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Phytochemical Profiling Coupled with Morpho-Anatomical Characterization of *Abutilon theophrasti* Medik. in Iraq

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ABSTRACT

This study aimed to investigate the morphological, anatomical, and phytochemical characteristics of *Abutilon theophrasti* Medik. (Malvaceae) to support its taxonomic identification and evaluate its bioactive compound profile. Morphological characteristics were examined through macroscopic observations, while permanent anatomical sections of the leaf and stem were prepared to study epidermal features, cortical organization, vascular tissues, and trichome distribution. The existence of calcium oxalate crystals in parenchyma cells was noted. The major phenolic compounds in the plant extract were identified and quantified by high-performance liquid chromatography (HPLC) as phytochemical analysis. To validate the results, an external standard method was utilized for calibration and quantification. Pseudostratified epidermis as well as dense glandular and non-glandular trichomes and calcium oxalate crystals with identification also reveal remarkable diagnostic significance. The HPLC analysis revealed the presence of gallic (95.9 ppm), rutin (85.4 ppm) and quercetin (82.9 ppm) acids alongside caffeic, syringic, apigenin and ferulic acids in the sample. These studies, which combined morphological, anatomical and phytochemical data support the proposed recognition of the genus A. and its placement within family Malvaceae. Further studies will need to examine potential biological activity underlying its medicinal use.

1. INTRODUCTION

The family Malvaceae is cosmopolitan in distribution and contains greater than 243 genera with more than 4,200 species distributed mostly in tropical and subtropical regions [1]. This family is of great economic and medicinal significance, containing many major crop plants (cotton Gossypium spp. Many genera of importance for medicinal and ornamental crops including Malva, Hibiscus and Althaea. Abutilon is one of the largest genera of Malvaceae, which encompasses more than 150 species of herbaceous and woody perennials and small trees, most common in tropical and subtropical regions [2]. Members of this genus have typical floral and fruit features that are characteristic of the family Malvaceae, and they have a long history in traditional herbalism.

The plant *Abutilon theophrasti* Medik (velvetleaf or Abutilon) is an annual or perennial herb which has height 0.5–2.5 m during the growing season, but over this species mainly depends on environmental factors, e.g., light intensity. The species is native to Asia but has widely naturalized in many areas of the world including Iraq [3]. *Abutilon theophrasti* is known as a global invasive and ecologically adaptable species, which has drawn considerable attention in ecological and pharmaceutical studies. *A. theophrasti* is recognized as a source of various bioactive phytochemical constituents including acts against phenolic compounds, flavonoids, terpenoids, anthocyanins and fatty acids contributing its biological activity to some extent [4, 5]. Roots had traditionally been used as an expectorant and antipyretic agent, while the leaves and flowers are used for treating abscesses and ulcers. Besides, the fruits are also consumed and used as animal feed. The species was also shown to be resistant to extreme environments, reflecting ecological hardiness in addition to economic importance [6]. Despite its wide distribution and the well-known medicinal properties of *A. theophrasti*, little scientific attention has been given to this relevant species in Iraq other than that focusing on nutritional aspects, including morphological, anatomical and chemical characterization.

In that regard, this paper aims to (a) document the diagnostic morphological and anatomical characteristics of *A. theophrasti* in Iraq; (b) determine and quantify some phenolic compounds in their air parts using high-performance liquid chromatography (HPLC); and (c) correlate putative structural characters observed with their possible ecological adaptations as well as chemotaxonomic significance.

2. MATERIALS AND METHODS

2.1 Material voucher specimen and collection information

Two entries for *Abutilon theophrasti* Medik. (Malvaceae) from roadside locations along sidewalk edges in Baghdad, Iraq

during the 2025 growing season. Plant samples were identified using standard taxonomic keys and authenticating relevant floristic references. Voucher specimen was deposited at Herbarium of the University of Baghdad, Baghdad, Iraq (voucher no.: 76664). The 5 healthy ripe adults were isolated. Typical plant parts (leaves, stems, roots and flowers) were collected from each of the plants for further morphological, anatomical and phytochemical analysis. A composite sample (from pooled aerial parts of the collected individuals) was used for phytochemical analysis. Plant specimens were frozen using storage at −40℃ until for analyses.

2.2 Methods

2.2.1 Morphological analysis

Plant organ morphological features were observed with a stereomicroscope (Leica M60 Stereo Microscope, Germany). They analyzed external features, such as leaf shape and arrangement; flower type; and fruit morphology. A similar coloration study of several plant segments refers to classification rules in modern botany [7].

