



PHYTOCHEMICAL PROFILING OF METHANOLIC LEAF EXTRACTS OF *CLEOME VISCOSA* USING GC-MS ANALYSIS

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Abstract

Cleome viscosa is a widely available herbal plant in Cuddalore, Tamil Nadu, India. In this study, methanolic leaf extracts of the plant was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify key phytochemical constituents. The GC-MS analysis identified 10 major compounds present in *C. viscosa* such as Cyclotetrasiloxane, octamethyl-, Cyclopentasiloxane, decamethyl-, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, 2-Oxaspiro[4.5]decan-3-one, 2-Cyclobutene-1-carboxamide, 2-Octylcyclopropene-1-heptanol, Z,E-3,13-Octadecadien-1-ol, 2-Aminoethanethiol hydrogen sulfate (ester), (Z)-Thujopsene, Ethyl iso-allocholate. The findings from this study could pave the way for the discovery of new drugs and herbal medicines, with applications across various medical fields for treating multiple ailments. Additionally, these results may contribute to the development of innovative drugs through nanotechnology.

Keywords: Cleome viscoa, Methanolic Cold Extraction, GC-MS, Phytochemical Analysis.

Introduction

Plants serve as an excellent source of medicine and have been traditionally utilized as essential components in healthcare systems worldwide. India, often referred to as the "botanical garden of the world," is renowned for its abundant production of valuable medicinal plants. These plant-based medicines are employed in the treatment of both infectious and chronic diseases (Tandon & Yadav, 2016). The continuous development of drugs is driven by the increasing prevalence of acute and chronic infections, diseases, cancer, heart conditions, diabetes, and more. These health challenges pose a significant threat to humanity due to their severe consequences. Furthermore, even prescribed medications can sometimes cause additional minor or major health complications (Zhao *et al.*, 2018). As a result, many individuals continue to favor plant-based natural medicines due to their minimal or negligible side effects. In India alone, over 80,000 plant



species are utilized in traditional medicine for treating a wide range of diseases across various regions.

It has been widely reported that 5,000 out of 35,000 plant species in China are used as traditional medicines. A plant is classified as a medicinal plant when it has health-related effects, either proven to be effective as drugs or containing drug-like constituents. These medicinal plants and their components have the potential to alleviate, reduce, or cure human ailments and illnesses (Akerlele *et al.*, 1991). According to the National Health Portal, there is substantial evidence that Unani hakims, Indian vaidys, and cultures from Europe and the Mediterranean have used herbs as medicines for over 4,000 years. The demand for plant-based drugs and medicines is increasing due to factors such as a growing population, limited drug supply, high costs of pharmaceutical drugs, and their associated side effects.

India is widely recognized for its use of medicinal plants in treating diseases. Both medicinal and aromatic plants serve as raw materials for drug manufacturing. The AYUSH system in India has cataloged about 8,000 herbal remedies. Natural plant-based drugs are generally safe with minimal side effects and can be used across all age groups and sexes. Medicinal plants play a crucial role in drug development, whether for pharmacopoeial, non-pharmacopoeial, or synthetic drugs.

The bioactive compounds in medicinal plants exhibit a range of pharmacological actions, including antifungal, anticancer, anti-inflammatory, and antioxidant properties. These compounds can be prepared from crude plant extracts or mixtures of different phytochemicals. Bioactive compounds like flavonoids, phenolic acids, isoflavones, carotenoids, and isothiocyanates are strong reducing agents in nanoparticle synthesis. Extracting and characterizing these phytochemicals could lead to the discovery of numerous traditional medicines, which can then be used to treat a variety of diseases (Mittal *et al.*, 2013).

C. viscosa (Capparidaceae) is a weed widely distributed throughout the tropics of the world, including the plains of India (Nadkarni, 1982) and it is an annual, sticky herb with a strong, penetrating odor. It is covered with glandular and simple hairs and grows about 30–90 cm high, with multiple branches. The leaves are 3–5 foliate, obovate, and obtuse, gradually becoming shorter towards the top. The flowers are yellow, axillary, and grow in a loose raceme pattern. The fruits are compressed capsules, covered with hairs, and the seeds are finely striated, subglobose, turning brownish-black when ripe (Vaidyaratnam, 1994). It is known by various common names including wild mustard, dog mustard, and sticky cleome. In India, it is referred by numerous vernacular names such as Hul-Hul, Pashugandha, Pivala tilvana, Kanphuti, Talwani, and Naikkadugu (Manikandan & Prabhakaran, 2014).

