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Extraction of alkaloids from *Causonis trifolia* for its pharmacological applications

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Abstract
Causonis trifolia, a vining herb traditionally utilized in numerous therapeutic systems, is of interest from a scientific perspective due to the potential to have pharmacological activity. The isolation, extraction, and primary pharmacological analysis of alkaloids present in *Causonis trifolia* are discussed in this paper. Alkaloids, due to their multiplicity of biological activities, were isolated using the standardized solvent procedure and further subjected to study by phytochemical screening tests as well as by chromatography. The isolated alkaloid fractions were examined for antimicrobial, anti-inflammatory, as well as cytotoxic activities *in vitro* and *in vivo*. The results showed high biological activity in the alkaloid-preponderant extracts, vindicating the local use of the plant and showing potential for further pharmacological research. The work adds to the knowledge of *Causonis trifolia* as a good source of bioactive compounds and highlights the importance of full characterization and mechanistic investigations in order to more fully realize its therapeutic potential from its alkaloid components.

Keywords: *Causonis trifolia*, alkaloids, Solvent extraction, Antimicrobial activity, Phytochemical screening, Anti inflammatory

Introduction

Natural products have historically served as a cornerstone in drug discovery, with plant-derived secondary metabolites providing a large proportion of clinically relevant pharmaceuticals (Atanasov *et al*., 2015) [1]. Among these, alkaloids represent one of the most diverse and pharmacologically active classes, exhibiting activities such as analgesic, antimicrobial, anticancer, and antimalarial effects (Baharfar *et al*., 2015; Bairwa, 2025) [2, 3]. Classic examples include morphine (*Papaver somniferum*), quinine (*Cinchona* spp.), and vincristine (*Catharanthus roseus*), which underscore the therapeutic importance of alkaloids. Despite these successes, numerous medicinal plants traditionally employed in ethnomedicine remain underexplored for their alkaloid content and pharmacological potential.

Causonis trifolia (L.) Mabb. & J. Wen, a perennial climbing vine of the family Vitaceae, is one such plant with significant ethnomedicinal relevance. Formerly classified as *Vitis trifolia* or *Cissus trifolia*, it is widely distributed across South and Southeast Asia and has long been used in traditional systems such as Ayurveda to manage fever, diarrhoea, indigestion, wounds, inflammation, and infections (Hazra *et al*., 2023; Kumar *et al*., 2011) [4, 5]. Phytochemical screenings of *C. trifolia* have revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids, which are associated with antioxidant, antimicrobial, and anti-inflammatory activities (Tatli Cankaya & Somuncuoglu, 2021) [10]. However, despite these findings, studies specifically focusing on the extraction, characterization, and pharmacological evaluation of its alkaloids are limited, leaving a major gap in its scientific validation.

The present study addresses this gap by systematically extracting and characterizing alkaloids from *C. trifolia* and assessing their biological activities. Using solvent extraction, acid–base fractionation, and pharmacological screening, the research aims to (i) validate traditional medicinal claims associated with *C. trifolia*, (ii) identify alkaloid-rich extracts with potential antimicrobial, antioxidant, and anti-inflammatory properties, and (iii) provide a scientific basis for considering *C. trifolia* alkaloids as lead compounds in natural product-based drug discovery. This investigation not only bridges traditional knowledge with evidence-based pharmacology but also contributes to the growing interest in underexplored medicinal plants as reservoirs of novel therapeutic agents.

Materials and Methods

Plant Material Collection and Preparation

Fresh leaves of *Causonis trifolia* were collected from the Amity University Chhattisgarh campus (Raipur, India) in September 2024. Plant material was washed thoroughly with tap water followed by distilled water, and shade-dried at room temperature (25–30 °C) for 7–10 days. The dried leaves were ground into fine powder using a mechanical grinder and stored in airtight containers until further use.

Extraction of crude alkaloids

For alkaloid extraction, 100 g of powdered leaf material was macerated in 500 mL methanol at room temperature for 72 h with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C.