2.2.2 Anatomical analysis

For the anatomical studies, small pieces of leaves and stems (2–3 cm long) were removed. These pieces were then placed directly into 30 mL glass vials containing a fixative solution [8] prepared according to the protocol described previously. The fixative was a mix of 90 mL of either 70% or 50% ethanol, 5 mL formol and (5 mL) glacial acetic acid. This fixative is commonly used in laboratory. Afterward, samples were washed in 70% ethanol to remove any excess of fixative and stored at 4℃ in 70% ethanol until sectioning. Transverse sections of leaves and stems were prepared from dried organs following the paraffin technique [9], which includes dehydration, clearing, infiltration of paraffin, and embedding. Tissue separation was achieved by staining sections with a Safranin-Fix Green solution, using standardized protocols [9] established for studies of plant anatomy. The stained sections were mounted on glass slides and permanently with Canada balsam. For the anatomical observations (n = 10), the samples were observed using a compound light microscope (40×) coupled with digital camera for images. Observations and recordings were made for structural features such as epidermal type, vascular bundles arrangement, collenchyma, sclerenchyma and other tissues. These observations were further confirmed by using an ocular micrometer.

2.2.3 Extraction of plant samples

To extract the plant materials, 3 g of powdered samples were continuously stirred with 12 mL of chloroform for 8 h at room temperature. Then, 15 min of ultrasonication was applied to the extract then added 100 mL of butanol and transferred into a separatory funnel. The written instructions in bullet format are: The polar organic phase (butanol layer) was collected, and concentrated to dryness under reduced pression using a rotary evaporator. This extraction method was repeated three times, and enough extract was isolated for further analysis [10].

2.2.4 High-performance liquid chromatography analysis

They were measured by HPLC SYKAM (Germany). Separation of each sample was performed on a C18-ODS column (25 cm × 4.6 mm). The mobile phase was composed of 70:25:5 (v/v/v) methanol, distilled water and formic acid with a flow rate of 1.0 mL/min. Detection was performed using a UV detector set at 280 nm. Gallic acid, ferulic acid, rutin, apigenin, syringic acid, caffeic acid, and quercetin standards (≥ 98% purity) were purchased from Sigma-Aldrich (Germany). Standard stock solutions were prepared in methanol and stored at 4℃ until use. Phenolic compounds were quantified using the external standard method. Concentration, in plant extract per compound was calculated as:

$$C_{sample} = \left(\frac{C_{st} \times Area_{sample}}{Area_{st}}\right) \times \left(\frac{DF}{wt}\right)$$

where, C_{sample} (mg/kg DW) represents the concentration in the sample, C_{st} is the concentration of the standard solution, $Area_{sample}$ and $Area_{st}$ are the chromatography peak areas for analyzed sample and standard respectively, DF is the dilution factor, wt is dry weight of plant material for extraction. Identification of the compounds was validated through retention times comparison with authentic standards.

Calibration and Linearity

Sharpness of the curve was evaluated for seven phenolic standards (gallic acid, ferulic acid, rutin, apigenin, syringic acid, quercetin and caffeic acid) at five concentration levels from 3 to 12 µg/mL.

Peak determination of the developed method Each concentration was sampled in triplet and peak area is then plotted against that concentration. The external standard method was used for linear regression analysis.

All analytes displayed very good linearity of relation across this investigated range, with correlation coefficients (R^2) > 0.99, indicating a very good proportional relationship between concentration and detector response.

Method Validation

The R^2 values were >0.99 for all phenolic standards, demonstrating that the method was appropriate for quantification. International Conference on Harmonisation (ICH) guidelines were used to validate the analytical method.

Linearity

All investigated compounds were linear over the 3–12 µg/mL concentration range.

Sensitivity (LOD and LOQ)

The experimental limit of detection (LOD) and limit of quantification (LOQ) were determined as 2 ppm and 6.6 ppm, respectively. These results suggest that the developed method has satisfactory sensitivity for phenolic compound determination. The validation parameters yielded substantiate that the developed procedure is simple, accurate, precise and reliable for the HPCL estimation of quercetin.