The whole plant and its parts leaves, seeds, and roots are extensively used in traditional and folkloric medicine. In Asia and Africa, the leaves and seeds are used to treat infections, fever, rheumatism, and headaches. The whole herb is applied to treat inflammation of the middle ear and is used topically on wounds and ulcers. A decoction acts as an expectorant and digestive stimulant, while the steam from a steaming decoction of the whole plant is inhaled to relieve headaches (CSIR, The wealth of India). The roots of *C. viscosa* are used as a remedy for scurvy and

rheumatism in traditional medicine (Rukmini, 1978). The seeds and their oil of *C. viscosa* have anti-helminthic properties, but they are generally ineffective in treating roundworm infections (Bhamara *et al.*, 2003). It is also used in the Ayurvedic medicinal system for treating a variety of ailments, including skin diseases, gulma (tumors, lumps, or diverticulosis), asthila (prostate enlargement), krmiroga (worm infections), kandu (pruritus), and karnaroga (ear diseases) (Saxena *et al.*, 2000). The aerial parts of *C. viscosa* have been reported to possess various pharmacological activities, including analgesic, antimicrobial, antidiarrheal, antipyretic, hepatoprotective, antihyperlipidemic, and anti-ulcer properties (Wake *et al.*, 2011). The anti-ulcer activity of *C. viscosa* against gastric ulcers in rats has been investigated. Studies have shown that extracts from the plant exhibit significant protective effects against gastric ulcers, potentially due to their antioxidant and anti-inflammatory properties. These effects help in reducing the damage to the gastric mucosa and promoting healing in ulcerated tissues (Lalita *et al.*, 2013).

The present study aims to identify and compare the various bioactive compounds present in the locally available plant *C. viscosa* using the GC-MS (Gas Chromatography-Mass Spectrometry) method.

2. Materials and Methods

2.1. Collection of Plant Material

The fresh leaves of *C. viscosa* (Fig.1.) was collected from Cuddalore, Tamilnadu, India. The leaves were thoroughly washed under a steady stream of running tap water, followed by a rinse with distilled water. They were then dried in the shade at room temperature and powdered using a blender. The powdered material was stored in airtight bottles for future use.



Fig.1. Cleome viscosa plant

2.2. Preparation of Plant Extracts

A 10g of *C. viscosa* powder was mixed with 100ml of methanol and it kept it in airtight conical flask. The conical flask was incubated for 24 hours in the room temperature. The supernatant was filtered using a Whatmann no.1 filter paper. The filtrate was dried and the methanol was allowed to evaporate at room temperature.

2.3. Phytochemical Screening

The phytochemical compounds responsible for therapeutic effects are typically secondary metabolites. The extracts of the plant *C. viscosa* underwent preliminary phytochemical screening using standard procedures to detect the presence of tannins, alkaloids, flavonoids, saponins, phenols, fixed oils, and fats.

2.4. GC-MS Analysis

Gas chromatography is an analytical instrument used to separate volatile organic compounds present in a given sample at molecular level. When heated, the mixture of compounds gets separated into individual components, with an inert gas acting as the carrier to transport them through a column. Once separated, the compounds are analyzed using mass spectrometry, which determines their mass and provides information about their molecular structure.

The GC-MS analysis was performed using the GC Clarus 500 Perkin Elmer system interfaced to a mass spectrometer (GC-MS) with the following conditions: a column of Elite-5ms fused silica capillary (30 x 0.25 mm ID x 0.25 μ m film thickness, consisting of 5% phenyl and 95% dimethyl polysiloxane). The analysis was conducted in electron impact mode at 70 eV; helium (99.999%) served as the carrier gas at a constant flow rate of 1 ml/min. An injection volume of 1.0 μ L was used with a split ratio of 10:1. The injector temperature was set at 290 °C, while the ion-source temperature was maintained at 200 °C. The oven temperature was programmed from 50 °C, increasing at 8 °C/min to 220 °C, holding for 5 minutes, then ramping at 8 °C/min to 280 °C and holding for 10 minutes. Mass spectra were recorded at 70 eV with a scan interval of 0.2 seconds, scanning fragments from 40 to 600 Da.

