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Phytochemical Screening and Total Phenolic Content of Leaf and Seed Extracts of *Acacia nilotica* (Al-Sibini) from Al-Khums, Libya

Mahmoud Mohamed Howas[1], Ebtehal El-Ghezlani [2], Fatma Kahel[3] and Entsar Mohammed Al-shokri[4]

[1]Chemistry Department, Faculty of Science, Elmergib University, Alkomes, Libya

[2.3.4]Faculty of Education – Department of Chemistry, Elmergib University, Alkomes, Libya

*Corresponding author: Mahmoud87336@gmail.com.

الفحص الكيميائي النباتي والمحتوى الفينولي الكلي لمستخلصات الأوراق والبذور لنبات السنط النيلي (السبيني) من مدينة الخمس، ليبيا

محمود محمد حواس[1], ابتهال الغزلاني[2], فاطمة كحل[3] , انتصار محمد الشكري[4]

[1]قسم الكيمياء، كلية العلوم، جامعة المرقب، الخمس، ليبيا

[2]، [3]،[4] كلية التربية – قسم الكيمياء، جامعة المرقب، الخمس، ليبيا

المؤلف المسؤول : Mahmoud87336@gmail.com

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الملخص:

يُستخدم نبات السنط النيلي المعروف محلياً في ليبيا باسم "السبيني" تقليدياً لأغراض طبية متعددة، تشمل علاج مرض السكري والقرحة والحالات الالتهابية. هدفت هذه الدراسة إلى إجراء فحص كيميائي نباتي وتحديد المحتوى الفينولي الكلي في المستخلصات المائية والإيثانولية (بتركيز 75%) لأوراق وبذور هذا النبات، الذي جُمعت عيّناته كعينة مجمعة من عشرة اشجار سليمة وناضجة بمدينة الخمس في ليبيا. أُجري التحليل الكيميائي النباتي النوعي باستخدام اختبارات قياسية، كما حُدِّد المحتوى الفينولي الكلي بطريقة "فولين-سيوكالتو "مع استخدام حمض الجاليك كمادة قياسية. وأظهرت النتائج أن جميع المستخلصات احتوت على القلويدات، والفلافونويدات، التانينات، والفينولات، الستيرويدات، الكربوهيدرات والفلوباتانينات. وقد تم الكشف عن البروتينات والأحماض الأمينية في مستخلصات الأوراق فقط، بينما وُجدت الصابونينات حصرياً في المستخلصات المائية، في حين تواجدت الجليكوزيدات القلبية في المستخلص الإيثانولي للبذور والمستخلص المائي للأوراق. وكان المحتوى الفينولي الكلي أعلى بشكل ملحوظ في الأوراق (285.8 ± 1.6 ملغ مكافئ حمض الجاليك/غرام مستخلص) مقارنة بالبذور (31.4 ± 1.3 ملغ مكافئ حمض الجاليك/غرام مستخلص). وتؤكد هذه النتائج أن نبات السنط النيلي يُعد مصدراً غنياً بالمركبات الكيميائية النباتية النشطة بيولوجياً، والتي تمتلك إمكانات واعدة في التطبيقات الصيدلانية والغذائية العلاجية .

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الكلمات المفتاحية: السنط النيلي ، السبيني، الفحص الكيميائي النباتي، المحتوى الفينولي الكلي، فولين- سيوكالتو.

Abstract

Acacia nilotica (A. nilotica), locally known in Libya as "Al-Sibini" (اسَّبْيِنِي), is traditionally used for various medicinal purposes, including the treatment of diabetes, ulcers, and inflammatory conditions. This study aimed to perform phytochemical screening and determine the total phenolic content of aqueous and 75% ethanolic extracts from the leaves and seeds of *A. nilotica* collected from Al-Khums city plant materials were collected as a pooled sample (Pooled sample) from ten mature, healthy trees selected randomly throughout the sampling zone, Libya. Qualitative phytochemical analysis was conducted using standard tests, and total phenolic content was determined by the Folin-Ciocalteu method using gallic acid as a standard. Results showed that all extracts contained alkaloids, flavonoids, tannins, phenols, steroids, carbohydrates, and phlobatannins. Proteins and amino acids were detected only in leaf extracts, while saponins were present exclusively in aqueous extracts, cardiac glycosides were present in ethanolic seed extract and aqueous leaf extract .The total phenolic content was significantly higher in leaves (285.8 ± 1.6 mg GAE/g extract) than in seeds (31.4 ± 1.3 mg GAE/g extract). These findings confirm that *A. nilotica* is a rich source of bioactive phytochemicals with potential pharmaceutical and nutraceutical applications.