3. RESULTS AND DISCUSSION

3.1 Morphological characteristics

The general morphology of *Abutilon theophrasti* is shown in Figure 1(A). The leaves are green, the adaxial surface darker than the abaxial surface. They are large and pubescent with a soft, downy texture and emit a faint odor. The lamina is cordate with an acute apex and crenate margins. Leaf length

ranges from 10–15 cm and width from 9–11 cm, with divergent palmately reticulate venation. As illustrated in Figures 1(B) and (C), the midrib is more prominent on the abaxial surface than on the adaxial surface. The petiole is cylindrical, green, and densely hairy, measuring 2–15 cm in length and 0.5–1.0 cm in diameter. The stem is cylindrical, green, and erect, bearing numerous trichomes. It is branched and gives rise to multiple lateral branches. The results also revealed that the root system is of the taproot type, with numerous lateral roots. The root is brown, cylindrical, and measures 7–10 cm in length and 1–2 cm in diameter (Figure 1(D)). As revealed by the morphological characterization, the flowers are simple, small, solitary, yellow, axillary, and odorless, measuring 2.0–2.5 cm in length and 2–4 cm in diameter (Figure 1(E)) They are bisexual and actinomorphic, exhibiting radial symmetry and pentamerous organization (Figure 1(F)). The anthophore is cylindrical, green, and pubescent, measuring 0.7–2.0 cm in length and 0.2–1.5 cm in diameter. The corolla consists of five free, bright yellow, cubical-shaped petals, each measuring 1–2 cm in length and 2–4 cm in width (Figure 1(G)). The calyx is composed of five green, pentagonal sepals that are fused (Figure 1(H)). The androecium comprises stamens that are united into a single bundle, forming a yellow staminal column measuring 1–2 cm in length and bearing yellow anthers approximately 1–1.5 mm long. The anthers contain pollen sacs with pollen grains. The gynoecium is superior and syncarpous, consisting of ten fused carpels, each containing 1–4 ovules arranged along two axes. The style connects the ovary to the stigma and measures approximately 0.5–0.7 cm in length.

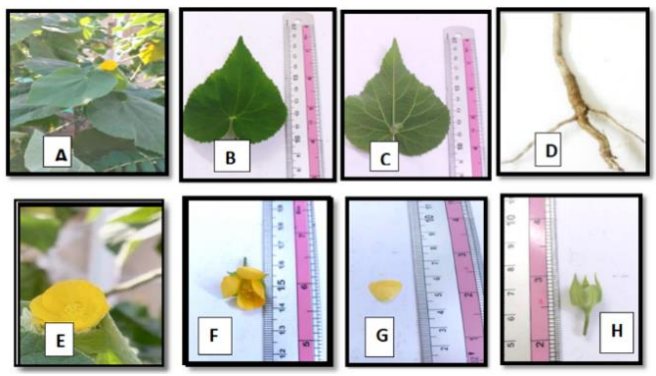


Figure 1. Morphological characteristics of *Abutilon theophrasti* Medik. (A) General habit of the plant in its natural habitat, (B) Upper (adaxial) surface of the leaf, (C) Lower (abaxial) surface of the leaf showing venation pattern, (D) External morphology of the root system, (E) Complete flower, (F) Corolla, (G) Petal, (H) Calyx

3.2 Anatomical characteristics of the leaf

The anatomical investigation revealed that the upper epidermis consists of a single layer of tightly packed, non-intercellular cells with a regular morphology, ranging from rectangular to cuboidal in shape. Both glandular and non-glandular trichome bases were observed. The thickness of the upper epidermis was approximately 14.7 µm. In contrast, the lower epidermis was thinner, measuring 8–12 µm in thickness, and was covered by a cuticle approximately 4 µm thick. Stomata were predominantly round and surrounded by 3–5 subsidiary cells (Figures 2(A) and 2(B)). Glandular trichomes were observed as uniseriate structures with distinct stalks and heads, both of which were covered by a cuticle. Branched glandular trichomes (Figures 2(C-D)) were predominant over most parts of the leaf, in agreement with previous observations [11]. Glandular trichomes occurred in two forms: unicellular and multicellular, each bearing a single head. In addition, long non-glandular trichomes with thick, multicellular walls composed of 2–14 cells were present. The epidermal surface was also densely covered with various types of elongated, needle-like trichomes. The mesophyll region was composed of fundamental tissues. It consisted of two layers of elongated palisade parenchyma cells rich in plastids and continuous with the midrib and a spongy parenchyma made up of 4–5 layers of loosely arranged, rounded cells containing green plastids. Abundant calcium oxalate crystals were observed within the vacuoles of parenchymal cells (Figures 2(E-H)). These findings are summarized in Table 1 and are consistent with previously reported studies [12].