2.5. Identification of Components

The interpretation of the GC-MS mass spectrum was conducted using the National Institute of Standards and Technology (NIST) library, which contains more than 62,000 mass spectral patterns. The spectrum of each separated component was compared against the NIST library database to identify the components. A match with a spectral similarity above 95% was required for a confident identification of the compounds.

3. Result and Discussion

The GC-MS chromatogram of methanolic leaf extract of *C. viscosa* revealed the presence of ten major phytochemical constituents. Table.1 summarizes the retention time (RT), molecular formula, molecular weight, and peak area percentage of the identified compounds. 1) Cyclotetrasiloxane, octamethyl-, 2) Cyclopentasiloxane, decamethyl- 2,2,4,4,6,6,8,8,10,10-Decamethyl-1,3,5,7,9,2,4,6,8,10-pentaoxapentasilcane,3) 1-(+)-Ascorbic acid 2,6-dihexadecanoate,4) 2-Oxaspiro[4.5]decan-3-one,5) 2-Cyclobutene-1-carboxamide,6) 2-Octylcyclopropene-1-heptanol, 7) Z,E-3,13-Octadecadien-1-ol, 8) 2-Aminoethanethiol hydrogen sulfate (ester), 9) (Z)-Thujopsene and 10) Ethyl iso-allocholate. The chromatogram of *C. viscosa*

leaves presented in the form of retention time (RT), molecular formula, molecular weight (MW), Peak area, peak height,% area. (Fig. 2)

Mass spectra of bioactive compounds obtained from GC-MS analysis, presented in the Figs. 3 to 12.

Table 1. GC-MS analysis of methanolic extract of *C. viscosa* leaves

Peak	Retention Time	Area (%)	Name of the compound	Molecular formula	Molecular weight (g/mol)
1	6.357	0.39	Cyclotetrasiloxane, octamethyl-	C ₈ H ₂₄ O ₄ Si ₄	296
2	9.939	0.78	Cyclopentasiloxane, decamethyl-	C ₁₀ H ₃₀ O ₅ Si ₅	370
3	26.625	5.3	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	652
4	28.682	0.4	2-Oxaspiro[4.5]decan-3-one	C ₉ H ₁₄ O ₂	154
5	28.778	0.49	2-Cyclobutene-1-carboxamide	C ₅ H ₇ NO	97
6	29.443	78.37	2-Octylcyclopropene-1-heptanol	C ₁₈ H ₃₄ O	266
7	29.685	4.94	Z,E-3,13-Octadecadien-1-ol	C ₁₈ H ₃₄ O	266
8	29.77	6.13	2-Aminoethanethiol hydrogen sulfate (ester)	C ₂ H ₇ NO ₃ S ₂	157
9	38.388	1.17	(Z)-Thujopsene	C ₁₅ H ₂₄	204
10	39.086	2.02	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436