Keywords: *Acacia nilotica, Al-Sibini, Phytochemical screening, Total phenolic content, Folin-Ciocalteu.*

Introduction

According to the World Health Organization (WHO), approximately 80% of the global population relies on traditional medicine for primary healthcare needs (Bashir et al., 2014). Medicinal plants are important sources of bioactive compounds, including polyphenols, flavonoids, alkaloids, and terpenoids, which exhibit various pharmacological activities (Baker et al., 1995; Kumar et al., 2023). Acacia nilotica (L.) Willd. ex Del., family Fabaceae, locally known as "Al-Sibini" in Libya, is a spiny tree widely distributed in arid and semi-arid regions. It has been traditionally used to treat diabetes, diarrhea, dysentery, and skin infections. The plant was first described by Linnaeus in 1753 (Tyagi et al., 2016). Previous studies have reported the presence of various secondary metabolites in A. nilotica; however, comparative phytochemical analysis of leaf and seed extracts from Libyan specimens is limited. Therefore, this study was conducted to screen the phytochemical constituents and quantify the total phenolic content in leaf and seed extracts of *A. nilotica* from Al-Khums, Libya.

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Table 1: Taxonomic classification of *Acacia nilotica* (Delile, 1813)

Classification	Taxonomic name
Kingdom	Plantae
Order	Fabales
Family	Fabaceae
Genus	Acacia
Species	Acacia nilotica
Binomial name	Acacia nilotica (L.) Willd. ex Del.
Local name	Al-Sibini (اسبيني)



Figure 1: Morphology of *Acacia nilotica* (Al-Sibini) - leaves, pods (seeds), and flowers collected from Al-Khums city

Materials and Methods

1. **Plant Collection and Preparation**

Fresh leaves and mature pods (containing seeds) of *Acacia nilotica* were harvested on October 1, 2025, from the Souq Al-Khamis area in Al-Khums City, Libya (32.65°N, 14.26°E). To ensure biological and demographic representation across the local plant population and minimize genetic variation from individual trees, plant

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materials were collected as a pooled sample (pooled sample) from ten mature, healthy trees selected randomly throughout the sampling zone.

Taxonomic authentication was conducted by the Department of Biology, Faculty of Science, Elmergib University. Leaves and seeds were separated from the pods, washed thoroughly with tap water to remove dust and debris, followed by a final rinse with distilled water. The samples were shade-dried at room temperature (25±2∘C25 \pm 2^\circ\text{C}25±2∘C) for 14 days with daily stirring to prevent fungal growth. Dried plant parts were pulverized into fine powder using an electric grinder, passed through a 425 µm425\ \mu\text{m}425 µm mesh sieve, and preserved in airtight glass containers stored in a dark environment at 4∘C4^\circ\text{C}4∘C until extraction.

2. Preparation of Extracts

Each powdered plant sample (50 g) was individually macerated in 500 mL of either distilled water or 75% ethanol (1:10 w/v ratio) for 72 hours at room temperature, accompanied by periodic shaking. Following extraction, the mixtures were filtered through Whatman No. 42 filter paper and evaporated to dryness using a rotary evaporator under reduced pressure at 40°C. The resulting extracts were weighed to determine the percentage yield and subsequently kept at 4°C until further analysis.

3. Phytochemical Screening

Qualitative phytochemical tests were performed using standard procedures to detect alkaloids (Mayer's and Dragendorff's tests), flavonoids (alkaline reagent and Shinoda tests), tannins (ferric chloride test), saponins (Foam test), steroids (Salkowski and Lieberman-Burchard tests), cardiac glycosides (Keller-Killiani test), proteins (Ninhydrin and Xanthoproteic tests), carbohydrates (Benedict's and Molisch's tests), and phlobatannins (HCl test), as described by Harborne (1998), Sofowora (1993), and Evans (2009).

4. Determination of Total Phenolic Content

Total phenolic content was determined using Folin-Ciocalteu method (Sembiring et al., 2018). Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of 7.5% Na_2CO_3. After 2 hours incubation at 25°C in the dark, absorbance was measured at 765 nm using UV-Vis spectrophotometer. Gallic acid (15.62-500 µg/mL) was used as standard. Results

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were expressed as mg gallic acid equivalent (GAE) per gram of dry extract. All determinations were performed in triplicate.

5. Statistical Analysis

Data are expressed as mean ±\pm± standard deviation (SD) of triplicate measurements. Relative standard deviation (RSD%) was calculated to assess precision. Standard Error (SE) was derived using the formula $SE = \frac{SD}{\sqrt{n}}$ (n=3). Statistical evaluation was conducted using One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test in SPSS version 26.0. Differences were deemed statistically significant at $p<0.001$.