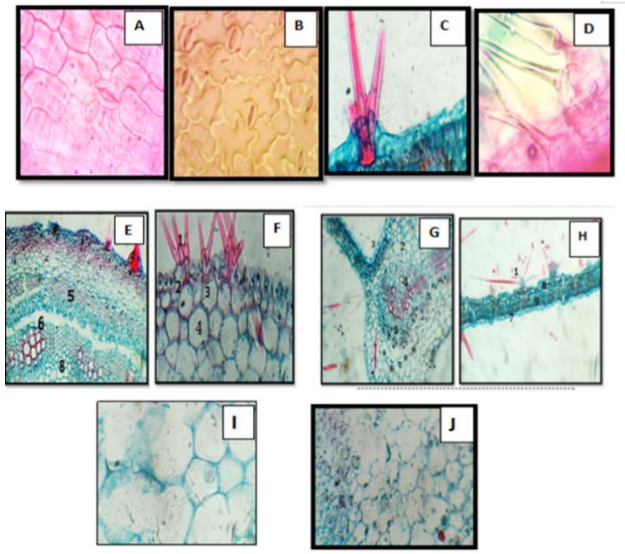


Figure 2. The anatomical features of the stem of *Abutilon theophrasti.* (A & B) Stomata on the surface of the upper and lower epidermis, (C & D) Morphological variations in the forms of glandular and non-glandular trichomes, (E & F) Cross-section in the stem, where 1) non glandular hair, 2) epidermis, 3) collenchyma, 4) parenchyma, 5) pericycle fibers, 6) xylem vessels, 7) phloem, 8) parenchyma (40×), (G & H) Cross-section in the leaf, where 1) cuticle and glandular and non-glandular trichomes, 2) parenchyma, 3) collenchyma, 4) xylem vessels, 5) cambium phloem, 6) parenchyma, 7) palisade cell, 8) spongy tissue, 9) lower epidermis (40×); (I & J) Calcium oxalate 10× and 40×, respectively

3.3 Anatomical characteristics of the stem

The stem cross-section was approximately circular in outline. The epidermis consisted of a single layer of closely packed cells without intercellular spaces and bore both glandular and non-glandular trichomes [13], as shown in Figures 2(E-F). The cortex was relatively narrow and composed of parenchymal cells with polyhedral walls. The vascular tissue was arranged in 22–30 semicircular vascular bundles with varying sizes, as clearly shown in Figure 2(E). The pericycle is made of 5–6 layers of lignified cells and the pith consist of vast, polygonal, thin-walled parenchymatous cells many of which were hollow and contained calcium

oxalate crystals (Figures 2(I) and (J)). Secretory structures (that contained plant mucilage), consistent with previous findings [14], were also noticed. The anatomically significant features listed in Table 2 provide not only structural support and protective functions, but may contribute to the capacity of this plant to produce and sequester bioactive compounds.

Table 1. Calibration and validation parameters of phenolic standards

Compound	Retention Time (min)	Linear Range (µg/mL)	Regression Equation	R^2	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	2.7	3–12	Y = 250.31494X	0.9998	2	6.6
Rutin	4.2	3–12	Y = 397.96429X	0.9999	2	6.6
Apigenin	5.9	3–12	Y = 334.57143X	0.9999	2	6.6
Quercetin	7.8	3–12	Y = 311.25714X	0.9997	2	6.6
Caffeic acid	9.9	3–12	Y = 334.37987X	0.9995	2	6.6

LOD: limit of detection, LOQ: limit of quantification.

Table 2. Quantitative anatomical measurements of the leaf of *Abutilon theophrasti* (µm)

Leaf Structure	Length (µm) (n = 10)	Width (µm) (n = 10)	Thickness (µm)	Diameter (µm)
Cuticle	-	-	4.0	-
Upper epidermis	56 ± 2.10	42.6 ± 2.10	14.7 ± 0.80	-
Lower epidermis	45 ± 2.22	40 ± 3.17	12.8 ± 0.20	-
Stomata	35 ± 2.10	29 ± 2.15	-	-
Nonglandular trichomes	150 ± 9.12	40 ± 2.19	-	-
glandular trichomes stalk	10 ± 0.80	55 ± 6.17	-	70-80
glandular trichom-head	21 ± 0.16	20 ± 0.20		
Palsied Layer	-	-	110 ± 7.39	-
Spongy layer	-	-	68 ± 8.10	-
Xylem	75 ± 20.17	80.5 ± 5.10	-	-
Phloem	55 ± 4.44	120 ± 9.10	-	-

Values are expressed as mean ± Standard deviation; Dash (-): parameters not applicable or not measured. All the measurements are presented in micrometers (µm).

