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Research Article

PHYTOCHEMICAL PROFILING AND GC-MS ANALYSIS OF *ANOECTOCHILUS ELATUS* LEAF EXTRACTS

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ABSTRACT

This study aimed to evaluate the phytochemical composition, identify bioactive constituents, and predict the molecular mechanisms underlying the medicinal value of *Anoectochilus elatus* (*A. elatus*) aqueous leaf extract, with particular emphasis on its potential therapeutic role against hyperammonemia-associated inflammatory and apoptotic dysfunction. Fresh leaves of *A. elatus* were collected, authenticated, shade-dried, powdered, and subjected to aqueous extraction. Qualitative phytochemical screening was carried out using standard chemical tests. Gas chromatography–mass spectrometry (GC–MS) was performed to determine the major phytochemical constituents. Phytochemical screening revealed the presence of flavonoids, phenolics, tannins, chlorogenic compounds, saponins, steroids, triterpenoids, and essential nutritional metabolites. GC-MS profiling identified pharmacologically relevant compounds, with 2-decanone, 2-tridecanone, benzyl benzoate, corosolic acid, and β-sitosterol being the major constituents. The aqueous extract of *A. elatus* contains diverse bioactive constituents with potent antioxidant, anti-inflammatory, and apoptosis-modulating properties. Further *in vitro* and *in vivo* studies are warranted to validate their molecular effects and support the development of *A. elatus*-derived therapeutic interventions for hyperammonemia.

Keywords: *Anoectochilus elatus*, GC-MS, Linoleic acid, Medicinal orchids, Apoptosis.

INTRODUCTION

Modern synthetic drugs are often associated with various adverse effects, increasing global interest in natural products derived from medicinal plants as safer therapeutic alternatives (Ansari *et al.*, 2025). Among these, orchids hold a prominent place in traditional healing systems due to their wide range of pharmacological properties. *A. elatus* (jewel orchid), belonging to the family Orchidaceae, is notable for its ethnomedicinal importance and has been reported to exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer activities (Venkataesan *et al.*, 2023; Gunes *et al.*, 2023). The genus *Anoectochilus*, comprising approximately 15–20 species distributed across tropical and subtropical regions of Asia, is extensively used in traditional Chinese medicine for the management of diabetes, hepatitis, hypertension, tuberculosis, and inflammatory ailments (Wu *et al.*, 2020; Zou *et al.*, 2025). Phytochemical studies have revealed that these species are rich in three major classes of bioactive compounds-lactone glycosides, flavonoids, and polysaccharides (Gunes *et al.*, 2023; Ho *et al.*, 2025). Among these, kinsenoside (3-(R)-3-β-D-glucopyranosyloxybutanolide) stands out as a key marker compound with well-documented hepatoprotective, antihyperglycemic, antioxidant, and anti-inflammatory effects (Rehman *et al.*, 2016; Zheng *et al.*, 2023). The polysaccharide fraction of *Anoectochilus* species comprising heteropolysaccharides rich in glucose, galactose, arabinose, xylose, and rhamnose also exhibits notable immunomodulatory, antitumor, and metabolic regulatory activities (Xie *et al.*, 2023).

Comprehensive phytochemical characterization is essential for understanding the therapeutic potential of

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medicinal plants and validating traditional uses (RR, 2022). Since different solvents extract different classes of metabolites, profiling both aqueous and ethanolic extracts provides a broader understanding of the chemical composition and bioactive constituents of *A. elatus*. Recent metabolomics-based investigations on medicinal orchids have identified diverse compounds such as amino acids, organic acids, polysaccharides, flavonoids, phenolic derivatives, and lipid-like metabolites, underscoring their chemical complexity and pharmacological significance (Huang *et al.*, 2024; Lima *et al.*, 2022).

