Fig. 2d) and uniseriate multicellular trichomes with warty cuticle and tapered apical cells (Fig. 2e).

Stems: The powdered stem showed glandular trichomes with an uniseriate multicellular stalk and a multicellular head (Fig. S2a). The top views of glandular trichomes were abundant along the longitudinal section of the stem (Fig. S2b). Fibers were short with a wide lumen (Fig. S2c). The vessels were bordered and pitted (Fig. S2d).

Inflorescences: The powdered inflorescence showed a stigma that was papillosed with rectangular cells having

straight walls (Fig.S3a). The pollen grains were spherical with three germinal pores and had a spiny exine (Fig.S3b and c).

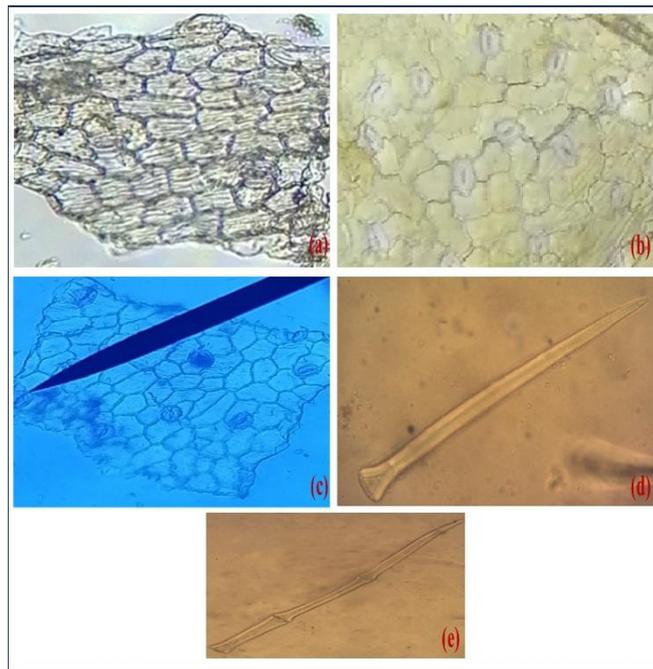


Figure 2: Photos of powdered *Felicia abyssinica* leaves. **a.** Upper epidermal cells showing a striated cuticle (100×). **b.** Lower epidermal cells (400×). **c.** Upper epidermis with anisocytic stomata (100×). **d.** Unicellular trichome (400×). **e.** Uniseriate multicellular non-glandular trichome with warty cuticle with tapered apical cell (100×).

3.3 Histochemistry

Leaves: The alkaloids and/or nitrogenous compounds were present in the mesophyll of the leaf as a reddish-brown content (Fig. 3a). Phenolic compounds and lignins were localized in vessels of the leaf (Fig. 3b), (Fig. S3). Flavonoids were located in the epidermis and the vessels of the leaf as appeared in the transverse section of the leaf (Fig. 3c).

Stems: Dragendorff's reagent revealed an abundance of alkaloids and/or nitrogenous compounds in the hypodermis and the area of vascular bundles, including the xylem vessels and fibers, in the *F. abyssinica* stem (Fig.4a).

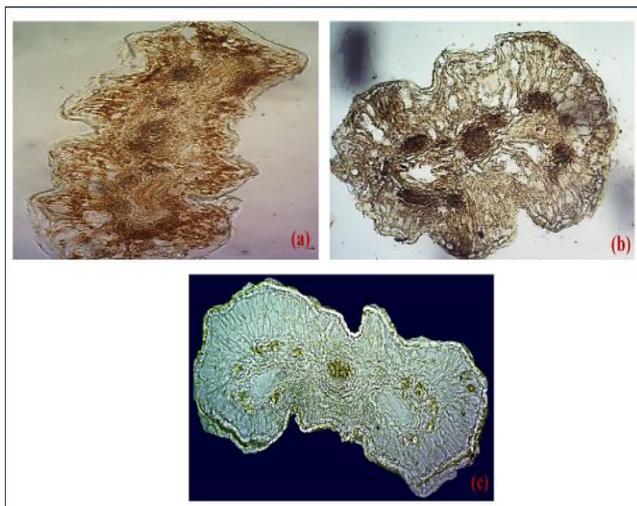


Figure 3: Transverse section of *Felicia abyssinica* L. leaf stained with different reagents **a.** Dragendorff's reagent. **b.** Ferric chloride (100×). **c.** Aluminum chloride (100×).

Phloroglucinol/hydrochloric acid stained the lignified elements of the stem hypodermis, fibers, and xylem vessels with red color (Fig.4b). Flavonoids were observed as a distinct yellow layer in the hypodermis and the area of vascular bundles, including the xylem vessels and fibers (Fig.4c).