Table 3. Quantitative anatomical measurement of the stem of *Abutilon theophrasti* (µm)

Stem Structure	Length (µm) (n = 10)	Width (µm) (n = 10)	Thickness (µm) (n = 10)
Cuticle	-	-	6.5 ± 2.5
Epidermis	55 ± 5.17	45 ± 2.22	-
Non-glandular trichomes	140 ± 22.12	80 ± 6.13	-
Glandular hair-stalk	45 ± 5.70	45 ± 1.12	-
Glandular hair-head	70 ± 4.20	50 ± 2.01	
Collenchyma tissue	-	-	60 ± 3.30
Parenchyma tissue	-	-	80 ± 7.11
Cortex	-	-	135 ± 17.90
Phloem and cambium thickness	-	-	65 ± 3.70
Xylem vessels	97.5 ± 15.18	250 ± 10.45	-

Values are expressed as mean ± Standard deviation; Dash (-): parameters not applicable or not measured; measurements were taken from transverse stem sections and expressed in micrometers (µm).

3.4 Chemical composition and biological activity

The rich profile of phenolic acids and flavonoids with significant biological activities that has been found, with *A. theophrasti* cultivated under Iraqi agricultural conditions, helped illustrate this plant as a source of biologically active products. These marked compounds suggest that *A. theophrasti* contains numerous phytochemicals of possible medicinal interest as shown in Table 3. The main phenolic compound is gallic acid (95.9 ppm) which probably plays an important role in the extract antioxidant activity. In accordance with previous data on the phenolic-rich extracts from plant sources [9], this extract was noticed for its strong radical scavenging utility, coupled to its protective usefulness in oxidative stress-related diseases. They were also abundant in rutin (85.4 ppm) and quercetin (82.9 ppm) as the compounds associated with antioxidant capability. In addition to this, these flavonoids have been reported to promote lipid peroxidation [15] and alter inflammatory responses while protecting cellular components against damage by reactive oxygen species [16, 17]. Its high levels of quercetin and its glycosides (also known as quercitrin, rutin) can justify its contribution to the overall antioxidant activity, when compared with other polyphenolics [18-20]. Other bioactive compounds were detected in moderate quantities, including ferulic acid (58.7 ppm), apigenin (63.0 ppm) and syringic and caffeic acids; their characteristic HPLC retention times help us to identify them as shown in Table 4 with known antibacterial, antiviral, neuroprotective and antioxidant activities [21].

The broad range of phenolic acids and flavonoids indicates that the biological activity of *A. theophrasti* is not due to a single compound [22] but rather results from multiple activities by these secondary metabolites acting in concert. The active constituents in *A. theophrasti* are rationalized with its traditional uses in folk medicine as a source of an anti-inflammatory [23], medicinally important [24] and

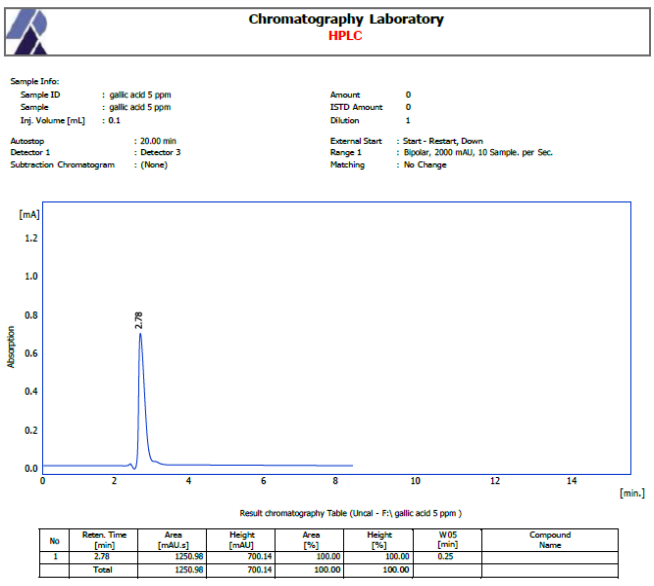
antimicrobial agent. The therapeutic interest over this species is also supported by similar profiles of phytochemicals demonstrated on other phenolic rich medicinal plants. This indicates that *A. theophrasti* is a bioactive metals production goldmine, which will contribute its value in pharmacotherapeutics and antioxidant activity [24]. These findings confirm that plant biological activities seem to be due not only or primarily to a single key compound but rather to the synergic interaction of phenolic and flavonoid constituents [25].