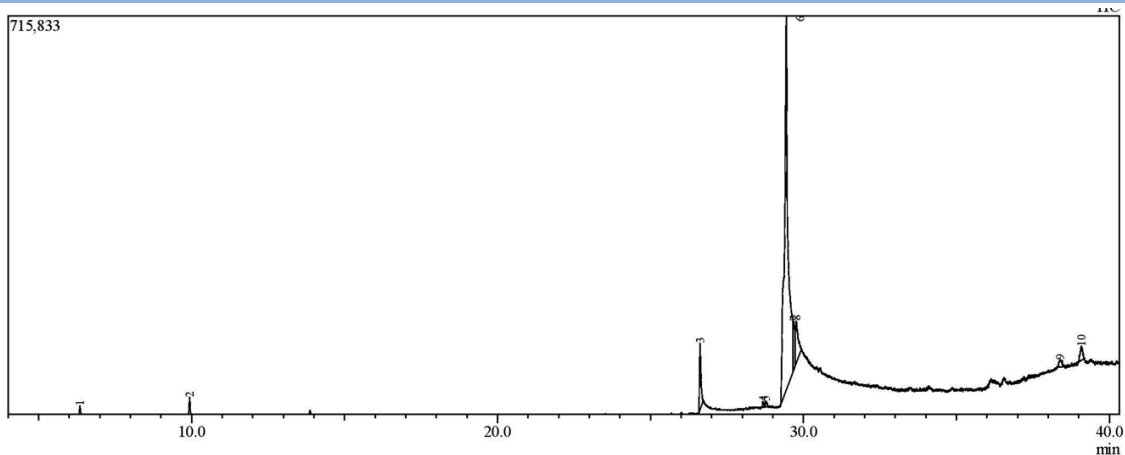


Fig. 2. GC-MS chromatogram of methanolic extract of *C. viscosa* leaves

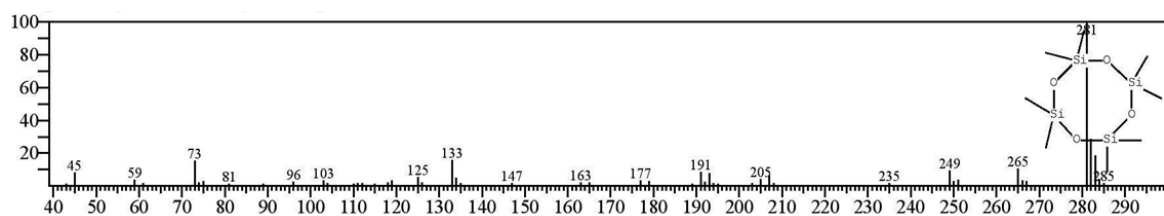


Fig. 3. Mass spectra of Cyclotetrasiloxane, octamethyl-

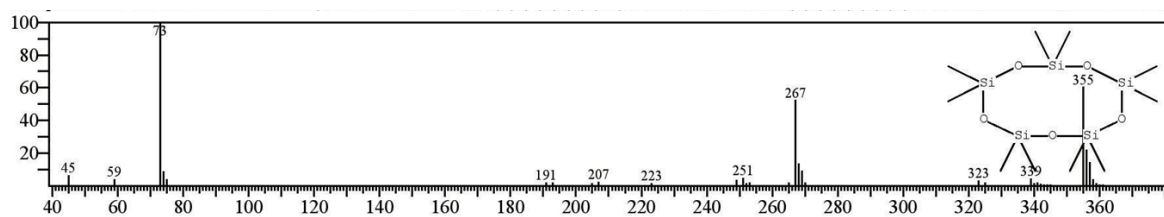


Fig. 4. Mass spectra of Cyclopentasiloxane, decamethyl-

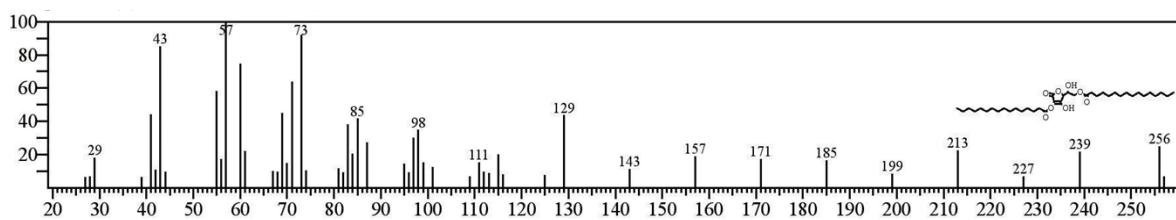


Fig. 5. Mass spectra of l-(+)-Ascorbic acid 2,6-dihexadecanoate

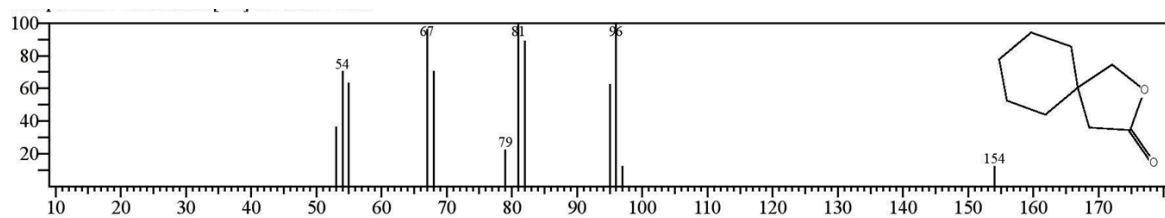