Results and Discussion

1. Extract Yield

The percentage yield and physical characteristics of *A. nilotica* extracts are presented in Table 2. The aqueous seed extract gave the highest yield (16.96%), while the ethanolic seed extract gave the lowest yield (8.19%). Color variations were observed: aqueous leaf extract was brown, ethanolic leaf extract was dark green, aqueous seed extract was reddish-brown, and ethanolic seed extract was light green. These differences may be attributed to the polarity of the solvents and the nature of the extracted phytochemicals.

Table 2: Percentage yield and physical characteristics of *A. nilotica* extracts

Plant Part	Solvent	Colour	Yield (%)
Leaves	Aqueous	Brown	11.48
Leaves	Ethanol 75%	Dark green	11.25
Seeds	Aqueous	Reddish brown	16.96
Seeds	Ethanol 75%	Light green	8.19

2. Phytochemical Screening

Qualitative phytochemical analysis revealed the presence of diverse secondary metabolites in all extracts (Table 3). Alkaloids (Dragendorff's test), flavonoids, tannins, phenols, steroids (Salkowski's test), and carbohydrates were detected in all

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four extracts. Cardiac glycosides were present in the ethanolic seed extract and aqueous leaf extract. Xanthoproteic tests confirmed proteins exclusively in leaves. Phlobatannins showed strong positive reactions in all extracts. Notably, saponins were detected only in the aqueous extracts of both leaves and seeds but were absent in the ethanolic extracts, indicating their hydrophilic nature. These phytochemicals are known to possess antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties (Shraim et al., 2021; Barnawi et al., 2021; Kumar et al., 2023).

Table 3: Phytochemical constituents of *A. nilotica* leaf and seed extracts

Phytochemical	Test	Seeds Aqueous	Seeds Ethanol	Leaves Aqueous	Leaves Ethanol
Alkaloids	Mayer's	-	-	-	-
Alkaloids	Dragendorff's	+++	+++	+++	+++
Flavonoids	Alkaline reagent	++	+++	+	+
Flavonoids	Shinoda	++	+	+	-
Tannins/Phenols	$FeCl_3$	++	+++	+	+
Fixed oils	Spot test	+++	+++	+	+
Steroids	Salkowski	+++	+++	+++	+++
Steroids	Lieberman-Burchard	-	-	++	++
Cardiac glycosides	Keller-Killiani	-	++	++	-
Proteins	Ninhydrin	+	+	-	-
Proteins	Xanthoproteic	-	-	+++	++
Carbohydrates	Benedict's	+	++	+	++
Carbohydrates	Molisch's	++	-	++	-

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Saponins	Foam test	+++	-	++	-
Saponins	Frothing test	+++	-	++	-
Phlobatannins	HCl	+++	+++	+++	+++

Note: +++ = Strongly present, ++ = Moderately present, + = Weakly present, - = Absent

3. Total Phenolic Content (TPC)

The total phenolic content was quantified using the Folin-Ciocalteu method with gallic acid as the standard (Tables 4 and 5 and Figure 2). The calibration curve exhibited excellent linearity with the equation $y = 0.00185x + 0.080$ ($R^2 = 0.9923$). The leaf extracts exhibited a significantly higher phenolic content (285.8 ± 1.6 mg GAE/g) than the seed extracts (31.4 ± 1.3 mg GAE/g), representing an approximately 9-fold difference. This substantial variation indicates that leaves are the primary site of phenolic accumulation in *A. nilotica species*. Phenolic compounds are major contributors to antioxidant activity because of their ability to donate hydrogen atoms or electrons to free radicals (Camacho et al., 2022). The high phenolic content of the leaves suggests their superior potential for therapeutic applications compared to that of the seeds.

Table 4: Total phenolic content of *A. nilotica* extracts (mg GAE/g extract)

Plant Part	Extraction Solvent	Replicates	Mean ± SD	RSD%	SE
Leaves	75% Ethanol Extract	284.15, 287.40, 285.91	285.82 ± 1.64[a]	0.57%	0.95
	Water Extract	208.35, 212.55, 210.45	210.45 ± 2.10[b]	1.00%	1.21
Seeds	Water Extract	32.47, 34.08, 31.40	32.65 ± 1.35[c]	4.13%	0.78
	75% Ethanol Extract	17.35, 19.05, 18.20	18.20 ± 0.85[d]	4.67%	0.49

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Values are expressed as mean standard deviation (SD) for three replicates (n = 3). Means in the same column followed by different superscript letters (a–d) indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$). One-way ANOVA revealed a highly significant overall effect ($F = 21,895$, $p < 0.001$).