Table 4. Concentrations of some phenolic and flavonoid compounds identified in the *Abutilon theophrasti* plant extract as determined by high-performance liquid chromatography (HPLC)

Compound	Concentration (mg/kg DW)
Gallic acid	95.9
Ferulic acid	58.7
Rutin	85.4
Apigenin	63.0
Syringeic acid	67.7
Quercetin	82.9
Caffeic acid	77.7

The current study findings regarding anatomical and phytochemical characteristics of *Abutilon theophrasti* are broadly consistent with several previous works on other Abutilon species [26, 27]. Similar studies on Abutilon indicum and Abutilon pannosum have also allowed for the comparison of key diagnostic features (e.g., presence of glandular and non-glandular trichomes) in both taxa which are crucially important to herbivore defence and tolerance to environmental stress. In addition, there are other species in the Malvaceae with similar epidermal structures and a high density of indumentum; these features have also been noted as important taxonomically [28, 29]. The presence of calcium oxalate crystals in the parenchymatous tissues of the present samples has been observed in other Abutilon species; these crystals are also viewed as adaptive for ion regulation and structural protection.

Overall, these results substantiate the taxonomic and medicinal significance of *Abutilon theophrasti*.



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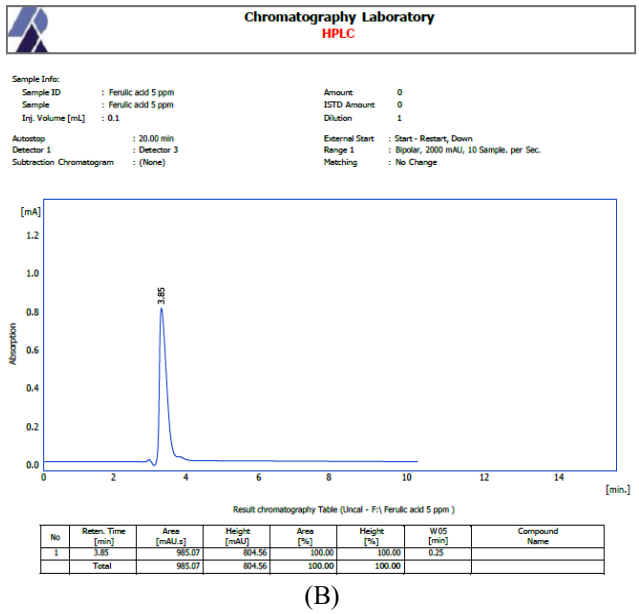

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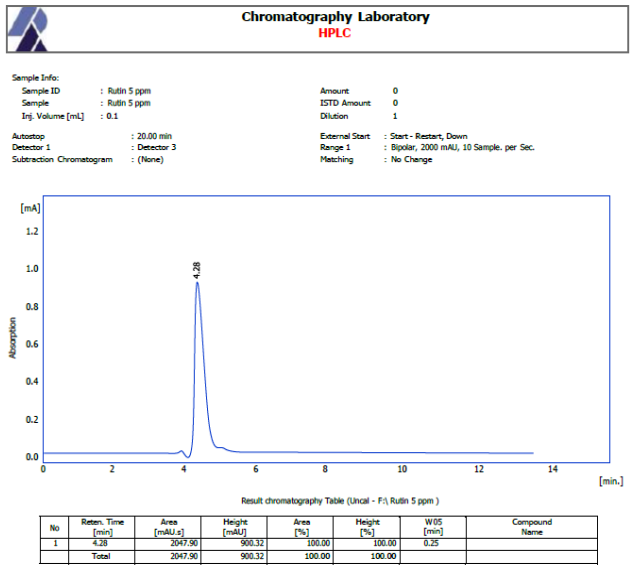

