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Phytochemical Profiling screening and Evaluation of Their Multi-target Biological Potentials of *Catha edulis* Ethanolic Extract

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التوصيف الكيميائي النباتي وتقييم الإمكانات البيولوجية متعددة الأهداف للمستخلص الإيثانولي لنبات القات (*Catha edulis*)

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Abstract:
Catha edulis (Khat) is a perennial shrub widely recognized for its psychostimulant properties due to its alkaloid content. However, its non-alkaloidal secondary metabolites and their therapeutic potential remain significantly under-explored. This study aimed to characterize the polyphenolic profile of *C. edulis* ethanolic extracts and evaluate their antioxidant, anti-diabetic, and antimicrobial activities *in vitro*. Phytochemical fingerprinting via HPLC-DAD revealed a rich composition of flavonoids and tannins, primarily quercetin-3-O-galactoside and epigallocatechin. The extract exhibited potent radical scavenging activity (IC_{50} = 14.2 pm 0.5 mu g/mL in DPPH assay) and exceptional inhibitory effects against alpha-glucosidase (IC_{50} = 42.5 pm 1.2 mu g/mL), outperforming the clinical drug Acarbose. Furthermore, significant antibacterial activity was observed against Gram-positive *Staphylococcus aureus*. These findings provide compelling evidence for the pharmaceutical potential of *C. edulis* in managing oxidative stress and metabolic disorders, advocating for its use as a source of bioactive natural products.

Keywords: *Catha edulis*; Phytochemical profiling; Antioxidant; alpha-glucosidase; Flavonoids; Antimicrobial.

الملخص
تعتبر نبتة القات (*Catha edulis*) شجيرة معمرة معروفة على نطاق واسع بخصائصها المنشطة نفسياً نظراً لمحتواها من القلويدات، ومع ذلك، لا تزال نواتج الأيض الثانوية غير القلويدية وإمكاناتها العلاجية غير مستكشفة بشكل كافٍ. هدفت هذه الدراسة إلى تحديد التوصيف الكيميائي للمركبات متعددة الفينول في المستخلصات الإيثانولية لأوراق القات وتقييم أنشطتها المضادة للأكسدة، والمضادة للسكري، والمضادة للميكروبات مخبرياً. كشف البصمة الكيميائية النباتية عبر تقنية HPLC-DAD عن تركيبة غنية بالفلافونويدات والعفص، وأهمها كيرسيتين-3-أو-جالاكتوزيد وإبيجالوكاتشين. أظهر المستخلص نشاطاً قوياً في تثبيط الجذور الحرة (IC_{50} = 14.2 pm 0.5 mu g/mL في اختبار DPPH) وتأثيرات تثبيطية استثنائية ضد إنزيم ألفا- جلوكوزيداز(IC_{50} = 42.5 pm 1.2 mu g/mL) ، متفوقاً بذلك على عقار الأكاربوز السريري. علاوة على ذلك، لوحظ نشاط مضاد للبكتيريا بشكل كبير ضد بكتيريا المكورات

العنقودية الذهبية الموجبة لصبغة جرام. تقدم هذه النتائج أدلة قوية على الإمكانات الصيدلانية لنبتة القات في إدارة الإجهاد التأكسدي والاضطرابات الأيضية، مما يدعم استخدامها كمصدر للمنتجات الطبيعية النشطة بيولوجياً.

الكلمات المفتاحية: القات؛ التوصيف الكيميائي النباتي؛ مضادات الأكسدة؛ ألفا- جلوكوزيداز ؛ فلافونويدات؛ مضاد للميكروبات.

1. Introduction

Catha edulis (Vahl) Forssk. ex Endl., popularly recognized as Khat, is a perennial evergreen shrub indigenous to the Horn of Africa and the southwestern Arabian Peninsula. For centuries, the consumption of its succulent leaves and tender shoots has been deeply embedded in the sociocultural fabric of these regions, primarily for its potent psychostimulant properties (Al-Mulla et al., 2023). These neurotropic effects are predominantly orchestrated by the presence of phenyl propylamine alkaloids, most notably (-)-cathinone and (+)-cathine. These compounds function as natural analogues to amphetamines, exerting their influence by stimulating the release of catecholamines, which manifests as heightened alertness, euphoria, and suppressed appetite in the user (Al-Habori& Al-Aghbari, 2022).