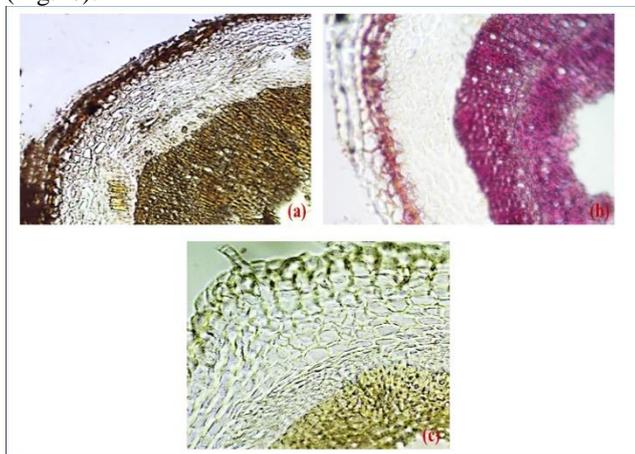


Figure 4: Transverse section of *Felicia abyssinica* L. stem stained with different reagents **a.** Dragendorff's reagent (100x), **b.** Phloroglucinol /HCl (100x), **c.** Aluminum Chloride (100x).

4. DISCUSSION

Although the Asteraceae family is very large, most members display the same anatomical features. In this study, we showed that *F. abyssinica* L. also exhibits the characteristic features of this family: anomocytic (predominantly) and anisocytic stomata²⁰, single epidermal layer, and collateral vascular bundle²¹. Leaves of plants belonging to Asteraceae usually exhibit a mesophyll differentiated into palisade and spongy parenchyma²².

The key characteristics of *F. abyssinica* powdered aerial parts include multicellular, uniseriate non-glandular trichomes with tapered apical cells, which are also found in the *Calea* genus²³. Uniseriate multicellular stalk, multicellular head glandular trichome, and polygonal epidermal cells showing anomocytic and anisocytic stomata were observed.

Phloroglucinol/hydrochloric acid and TB staining revealed a high density of lignin in the xylem vessels of the leaf and the hypodermis, fibers, and xylem vessels of the stem (Fig. 1b and 3b).

The presence of flavonoids and phenolic compounds has been reported in the family Asteraceae²⁴. Flavonoids have been detected in epidermal cells^{25,26} and as a secretion of glandular trichomes²⁷. In this study, aluminum chloride staining revealed flavonoids in the leaf epidermal cells and vessels and in the stem in the hypodermis and area of vascular bundles (Fig. 3c and 4c).

The type and quantity of secondary metabolites produced by a plant are affected by many factors, such as species, genotype, physiology, developmental stage, and environmental factors during growth²⁸. We could identify the types of secondary metabolites that aid *F. abyssinica* L. to adapt to drastic conditions of hot climates and high altitudes.

In the present study, the plant *F. abyssinica* was collected from high-altitude regions with sandy and arid soil. The plants that grow in this drastic environment employ many strategies to tolerate these habitats, which manifest as morphological, anatomical, and phytochemical alterations²⁹. Morphologically, the *F. abyssinica* stem is elastic and shrubby to resist the wind found in this environment with a deep root to allow it to reach water from deeper soil layers. *F. abyssinica* has a short life allowing it to grow and produce seeds before the dry season. The seeds lie dormant until the rainy season begins when they will germinate and grow. The leaf is fleshy, which may be due to the high content of mucilage, so it can store a high volume of water³⁰ and is small in size to reduce excessive transpiration.

Anatomically, both the leaf and stem are covered with thick cuticles to decrease the loss of water. Plants produce secondary metabolites as a defense mechanism against drastic conditions such as drought³¹. Phytochemically, *F. abyssinica* contains flavonoids and polyphenolic compounds that help overcome oxidative stress in response to drought through their antioxidative properties³².

We were able to localize and demonstrate sites of accumulation and production of some secondary metabolites in the plant tissues. The phytochemical screening and the histochemical localization that we demonstrated helped enlarge our pharmacognostic knowledge about this species.

5. CONCLUSION

This study represents the first phytochemical, anatomical, and histochemical investigation of the aerial parts of *F. abyssinica* L. Our findings are consistent with the reported data on plants from the Asteraceae family. Phytochemical screening of *F. abyssinica* aerial parts revealed the presence of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, coumarins, mucilage, and reducing sugars. Anthraquinone glycosides, free anthraquinone, and cardiac glycosides are absent. Anatomically, *F. abyssinica* powdered leaves showed anomocytic (predominantly) and anisocytic stomata, one layer of the epidermis, dorsiventral mesophyll, and a collateral vascular bundle. The multicellular, uniseriate non-glandular trichomes with tapered apical cells are characteristic of *F. abyssinica* leaves. The stem shows a uniseriate multicellular stalk and multicellular head glandular trichomes. All these anatomical features are in agreement with the characteristic features of the family Asteraceae. Histochemical localization of different secondary metabolites showed the presence of alkaloids in the hypodermis, fibers, and xylem vessels of the stem vascular bundles as well as the mesophyll of the leaf. Phenolic compounds are localized in the vessels of the leaf. The lignified elements of the stem are located in the hypodermis and area of the vascular bundles, while it was found in the xylem vessels of the leaf. Flavonoids were observed as a distinct yellow layer in the hypodermis and the area of the vascular bundles of the stem, the epidermis, and the vessels of the leaf. This study provides a tool for the identification of *F. abyssinica* aerial parts in their entire and powdered forms. Moreover, it sheds

light on the chemical nature and histochemical localization of the phytochemical constituents of this plant, hence, the possible predicted biological activities that can explain the traditional uses of plants from genus *Felicia*. This study opens the way for further investigation of this species, for studying phytochemical profiling, isolation, and structural elucidation of its phytoconstituents, besides examination of their biological activities.

COMPETING INTERESTS:

The authors declare no conflict of interest.

FUNDING:

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