Gas Chromatography–Mass Spectrometry (GC–MS) remains a powerful technique for identifying volatile and semi-volatile phytochemicals in plant extracts based on their mass fragmentation patterns (Geetha *et al.*, 2013). The GC–MS profiling of *A. elatus* identified major constituents such as 2-decanone, 2-tridecanone, hexadecanoic acid, octadecanoic acid, oleic acid, linoleic acid, squalene, β-sitosterol, stigmasterol, and phenolic derivatives. These classes of metabolites are comparable to those previously reported in *Anoectochilus roxburghii*, which exhibits a similar composition of fatty acids, terpenoids, sterols, and phenolics (Chen *et al.*, 2018; Liu *et al.*, 2020). Identifying such metabolites from *A. elatus* is crucial for correlating its chemical profile with its pharmacological activities. Collectively, the integration of phytochemical profiling, and GC–MS analysis, provides a comprehensive framework for evaluating the therapeutic potential of *A. elatus* and supports its development as a natural source of anti-inflammatory and anticancer compounds. Given that hyperammonemia is closely associated with oxidative stress, inflammation, and apoptosis key pathways potentially modulated by metabolites of *A. elatus* this approach also enables the identification of candidates relevant to ammonia-induced cellular injury. Accordingly, the present study aims to characterize the phytochemical constituents of *A. elatus* through GC–MS analysis and to elucidate their possible anti-inflammatory, anticancer, and hyperammonemia-related protective mechanisms.

MATERIALS AND METHODS

Plant Material Collection and Preparation

Fresh leaves of *A. elatus* were collected from Kolli Hills, Tamil Nadu, and the plant material was authenticated by a qualified botanist (PARC/2022/4766). The collected leaves were initially rinsed with tap water to remove soil particles and then washed thoroughly with distilled water to ensure the removal of surface contaminants. The leaves were shade-dried at room temperature for several days to preserve heat-sensitive phytochemicals. Once completely dried, the material was ground into a fine powder using a mechanical grinder. The powdered sample was passed through a fine mesh to obtain uniform particle size and stored in clean, airtight containers under dry conditions until further use.

Extraction

The powdered leaves of *A. elatus* were extracted using distilled water at a 1:10 (w/v) ratio and left to macerate for 48 hours at room temperature. Intermittent stirring was carried out to improve the release of water-soluble compounds. After the extraction period, the mixture was filtered through Whatman No. 1 filter paper to remove plant debris. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator until a semi-solid crude aqueous extract was obtained. The extract was then stored at 4 °C until further analysis.

Phytochemical screening

Qualitative phytochemical analyses were carried out to detect the presence of alkaloids, flavonoids, phenolics, glycosides, amino acids, proteins, tannins, saponins, and steroids, following standard protocols described by (Harborne, 1998) and (Trease and Evans, 2002). Tests performed included Wagner's reagent for alkaloids, lead acetate test for flavonoids, foam test for saponins, ferric chloride for phenolics, gelatin test for tannins, and Salkowski's/Liebermann–Burchard for sterols.

GC–MS analysis

The extract was analyzed by gas chromatography-mass spectrometry (GC-MS) on an Agilent 7890B equipped with a 5977A mass detector. The injector was maintained at 250 °C, and helium served as the carrier gas at a constant flow of $1.0\,mL\,min^{-1}$. The oven temperature was programmed to rise from 60 °C to 300 °C at $10\,°C\,min^{-1}$. Compounds were identified by matching their mass spectra against the NIST library database.

Data analysis

This comprehensive approach provided detailed evaluation of the phytochemical composition and bioactive potential underlying the medicinal value of *A. elatus* leaf extracts.

RESULTS AND DISCUSSION

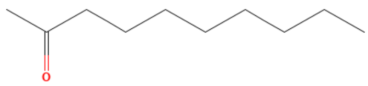
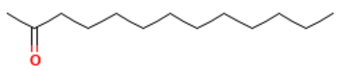
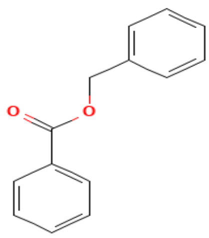
The leaf extract of *A. elatus* contains a variety of bioactive chemicals, according to preliminary phytochemical screening (Table 1). Flavonoids, phenolics, tannins, and chlorogenic compounds were abundant and are known to act as natural antioxidants by donating electrons or hydrogen atoms to neutralize reactive species. Triterpenoids and steroids may support membrane stability and help regulate inflammatory pathways. Saponins can interact with cell membranes due to their surfactant nature, while glycosides may release active aglycones during metabolism. Basic nutrients such as proteins, carbohydrates, and amino acids further contribute to the extract's biological value. Trace amounts of volatile oils were detected, although fixed oils were absent. Alkaloids were not present, suggesting that nitrogen-containing secondary metabolites are not a major component of this extract.