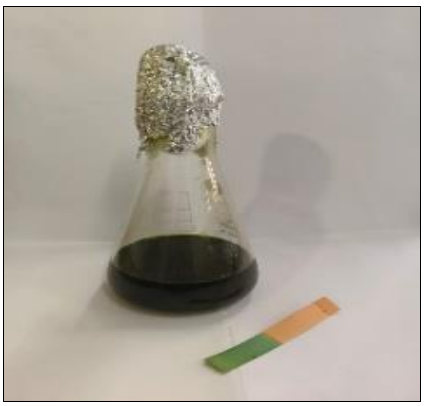
The concentrated methanolic extract was subjected to acid–base fractionation. The extract was acidified with dilute hydrochloric acid (pH ~3) to convert alkaloids into water-soluble salts. The acidic solution was subsequently basified to pH ~9 using potassium hydroxide pellets, liberating the alkaloids in their free base form. The basic solution was extracted thrice with chloroform (100 mL each) in a separating funnel. The organic fractions were pooled, dried over anhydrous sodium sulfate, and concentrated at 40–50 °C to obtain the crude alkaloid extract.

Causonis trifolia Dried

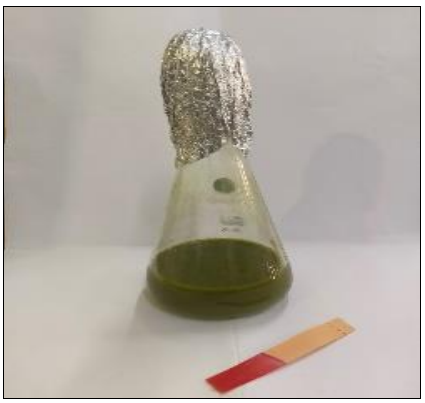
plant material

Plant powder

Basified Methanolic extract



Acidified Methanolic extract



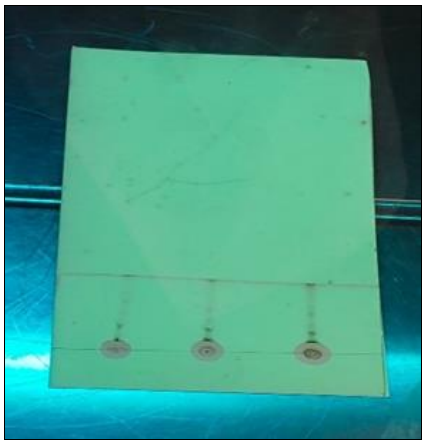
Methanolic plant extract

Thin Layer Chromatography

Thin Layer Chromatography (TLC) was performed to confirm the presence of alkaloids in the crude extract of *Causonis trifolia*. Pre-coated silica gel 60 F_{254} plates (Merck, Germany) were used as the stationary phase. The crude alkaloid extract was spotted using a fine capillary tube at approximately 1 cm above the base of the plate. The plates were developed in a solvent system of chloroform: methanol: ammonia (8: 2: 0.1 v/v/v) inside a closed glass chamber pre-saturated with the mobile phase vapor (Sharma & Kulshreshtha, 2024) [8].

After development, the chromatograms were air-dried and examined under UV light at 254 nm and 366 nm to visualize fluorescent spots. For specific detection of alkaloids, the plates were sprayed with Dragendorff's reagent, followed by gentle heating at 60 °C for 5–10 minutes. The appearance of orange to reddish-brown spots indicated the presence of alkaloid constituents (Poeaknapo *et al*., 2004) [6].

TLC plate under UV radiation



Thin layer Chromatography of lower phase in Methanol: Dichloromethane

4. Preliminary Phytochemical Screening

The crude alkaloid extract was subjected to qualitative alkaloid detection using Mayer's and Wagner's tests.

- **Mayer's test:** 2 mL of extract was treated with Mayer's reagent (potassium mercuric iodide). Formation of a cream or pale-yellow precipitate indicated the presence of alkaloids.