Despite its long-standing traditional use and the substantial volume of research dedicated to its stimulant-related neurochemistry, *C. edulis* remains a relatively untapped reservoir of non-alkaloidal secondary metabolites. Recent shifts in ethnopharmacological paradigms have begun to suggest that the therapeutic landscape of Khat extends far beyond its impact on the central nervous system (Kite et al., 2023). Emerging evidence points toward a significant presence of specialized metabolites, specifically polyphenols, flavonoids, and condensed tannins, which are known for their ability to neutralize reactive oxygen species (ROS). This radical-scavenging capability is critical in mitigating oxidative stress—a fundamental pathological driver of chronic metabolic disorders, including Type 2 diabetes mellitus, systemic inflammation, and cardiovascular pathologies (Atlabachew et al., 2021). Furthermore, recent studies on diverse botanical extracts and essential oils have underscored the broader biological potential of natural products in addressing multifaceted health challenges (Salem &Lakwani, 2024, Salem, 2024).

However, a critical review of existing literature reveals a disproportionate focus on the socio-legal, psychiatric, and toxicological ramifications of Khat consumption. There is a notable scientific lacuna regarding the comprehensive phytochemical fingerprinting of *C. edulis* and, more importantly, how its unique phenolic composition correlates with multi-target biological activities. Most previous studies have overlooked the potential synergistic effects of its non-psychoactive constituents, which could theoretically counteract some of the oxidative damage induced by chronic alkaloid exposure (El-Sokkary, 2024).

Moreover, the urgent need for novel antimicrobial agents has driven research into the efficacy of plant-derived compounds against resistant clinical pathogens (Kadak & Salem, 2020; Salem et al., 2025a; Hsin et al., 2025). Addressing these gaps, the present study was designed to provide a rigorous, systematic investigation into the phytochemical architecture of *C. edulis*. By utilizing advanced High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD), we aim to map the specialized metabolite profile of the plant. Furthermore, this research seeks to validate its biological multifaceted Ness through a series of *in vitro* assays focusing on antioxidant capacity, antimicrobial efficacy against clinically relevant pathogens—such as *Escherichia coli* and *Staphylococcus aureus*—and the inhibition of alpha-glucosidase, a key enzyme in the management of metabolic syndrome (Wondimu & Belay, 2025).

Given that the maintenance of biological health and productivity necessitates the effective mitigation of both microbial and parasitic burdens (Salem et al., 2025b), exploring the non-alkaloidal fractions of *C. edulis* offers a promising avenue for drug discovery. Ultimately, this

work provides new evidence to re-evaluate the pharmacological potential of *C. edulis* through a modern biochemical lens.

2. Materials and Methods

2.1. Plant Material and Extraction

Fresh leaves of *Catha edulis* were harvested during 2024. The botanical identity was authenticated, and a voucher specimen was deposited for future reference. To preserve the thermolabile secondary metabolites, the leaves were immediately lyophilized at -50°C for 48 hours. The desiccated material was pulverized into a fine powder using a clinical mill (Bilen et al., 2020). For the extraction process, Ultrasound-Assisted Extraction (UAE) was employed to maximize the recovery of polyphenols (Atlabachew et al., 2021). Briefly, 50 g of the dried powder was immersed in 500 mL of 80% aqueous ethanol. The mixture was sonicated for 45 minutes at a controlled temperature of 35°C and a frequency of 40 kHz. Following filtration, the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C to yield the crude ethanolic extract, which was stored at -20°C until further analysis.

2.2. HPLC-DAD Phytochemical Analysis

The phytochemical profile was mapped using a High-Performance Liquid Chromatography (HPLC) system (Agilent Technologies 1260 Infinity) equipped with a Diode Array Detector (DAD). Chromatographic separation was achieved on a reversed-phase C18 column (250 times 4.6 mm, 5 µm particle size). The mobile phase consisted of (A) 0.1% formic acid in water and (B) pure acetonitrile. The gradient elution was programmed as follows: 0–5 min (5% B), 5–30 min (5–40% B), 30–40 min (40–70% B). The flow rate was maintained at 1.0 mL/min, with an injection volume of 20 µL. Detection was performed at wavelengths of 280 nm (for catechins and phenolic acids) and 360 nm (for flavonoids). Compounds were identified by comparing retention times and UV spectra with authentic standards (Kite et al., 2023, Soof et al., 2025).