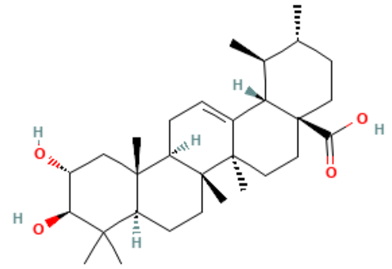
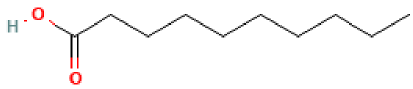
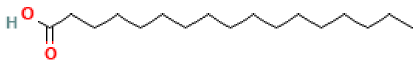
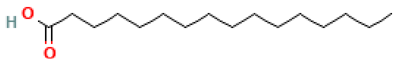
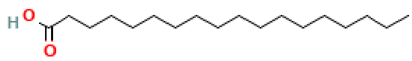
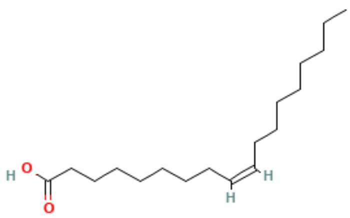
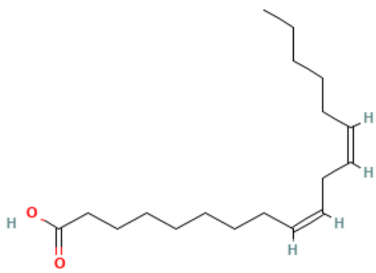
Table 1. Phytochemical constituents detected in the aqueous extract of *A. elatus*.

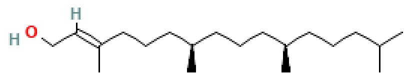
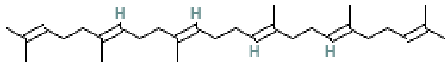
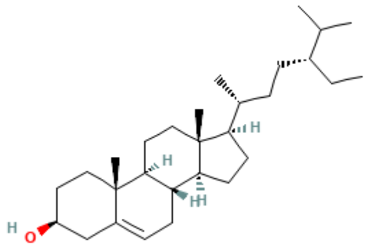
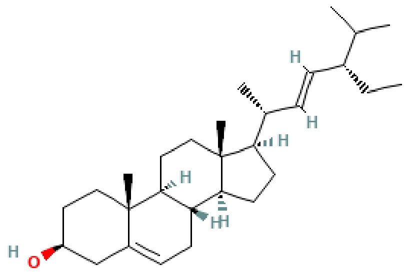
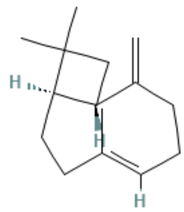
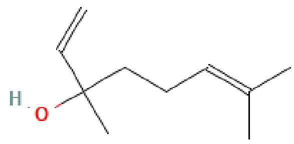
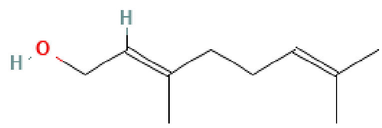
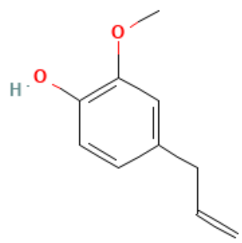
Phytochemical Class	Presence (+) / Absence (-)
Alkaloids	–
Flavonoids	+
Triterpenoids	+
Phenolic compounds	+
Proteins	+
Carbohydrates	+
Saponins	+
Steroids	+
Glycosides	+
Amino acids	+
Tannins	+
Oils (Fixed/Volatile)	– / + (trace)
Chlorogenic compounds	+