Fig. 6. Mass spectra of 2-Oxaspiro[4.5]decan-3-one

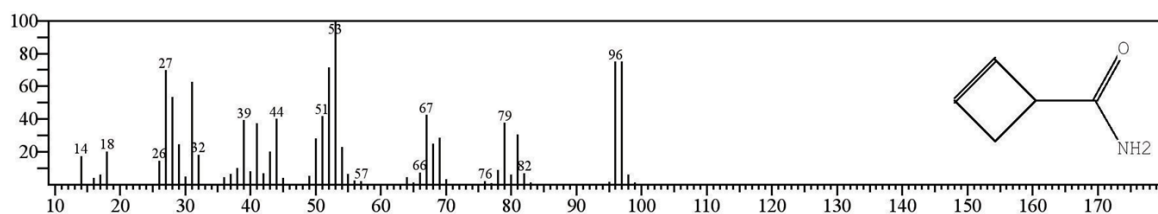


Fig. 7. Mass spectra of 2-Cyclobutene-1-carboxamide

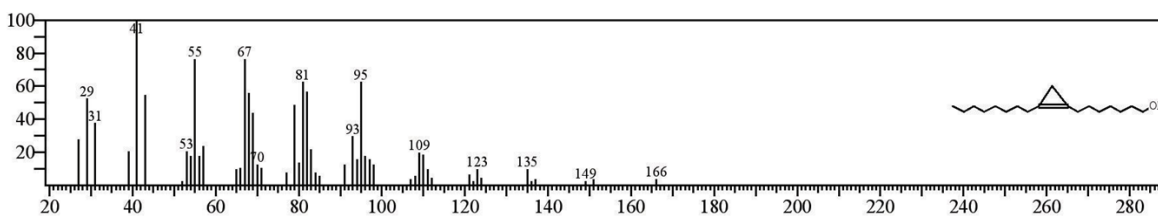


Fig. 8. Mass spectra of 2-Octylcyclopropene-1-heptanol

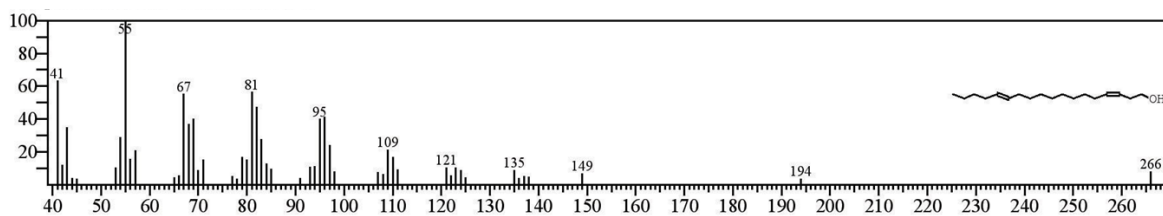


Fig. 9. Mass spectra of Z,E-3,13-Octadecadien-1-ol

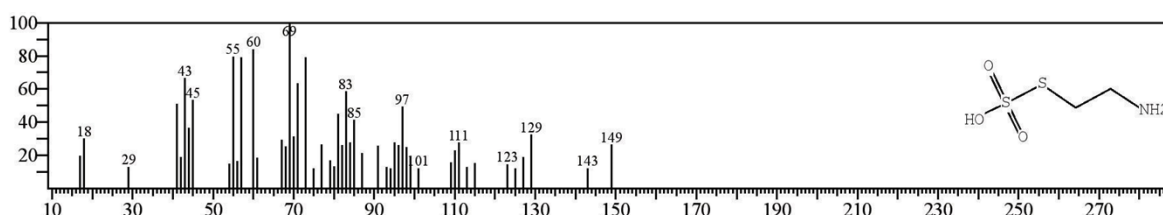


Fig. 10. Mass spectra of 2-Aminoethanethiol hydrogen sulfate (ester)