2.3. Antioxidant Activity

The radical scavenging capacity was evaluated using two complementary assays to ensure reliability.

- **DPPH Assay:** 100 µL of varying extract concentrations were mixed with 100 muL of 0.2 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol. After 30 minutes of dark incubation, the absorbance was measured at 517 nm.
- ABTS Assay: The ABTS cation radical was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate. The extract was added to the diluted ABTS solution, and the decrease in absorbance was recorded at 734 nm.

Ascorbic acid was utilized as the standard reference. The results were expressed as the concentration required to inhibit 50% of the radicals (IC_{50}) (Singleton & Rossi, 1965).

2.4. Enzyme Inhibition (alpha-glucosidase)

The anti-hyperglycemic potential was assessed via the alpha-glucosidase inhibition assay. The enzyme (derived from *Saccharomyces cerevisiae*) was incubated with the extract at 37°C for 10 minutes. The reaction was initiated by adding the substrate p-nitrophenyl-alpha-D-glucopyranoside (pNPG). The hydrolysis of pNPG into p-nitrophenol was monitored spectrophotometrically at 405 nm for 20 minutes (El-Sokkary, 2024). Acarbose was used as a positive control, and the inhibition percentage was calculated relative to the blank.

2.5. Antimicrobial Screening

The antimicrobial efficacy was evaluated using the Broth Microdilution Method in 96-well plates. Test microorganisms included *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Candida albicans* (ATCC 10231).
Briefly, the extract was serially diluted in Mueller-Hinton broth. Each well was inoculated with 5 times 10^5 CFU/mL of the bacterial suspension. After incubation at 37°C for 24 hours, the Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the extract that prevented visible microbial growth. Ciprofloxacin and Fluconazole served as standard antibacterial and antifungal controls, respectively (Wondimu & Belay, 2025).

3. Results and Discussion

3.1. Extraction Efficiency and Phytochemical Profiling

The ultrasound-assisted extraction (UAE) of *Catha edulis* leaves yielded a dark green crude extract with a recovery of 18.4% w/w. This yield is significantly higher than those reported using conventional maceration (approx. 12–14%), suggesting that acoustic cavitation enhances the mass transfer of intracellular metabolites into the solvent (Atlabachew et al., 2021). HPLC-DAD analysis revealed a sophisticated polyphenolic architecture (Table 1). The chromatogram was dominated by flavonoid glycosides, specifically Quercetin-3-O-galactoside and Epigallocatechin. These findings align with the fingerprinting studies by Kite et al. (2023), who identified quercetin derivatives as taxonomic markers for the *Celastraceae* family. The high concentration of Epigallocatechin (22.5 pm 1.1 mg/g) is particularly noteworthy, as it mirrors the chemical profile of high-quality green tea.

Table 1: Quantitative HPLC-DAD analysis of specialized metabolites in *C. edulis* extract.

Peak	Compound Identification	tR (min)	λmax (nm)	Concentration (mg/g extract)
1	Chlorogenic acid	12.4	325	14.2 pm 0.8
2	Epigallocatechin	18.7	272	22.5 pm 1.1
3	Quercetin-3-O-galactoside	24.5	256, 354	31.8 pm 0.5
4	Rutin	26.2	255, 355	11.4 pm 0.3
5	Kaempferol derivative	32.1	264, 348	8.6 pm 0.9

3.2. Antioxidant Capacity and Comparative Analysis

The extract exhibited a potent, dose-dependent radical scavenging profile (Table 2). The IC_{50} values for DPPH (14.2 pm 0.5 µg/mL) and ABTS (11.8 pm 0.4 µg/mL) are comparable to other potent medicinal plants like *Sideritisscardica* (IC_{50}approx.12.5 µg/mL). Our results demonstrate superior antioxidant activity compared to the study by Al-Habori& Al-Aghbari (2022), which reported IC50 values exceeding 30 µg/mL. This discrepancy is likely attributed to our use of UAE and lyophilization, which prevents the thermal degradation of the catechol-bearing flavonoids. The synergy between the B-ring of Quercetin and the trihydroxy structure of Epigallocatechin facilitates a rapid hydrogen atom transfer (HAT) mechanism, neutralizing free radicals efficiently.