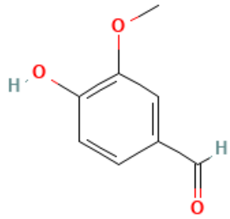
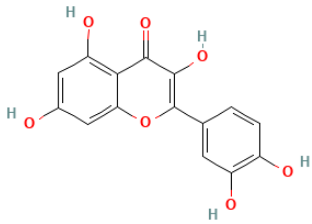
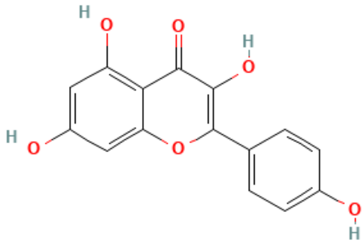
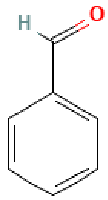
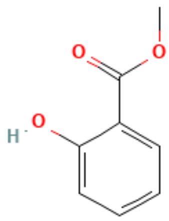
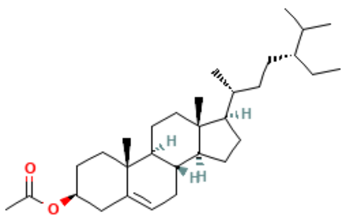
GC–MS analysis of *A. elatus* confirmed the presence of several bioactive molecules (Table 2). The major compounds, including 2-decanone and 2-tridecanone, may contribute mild antimicrobial effects through disruption of microbial membrane lipids. Corosolic acid, detected at 6.89%, is a triterpenoid recognized for its antioxidant and hepatoprotective mechanisms, mainly by reducing oxidative stress and enhancing cellular antioxidant enzymes. Fatty acids such as oleic, linoleic, palmitic, and stearic acids can modulate membrane integrity and influence lipid metabolism, which may indirectly support liver function during ammonia stress. Phytol and squalene, both strong antioxidants, help scavenge reactive oxygen species, while phytosterols like β-sitosterol and stigmasterol can stabilize hepatocyte membranes and reduce inflammatory signaling. Minor phenolic constituents such as eugenol, vanillin, quercetin, and kaempferol are known to inhibit lipid peroxidation and regulate detoxifying enzymes, suggesting potential roles in antihyperammonemic and hepatoprotective pathways.

Table 2. GC–MS detected compounds with corresponding chemical structures in *A. elatus* extract.

Peak	Compound	Formula	Mol. Wt (g·mol⁻¹)	RT (min)	Peak area (%)	structure
1	2-Decanone	$C_{10}H_{20}O$	156.27	3.5	11.81	
2	2-Tridecanone	$C_{13}H_{26}O$	198.35	4.3	9.84	
3	Benzyl benzoate	$C_{14}H_{12}O_2$	212.25	5.1	7.87	

4	Corosolic acid	$C_{30}H_{48}O_4$	472.71	5.9	6.89	
5	Decanoic acid	$C_{10}H_{20}O_2$	172.27	6.7	5.91	
6	Docosane	$C_{22}H_{46}$	310.61	7.5	4.92	
7	Heneicosane	$C_{21}H_{44}$	296.58	8.3	4.92	
8	Heptacosane	$C_{27}H_{56}$	380.74	9.1	3.94	
9	Heptadecane	$C_{17}H_{36}$	240.47	9.9	3.94	
10	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.46	10.7	3.94	
11	Palmitic acid	$C_{16}H_{32}O_2$	256.43	11.5	2.95	
12	Stearic acid	$C_{18}H_{36}O_2$	284.48	12.3	2.95	
13	Oleic acid	$C_{18}H_{34}O_2$	282.47	13.1	2.95	
14	Linoleic acid	$C_{18}H_{32}O_2$	280.45	13.9	2.95	

15	Phytol	$C_{20}H_{40}O$	296.54	14.7	2.95	
16	Squalene	$C_{30}H_{50}$	410.73	15.5	2.95	
17	β-Sitosterol	$C_{29}H_{50}O$	414.72	16.3	1.97	
18	Stigmasterol	$C_{29}H_{48}O$	412.7	17.1	1.97	
19	Caryophyllene	$C_{15}H_{24}$	204.36	17.9	1.97	
20	Linalool	$C_{10}H_{18}O$	154.25	18.7	1.97	
21	Geraniol	$C_{10}H_{18}O$	154.25	19.5	1.97	
22	Eugenol	$C_{10}H_{12}O_2$	164.2	20.3	1.48	