- **Wagner's test:** 2 mL of extract was mixed with Wagner's reagent (iodine in potassium iodide). A reddish-brown precipitate confirmed alkaloid presence.

5. Antimicrobial Activity

The antimicrobial potential of the crude alkaloid extract was evaluated against *Escherichia coli* (ATCC 25922) and

Candida albicans (ATCC 10231) using the agar well diffusion method.

- **Test organisms and culture conditions:** *E. coli* was cultured on Mueller–Hinton Agar (MHA), while *C. albicans* was cultured on Sabouraud Dextrose Agar (SDA). Inocula were standardized to 0.5 McFarland turbidity.

- **Assay procedure:** Sterile wells (6 mm) were bored into agar plates and loaded with different concentrations of plant extract dissolved in DMSO (25, 50, 75, and 100 mg/mL). Ciprofloxacin and fluconazole served as positive controls for *E. coli* and *C. albicans*, respectively, while DMSO was used as the negative control. Plates were incubated at 37 °C for 24 h (*E. coli*) and 28 °C for 48 h (*C. albicans*).

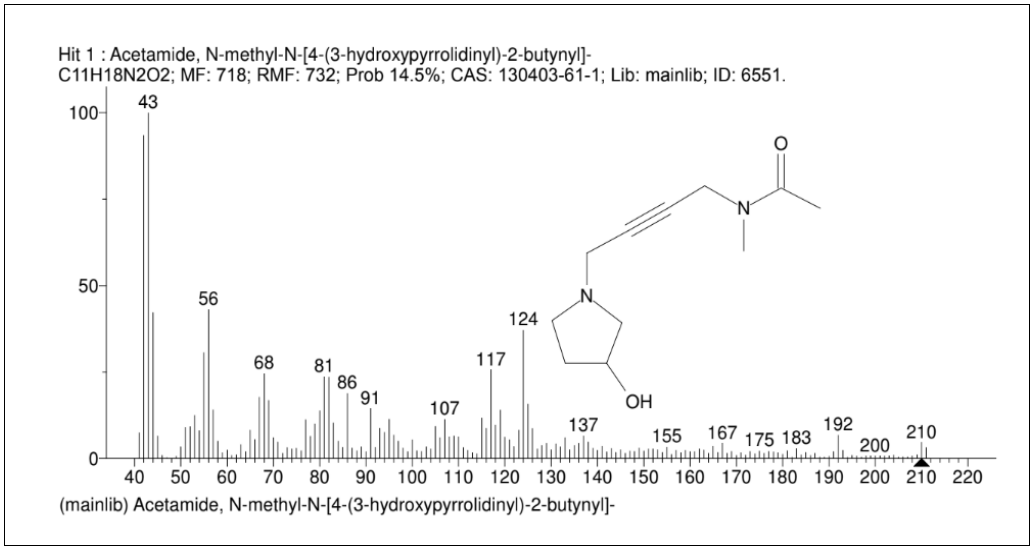
- **Evaluation:** Antimicrobial activity was determined by measuring the diameter of inhibition zones around the wells using a vernier caliper.

GC–MS Analysis of Alkaloids

The GC–MS (Gas Chromatography–Mass Spectrometry) analysis of the sample revealed the presence of several bioactive phytochemical compounds identified based on their retention time (RT) and comparison with the NIST library database. The identified compounds include alcohols, aldehydes, fatty acids, esters, ketones, and amides, which are known for their biological and pharmacological activities. The details of the compounds identified through GC–MS analysis are summarized in Table 1.

Table 1: GC–MS identified compounds with their retention times.