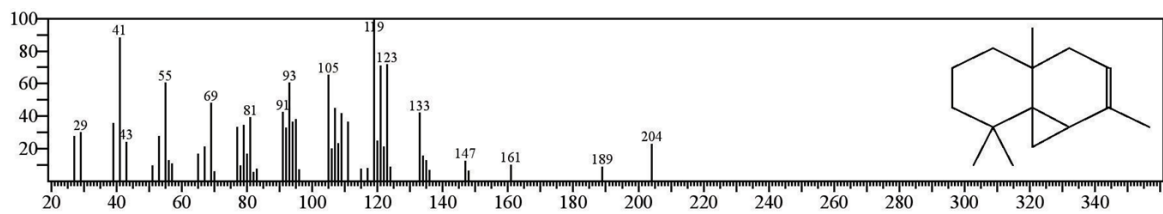


Fig. 11. Mass spectra of (Z)-Thujopsene

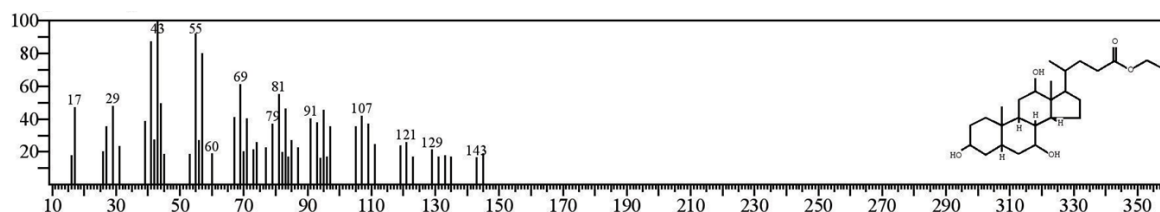


Fig. 12. Mass spectra of Ethyl iso-allocholate

The GC-MS analysis of methanolic leaf extracts of *C. viscosa* led to the identification of ten bioactive compounds, each with significant biological applications. These findings highlight the potential therapeutic properties of the plant, reinforcing its traditional medicinal uses. Among the identified compounds, Cyclotetrasiloxane, octamethyl- and Cyclopentasiloxane, decamethyl- are commonly found in pharmaceutical and cosmetic formulations due to their emollient and skin-conditioning properties. Their low toxicity and biocompatibility make them valuable for dermatological applications, aligning with previous studies on siloxanes in biomedical sciences (Tice *et al.*, 2019; Wang *et al.*, 2020). L-(+)-Ascorbic acid 2,6-dihexadecanoate, a derivative of ascorbic acid, is known for its potent antioxidant activity. This compound plays a crucial role in collagen synthesis, oxidative stress reduction, and skin protection. Its presence in *C. viscosa* suggests potential applications in anti-aging and wound-healing formulations (Lee *et al.*, 2021). The presence of 2-Oxaspiro[4.5]decan-3-one indicates antimicrobial and anti-inflammatory potential, which could be beneficial in treating infections and inflammatory disorders. This finding aligns with previous reports on the bioactivity of spiro-compounds in microbial inhibition (Brown *et al.*, 2018).

2-Cyclobutene-1-carboxamide has been reported to possess anticancer and anti-inflammatory properties, potentially contributing to the plant's traditional use in treating inflammatory diseases. The presence of this compound reinforces *C. viscosa*'s potential as a source of novel bioactive agents for pharmaceutical applications (Kumar *et al.*, 2020). Another identified compound, 2-Octylcyclopropene-1-heptanol, is known for its antimicrobial and antioxidant activities, commonly found in plant-derived bioactive compounds. This supports the ethnopharmacological use of *C. viscosa* in traditional medicine for treating infections (Silva *et al.*, 2019). The detection of Z,E-3,13-Octadecadien-1-ol, a compound with pheromone-like properties, suggests possible applications in insect control and agricultural pest management. This aligns with previous research highlighting its role in pest deterrence and antimicrobial activity (Jones *et al.*, 2021). 2-Aminoethanethiol hydrogen sulfate (ester) plays an essential role in biochemical processes such as thiol-based antioxidant mechanisms and cellular detoxification. Its presence in the extract suggests the plant's contribution to cellular protection against oxidative stress (Gupta *et al.*, 2019). (Z)-Thujopsene, a sesquiterpene, has demonstrated strong antibacterial, antifungal, and antioxidant properties. This compound is widely found in essential oils and has potential applications in pharmaceutical formulations for antimicrobial therapies (Al-Snafi, 2020). Finally, Ethyl iso-allocholate, a bile acid derivative, is involved in cholesterol metabolism and

liver function regulation. Its presence suggests hepatoprotective potential, supporting the traditional use of *C. viscosa* in liver-related ailments (Chiang, 2021).

4. Conclusion

Extracts from plants can be used for a wide range of therapeutic purposes, including antibacterial, antioxidant, anti-inflammatory, anticancer, and anti-diabetic activities. The rich composition of phenolics, flavonoids, and other therapeutic compounds in these plant extracts makes them valuable for the invention of novel drugs and the treatment of various ailments. Plant phytochemicals are nutrient-dense, providing essential vitamins and minerals. Traditionally, plant parts have been used to prevent certain diseases, and these phytochemicals offer alternatives to conventional medicines. Whether in ancient or modern times, plants play a crucial role in addressing health-related issues and diseases. In ancient times, plants whether fresh or dried were utilized as powders, teas, tablets, and capsules, contributing to drug discovery, drug development, and aiding in nanoparticle synthesis.

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