Table 5: Absorbance values of gallic acid standard at different concentrations

Concentration (µg/mL)	Absorbance (765 nm)
500	0.979
250	0.601
125	0.312
62.5	0.177
31.25	0.131
15.62	0.101

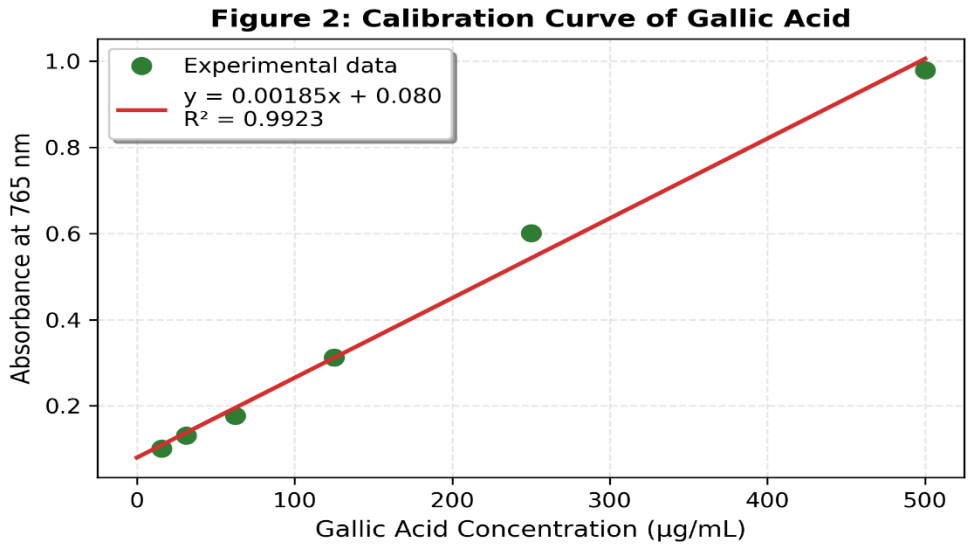


Figure 2: Calibration curve of gallic acid for total phenolic content determination (y = 0.00185x + 0.080, $R^2 = 0.9923$)

A comparative evaluation against the published literature emphasizes the unique phytochemical profile of Libyan *A. nilotica*. Regional studies from Sudan (El-Tahir et al., 2018) and Egypt (Sheded et al., 2020) reported leaf phenolic contents ranging

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between 140 and 195 mg GAE/ Furthermore, investigations in India (Singh et al., 2016) and Saudi Arabia (Al-Youssef et al., 2021) documented hydro-ethanolic leaf phenolic levels between 160 and 220 mg GAE/g .The significantly elevated phenolic concentration observed in Al-Khums leaves (285.82 mg GAE/g) can be attributed to the unique coastal arid microclimate of northwestern Libya. Intense solar radiation, elevated UV exposure, temperature fluctuations, and hyper-saline soil conditions in Al-Khums trigger metabolic up-regulation of the phenylpropanoid biosynthetic pathway. Polyphenols act as secondary defense antioxidants to prevent photo-inhibition and cellular oxidative stress. These findings confirm that Libyan *A. nilotica* leaves represent a superior natural source of phenolic antioxidants for pharmaceutical applications.

Conclusion

This study demonstrates that *Acacia nilotica* (Al-Sibini) leaves and seeds from Al-Khums, Libya contain diverse phytochemicals, including alkaloids, flavonoids, tannins, saponins, steroids, and phenolic compounds. The total phenolic content was approximately nine times higher in leaves (285.8 mg GAE/g) than in seeds (31.4 mg GAE/g). The presence of these bioactive compounds, particularly the high phenolic content in leaves, supports the traditional medicinal uses of *A. nilotica* and indicates its potential as a natural source of antioxidants for pharmaceutical and nutraceutical applications. Leaves represent a more promising source of phenolic antioxidants compared to seeds.

Recommendations

1. Quantitative analysis of individual phenolic compounds was performed using high-performance liquid chromatography with diode-array detection (HPLC-DAD) or liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2. Evaluate antioxidant activity using Di-Phenyl-1- Picrylhydrazyl Assay-2, 2 (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays to correlate with phenolic content.

3. Investigate the antimicrobial activity of extracts against pathogenic bacteria and fungi.

4. Conduct toxicity studies to establish the safety profile for potential therapeutic use.

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5. Explore the phytochemical composition of other plant parts, including bark, flowers, and roots.

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