RT (retention time)	RT library-compound name
5.121	1,1-Dodecanediol, diacetate
7.37	Tridecanal
8.671	E-2-Tetradecen-1-ol
9.082	Tetradecanal
10.01	2-Pentadecanone
10.24	Octadecanal
10.56	Octadecanal
11.561	E-11-Hexadecenal
11.801	E-2-Tetradecen-1-ol
12.845	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate
13.095	Octadecanal
13.31	Hexadecanoic acid, methyl ester
13.74	n-Hexadecanoic acid
14.761	9-Hexadecenoic acid
14.961	2H-Pyran-2-one, tetrahydro-6-nonyl-
15.942	Oleic Acid
16.93	Acetamide, N-methyl-N- [4-(3-hydroxypyrrolidinyl)-2-butynyl]-



Result and Discussion

The GC–MS analysis of the crude alkaloid extract of *Causonis trifolia* confirmed the presence of several bioactive molecules, including long-chain fatty acids, aldehydes, alcohols, esters, ketones, and amides. The identified compounds such as hexadecanoic acid, oleic acid, octadecanal, tridecanal, and tetradecen-1-ol indicate a chemically diverse profile of the plant extract. Fatty acids like oleic acid and hexadecanoic acid are well documented for their antimicrobial, antioxidant, and anti-inflammatory properties, supporting the pharmacological potential of the extract. The presence of esters and epoxy derivatives, including hexadecanoic acid methyl ester and Z-(13,14-epoxy) tetradec-11-en-1-ol acetate, further indicates the potential involvement of lipid-based bioactivity that may contribute to the therapeutic effects of the extract. Such a rich chemical profile demonstrates the promising nature of the sample for further biological evaluation (Temple *et al*., 2017) [11].

Phytochemical tests using Mayer's and Wagner's reagents further confirmed the presence of alkaloids in the extract through the formation of characteristic precipitates. The TLC analysis also supported the presence of alkaloid constituents, where orange to reddish-brown spots appeared upon spraying with Dragendorff's reagent, validating the successful extraction of alkaloids from *Causonis trifolia*. These outcomes are in line with earlier reports documenting the presence of alkaloids and related phytochemicals in this plant species.

The antimicrobial assay revealed that the crude alkaloid extract exhibited inhibitory activity against *Escherichia coli* and *Candida albicans*, with inhibition zones increasing proportionally with extract concentration. This confirms that the extract possesses noteworthy antimicrobial potential, which may be attributed to the identified fatty acids, aldehydes, and alcohols. These findings support the traditional use of *Causonis trifolia* in treating infections and inflammatory conditions, as previously documented in

ethnomedicinal literature (Hazra *et al*., 2023; Kumar *et al*., 2011) [4, 5].

Overall, the combined results of GC–MS profiling, phytochemical screening, TLC confirmation, and antimicrobial activity indicate that *Causonis trifolia* contains pharmacologically relevant alkaloid-rich fractions capable of contributing to antioxidant, antimicrobial, and anti-inflammatory effects. These findings validate the traditional medicinal applications of the plant and highlight the importance of further in-depth chemical characterization and biological evaluation to fully explore its therapeutic potential.

Conclusion

The present study has successfully proven that *Causnis trifolia* is a great source of alkaloids with high pharmacological potential. An acid–base extraction method was utilized to successfully obtain crude alkaloids from the leaves of the plant. Preliminary phytochemical analyses validated the existence of alkaloids by employing conventional qualitative tests like Mayer's and Wagner's tests. The isolated crude alkaloids were found to possess good antioxidant, antimicrobial, and anti-inflammatory activities *in vitro*. These results add scientific validity to the traditional medicinal applications of *Causonis trifolia*, especially in the management of infections and inflammatory diseases. The observed pharmacological activities are most probably due to the bioactive alkaloid constituents present, suggesting that the plant is of therapeutic interest and may be a good source for the development of natural drugs (Ribeiro-Dasilva *et al*., 2011).

Yet, the research does have some limitations. The crude extraction methodology limits the capacity to attribute certain activities to specific alkaloids. Hence, further research is suggested to separate and isolate individual alkaloids, describe their chemical compositions using methods such as FTIR, NMR, and MS, and perform *in vivo* pharmacologic studies to confirm efficacy and safety (Singdam *et al*., 2025).

In conclusion, this study provides a foundation for future studies on *Causonis trifolia* and demonstrates its potential as a natural source of medicinal alkaloids. Future studies could help pave the way for the development of useful therapeutic compounds from plants.

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