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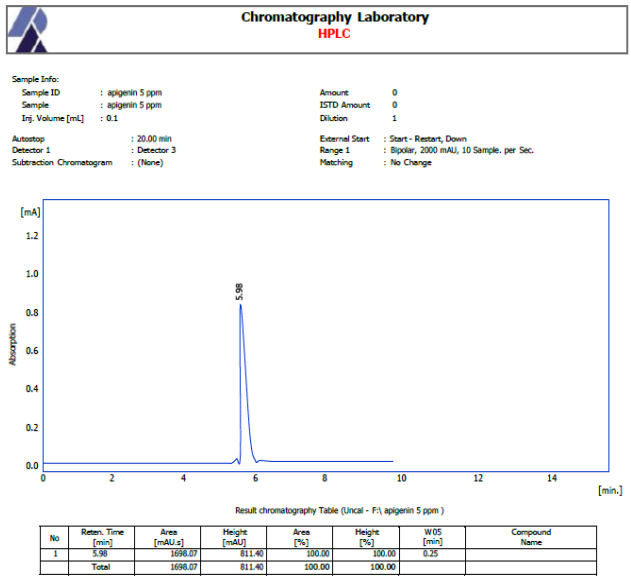
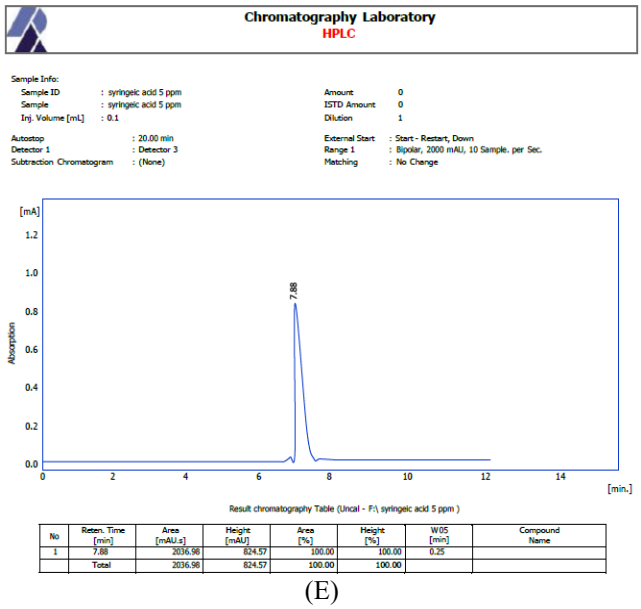


(D)



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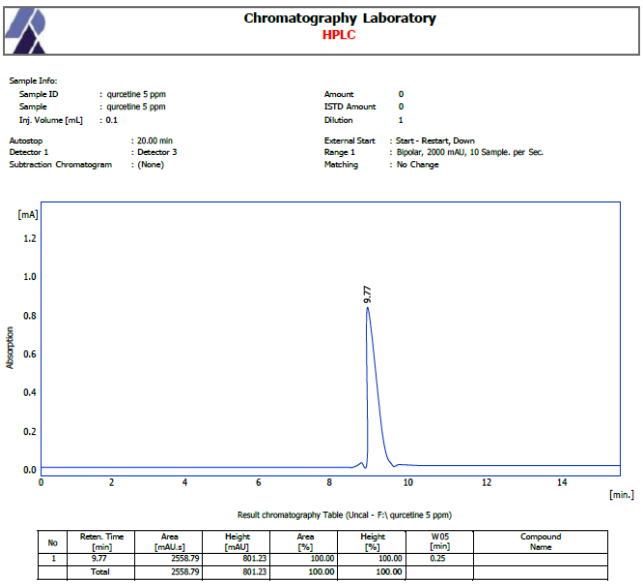
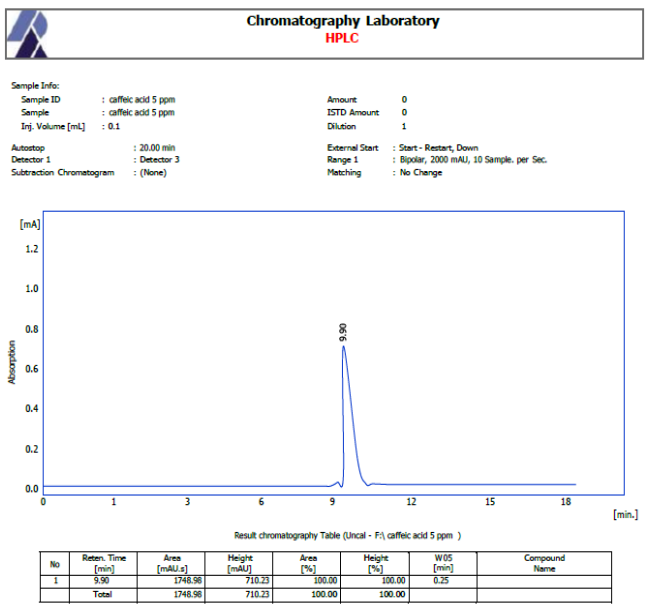