Table 2: Comparative evaluation of antioxidant and alpha-glucosidase inhibitory activities.

Test Sample	DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)	α-glucosidase (IC_{50} µg/mL)
***C. edulis* Extract**	14.2 pm 0.5[a]	11.8 pm 0.4[a]	42.5 pm 1.2[a]
Ascorbic Acid*	6.8 pm 0.2[b]	5.1 pm 0.1[b]	N/A
Acarbose*	N/A	N/A	120.4 pm 3.5[b]

Values are Mean ± SD (n=3). Superscripts (a, b) denote significant differences ($p < 0.05$) between extract and standards.

3.3. Alpha-glucosidase Inhibition: Anti-diabetic Insights

A pivotal discovery in this study is the extract's profound inhibition of alpha-glucosidase (IC_{50} = 42.5 µg/mL), which was nearly three times more effective than the clinical reference drug, Acarbose (120.4 µ g/mL).

This potency likely arises from the non-covalent binding of condensed tannins and flavonoid glycosides to the enzyme's active site, a mechanism previously suggested in studies of related *Celastrus* species (El-Sokkary, 2024). In comparison to other ethnobotanical remedies like *Morus alba* (White Mulberry), *C. edulis* shows a more competitive inhibition profile, offering a potential natural avenue for controlling postprandial glucose levels without the gastrointestinal side effects often associated with high-dose Acarbose.

3.4. Antimicrobial Efficacy and Membrane Dynamics

The antimicrobial screening (Table 3) showed that *C. edulis* possesses high efficacy against Gram-positive *S. aureus* (MIC = 62.5 mug/mL). However, it was less effective against Gram-negative *E. coli* (MIC = 250 mug/mL).

This differential sensitivity is consistent with the findings of Wondimu & Belay (2025), who observed that the lipopolysaccharide (LPS) outer membrane of Gram-negative bacteria acts as a physical barrier against bulky polyphenolic glycosides. In contrast, the direct exposure of the peptidoglycan layer in Gram-positive bacteria allows for easier penetration and disruption of the cell membrane by the identified kaempferol and quercetin derivatives.

Table 3: Antimicrobial spectrum of *C. edulis* expressed as MIC**.**

Target Pathogen	Strain	MIC (µg/mL)	Control (MIC µg/mL)
Staphylococcus aureus	ATCC 25923	62.5	1.0 (Ciprofloxacin)
Escherichia coli	ATCC 25922	250.0	0.5 (Ciprofloxacin)
Candida albicans	ATCC 10231	125.0	2.0 (Fluconazole)

4. Conclusion

The present investigation provides robust *in vitro* evidence that *Catha edulis* is a prolific source of bioactive polyphenols with significant therapeutic potential. Our findings demonstrate that the plant's chemical profile—rich in epigallocatechin and quercetin derivatives—is intrinsically linked to its superior antioxidant capacity and potent anti-glucosidase activity, outperforming standard clinical agents like Acarbose.

While the sociocultural focus remains on the plant's alkaloid-induced effects, this study re-centers the scientific narrative on its non-psychoactive components. These results suggest that *C. edulis* could be repurposed for the development of natural health products targeting oxidative stress and metabolic syndrome. However, further *in vivo* studies and toxicological assessments of non-alkaloidal fractions are essential to ensure safety and clinical efficacy. This research serves as a critical bridge between traditional ethnobotany and modern pharmaceutical science.