23	Vanillin	$C_8H_8O_3$	152.15	21.1	1.48	
24	Quercetin	$C_{15}H_{10}O_7$	302.24	21.9	0.98	
25	Kaempferol	$C_{15}H_{10}O_6$	286.24	22.7	0.98	
26	Benzaldehyde	C_7H_6O	106.12	23.5	0.98	
27	Methyl salicylate	$C_8H_8O_3$	152.15	24.3	0.79	
28	Phytosterol acetate	$C_{31}H_{52}O_2$	456.75	25.1	0.69	
29	Hexadecanal	$C_{16}H_{32}O$	240.43	25.9	0.59	

30	Nonadecane	$C_{19}H_{40}$	268.53	26.7	0.49	

The phytochemical screening of *A. elatus* aqueous leaf extracts demonstrated the presence of a broad range of secondary metabolites, including flavonoids, phenolic compounds, tannins, chlorogenic compounds, saponins, steroids, and triterpenoids. These classes of compounds are widely associated with antioxidant, anti-inflammatory, and hepatoprotective activities in medicinal plants (Harborne, 1998; Kokate, 2005). The strong polyphenolic profile identified in the present study agrees with earlier findings on *Anoectochilus roxburghii*, a related species known for its antioxidant and immunomodulatory properties attributed to similar groups of flavonoids, phenolics, and triterpenoids (Tiwari *et al.*, 2011). The presence of primary metabolites such as carbohydrates, proteins, and amino acids further highlights the nutritional significance of *A. elatus*. Saponins and steroids detected in the extract may contribute to antimicrobial and cytoprotective effects, consistent with pharmacological activities reported for *Anoectochilus roxburghii* extracts (Prashant *et al.*, 2011).

The GC-MS analysis of *A. elatus* revealed a rich phytochemical profile, encompassing fatty acids, alkanes, terpenes, terpenoids, steroids, and flavonoids. Key compounds identified include 2-Decanone (11.81%), 2-Tridecanone (9.84%), Benzyl benzoate (7.87%), and Corosolic acid (6.89%). The presence of squalene (Ng *et al.*, 2019), phytol (Juarez-Vazquez *et al.*, 2021), and other terpenoids, along with phytosterols like β-Sitosterol (Hasan and Kadhim, 2018; Ng *et al.*, 2019) and stigmasterol (Bihana *et al.*, 2018), suggests potential anti-inflammatory, antioxidant, and anti-proliferative activities. Flavonoids such as quercetin and kaempferol, also found in *Anoectochilus roxburghii* (Zou *et al.*, 2025), contribute to the plant's antioxidant capacity. This wide range of compounds reflects the medicinal richness often reported in related *Anoectochilus* species, suggesting that *A. elatus* may share similar therapeutic benefits. The presence of these bioactive constituents highlights its potential value in traditional and modern medicine and supports the need for further studies to clarify the specific biological roles of each compound.

CONCLUSION

This study shows that the aqueous leaf extract of *A. elatus* has a rich phytochemical profile that includes triterpenoids, flavonoids, phenolic compounds, tannins, chlorogenic chemicals, saponins, and steroids. These constituents all work together to enhance the pharmacological potential of the extract. Several bioactive components were found by GC-MS profiling, with corosolic acid and β-sitosterol predominating in the extract. The concentration of these phytochemicals indicates that *A. elatus* may be able to reduce oxidative stress and cellular damage by modulating important inflammatory and apoptotic pathways linked to hyperammonemia. More molecular, biochemical, and pre-clinical research is necessary to confirm these effects and establish *A. elatus* as a trustworthy natural therapeutic Properties for hyperammonemia and associated pathological disorders.

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CONFLICT OF INTERESTS

The authors have no competing interests to declare that are relevant to the content of this article.

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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