(F)



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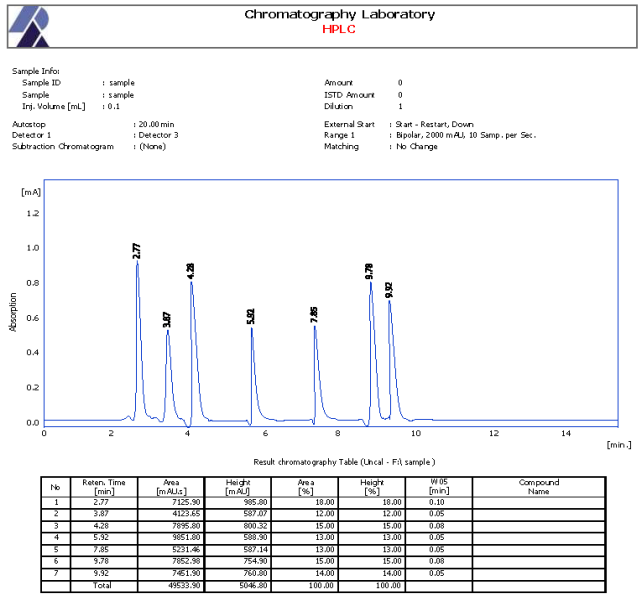

(H)

Figure 3. High-performance liquid chromatography (HPLC) chromatograms of phenolic standards (5 ppm) and phenolic profiling of *Abutilon theophrasti* (A) Gallic acid (retention time = 2.77 min), (B) Ferulic acid (3.87 min), (C) Rutin (4.28 min), (D) Apigenin (5.92 min), (E) Syringic acid (7.85 min), (F) Quercetin (9.78 min), (G) Caffeic acid (9.92 min), and (H) qualitative HPLC profile of phenolic constituents in the phenolic fraction of *A. theophrasti*

The retention times of the identified phenolic and flavonoid compounds are summarized in Table 5.

Table 5. Retention times of phenolic and flavonoid compounds identified in *Abutilon theophrasti* plant extract as determined by high-performance liquid chromatography (HPLC)

Compound	Chemical Class	Retention Time (min)
Gallic acid	Phenolic acid	2.77
Ferulic acid	Phenolic acid	3.87
Rutin	Flavonoid	4.28
Apigenin	Flavonoid	5.92
Syringic acid	Phenolic acid	7.85
Quercetin	Flavonoid	9.78
Caffeic acid	Phenolic acid	9.92

3.5 High-performance liquid chromatography profiling of the extract

The HPLC chromatogram of the methanolic extract of *Abutilon theophrasti* showed seven well-resolved peaks corresponding to the analyzed standards as shown in Figure 3. The retention times matched those of the reference compounds, confirming their presence in the extract.

3.6 Quantitative analysis

The quantitative analysis revealed that gallic acid was the most abundant compound (95.9 mg/kg DW), followed by rutin (85.4 mg/kg DW) and quercetin (82.9 mg/kg DW), and Caffeic acid 77.7 mg/kg DW) and Syringic acid (67.7 mg/kg DW), while ferulic acid showed the lowest concentration (58.7 mg/kg DW).

4. CONCLUSION

- *Abutilon theophrasti* Medik. shows specific morphological and anatomical characters, such as leaf and stem tissues organization, stomatal complexes, indumentum and calcium oxalate crystals for reliable identification at species level demonstrating adaptive responses.
- Results show a high presence of gallic acid, rutin, and quercetin in its alcoholic extract with moderate amounts of caffeic acid, syringic acid, apigenin, and ferulic acid. These phenolic acids and flavonoids are responsible for the antioxidant and antimicrobial effects of the extract.
- The biological effects are believed to be due not simply to any single agent but rather to the aggregative effect of a suite of phytochemicals.
- Conclusively, the structural and chemical properties of *A. theophrasti* showcase its biosynthetic potential for bioactive production with significant pharmacological implication mediated through antioxidant, antimicrobial activity.

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