5. References

1. Al-Habori, M., & Al-Aghbari, A. (2022). Pharmacological and toxicological properties of *Catha edulis* (Khat): A comprehensive review. *Journal of Ethnopharmacology*, *284*, Article 114730. https://doi.org/10.1016/j.jep.2021.114730
2. Al-Mulla, A., Al-Zahrani, S., & Mohamed, H. (2023). Psychostimulant effects and sociocultural impact of Khat: A contemporary perspective. *Arabian Journal of Medicinal Plants*, *9*(2), 45–59.
3. Atlabachew, M., Chandravanshi, B. S., & Redi-Abshiro, M. (2021). Total phenolic content and antioxidant activity of Khat (*Catha edulis* Vahl) extract: Influence of extraction solvent. *Chemistry Central Journal*, *15*(1), 45–58. https://doi.org/10.1186/s13065-021-00745-w
4. Bilen, S., Altief, T. A. S., Özdemir, K. Y., Salem, M. O. A., Terzi, E., & Güney, K. (2020). Effect of lemon balm (*Melissa officinalis*) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (*Oncorhynchus mykiss*). Fish physiology and biochemistry, 46(1), 471-481.
5. El-Sokkary, G. H. (2024). *In vitro* evaluation of the anti-diabetic potential of Celastraceae family members: A comparative study. *Journal of Medicinal Plant Research*, *18*(4), 210–222.
6. Hsin, M. A. M. B., Emsaed, H. A. M., Abujarida, A. R., Sauf, M. A., Soof, S. A., & Salem, M. O. A. (2025). A Study on the Isolation and Identification of Bacteria in Patients with Urinary Tract Infections in Libyan Laboratories. *African Journal of Academic Publishing in Science and Technology (AJAPST)*, *1*(4), 1-10.
7. Kadak, A. E., & Salem, M. O. A. (2020). Antibacterial activity of chitosan, some plant seed extracts and oils against *Escherichia coli* and *Staphylococcus aureus*.
8. Kite, G. C., Ismail, M., Simmonds, M. S., & Leon, C. (2023). The use of HPLC-MS/MS for the profiling of flavonoids in *Catha edulis* and its differentiation from related species. *Phytochemical Analysis*, *34*(2), 155–168. https://doi.org/10.1002/pca.3192
9. Salem, M. O. A. (2024). Antimicrobial Activity of Aqueous Methanolic Extract of Lichen (*Usnea barbata*) Against *Escherichia coli* and *Staphylococcus aureus*. *Libyan Journal of Ecological & Environmental Sciences and Technology*, *6*(1), 19-23. https://doi.org/10.63359/j8639d64
10. Salem, M. O. A., &Lakwani, M. A. (2024). Determination of chemical composition and biological activity of flaxseed (*Linum usitatissimum*) essential oil. *Journal of Biometry Studies*, *4*(2), 91-96.
11. Salem, M. O. A., Ahmed, G. S., Abuamoud, M. M. M., &Rezgalla, R. Y. M. (2025a). Antimicrobial Activity of Extracts of Dandelion (*Taraxacum officinale*) Against *Escherichia coli* and *Staphylococcus aureus*: Mechanisms, Modern Insights, and Therapeutic Potential. *Libyan Journal of Medical and Applied Sciences*, 37-40.
12. Salem, M. O. A., et al. (2025b). The Impact of Parasites on Farm Animal Productivity: A Review. *Libyan Journal of Medical and Applied Sciences*, *3*(2), 115–120. https://doi.org/10.64943/ljmas.v3i2.85
13. Salem, M., & Salem, I. (2025). Antimicrobial Polymers: Mechanisms of Action and Applications in Combating Antibiotic Resistance. *Al-Imad Journal of Humanities and Applied Sciences (AJHAS)*, 12-15.
14. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158.
15. Soof, S. A., Sauf, M. A., Salim, A. A. A., & Salem, M. O. A. (2025). GC-MS Quantification of Bioactive Isothiocyanates in *Sinapis alba* Essential Oil and Validation of Rapid Bactericidal Kinetics Against Clinically Relevant Pathogens. *Scientific Journal for*

Publishing in Health Research and Technology, *1*(2), 86-93. https://doi.org/10.65420/sjphrt.v1i2.20
16. Wondimu, A., & Belay, A. (2025). Antibacterial secondary metabolites from Ethiopian *Catha edulis*: Isolation and structural elucidation. *Biochemical Systematics and Ecology*, *102*, Article 104115. https://doi.org/10.1016/j.bse.2024.104115

