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RESEARCH ARTICLE

Isolation and Structural Characterisation of Phytoconstituents of *Coccinia indica* Extract

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ABSTRACT

Coccinia indica is a medicinal plant because of its bioactive compounds. The purpose of this study was to isolate and identify the phytoconstituents of Coccinia indica extract using chromatographic and spectroscopic methods. Powdered C. indica leaves were successively extracted with chloroform, methanol, and ethyl acetate. Purification was carried out by Soxhlet extraction and column chromatography using silica gel as the stationary phase. Compound separation and analysis were performed using thin-layer and high-performance thin-layer chromatography. Infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry were used to determine the structures of the isolated compounds. A light-yellow crystalline substance (470 mg) with a molecular formula of $C_{29}H_{50}O$ and a molecular weight 414.71 g/mol. The melting point was 145°C, and the compound was soluble in both alcohol and chloroform. The Rf value was 0.55. The IR spectrum revealed the presence of functional groups such as hydroxyl (-OH) and aliphatic C-H stretching. ^{1}H-NMR spectroscopy revealed characteristic peaks that facilitated the structural elucidation. Mass spectrometry yielded a molecular ion peak at m/z 414, confirming the molecular structure. Phytochemical screening revealed the presence of carbohydrates, amino acids, phytosterols, triterpenoids, alkaloids, fats, oils, and flavonoids. This also indicated the absence of tannins, phenols, vitamin C, and saponins. This is the first study to isolate and identify the bioactive phytoconstituents of Coccinia indica. Chromatographic and spectroscopic characterisation elucidated its structure and indicated its value as a pharmacologically active molecule, with implications for the development of future drugs and therapies.

Keywords: Coccinia indica; Phytochemical Characterisation; Chloroform Extract

INTRODUCTION

Most contemporary research on herbal medicine has focused on traditional folk remedies. Modern medicine has introduced a wide range of pharmaceuticals, none of which are non-toxic and safe for human consumption. Hundreds of medicinal plants have a long history of curative properties against various diseases and conditions. Nonetheless, it is crucial to thoroughly screen these plants for their efficacy, which requires immediate attention to determine their value. The screening process involves examining the biological activity of plants based on either chemotaxonomic analysis or ethnobotanical knowledge of a specific disease. Identifying a compound that is effective against a particular disease is complex and time-consuming. In India, approximately 2,500 plant species are utilised for medicinal purposes, with approximately 90% of these medicinal plants serving as the primary source of raw materials for herbal pharmaceuticals that are harvested from their natural habitats[1].

Coccinia indica (C. indica), commonly known as ivy gourd, belongs to the family Cucurbitaceae and grows abundantly and widely across India. Various parts of the plant have been used to relieve diabetes mellitus[2]. In the Indian subcontinent, C. indica has been utilised in both Ayurveda and Unani medicine[3]. It is also used as a wild vegetable by indigenous people of Southeast Asia and India. Every part of C. indica has valuable medicinal resources and is effective in treating ringworms, psoriasis, smallpox, scabies[4], and other itchy skin eruptions and ulcers. C. indica has antidiabetic[5–9], hypoglycaemic[8,10,11], anti-inflammatory[12–14], hepatoprotective[15–17], antioxidant[18,19], antibacterial[20,21], antitussive[22], and analgesic[13] activities. The juice extracted from the roots is used to treat diabetes, whereas a tincture made from leaves is used to treat gonorrhoea. Additionally, a paste derived from the leaves is applied

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to the skin to treat various diseases[23]. The dried bark of this plant has cathartic properties. Furthermore, the leaves and stems possess antispasmodic and expectorant properties. The fleshy green fruit of this plant is known for its bitter taste. It is also used to treat tongue sores by chewing[21,24]. The plant's active components are linked to its compounds, which are directly responsible for the plant's therapeutic properties, and are called active pharmaceutical ingredients (API). They are mainly secondary metabolites, such as alkaloids, steroids, tannins, and phenolic compounds, as well as flavonoids, resins, fatty acids, and gums, which can elicit specific physiological responses in the body[25].

The pharmaceutical industry is concentrating on the creation of novel medications and plant-based treatments by examining leads from the traditional Ayurveda medical system, which has been practiced for thousands of years. As the pharmacological importance and active phytochemicals involved have not been thoroughly investigated, our primary objective was to isolate the active compounds from the aerial parts of C. indica.

METHODOLOGY

Extraction of Plant Material: The C. indica leaves were successively extracted with chloroform, methanol, and ethyl acetate in an extractor. Preliminary small-scale extraction was performed to determine the yield prior to pilot-scale extraction.

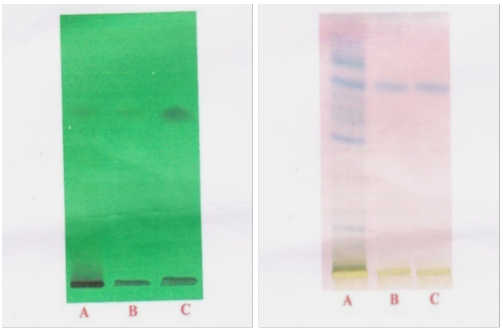
Extraction Process: Powdered leaves (200 g) were macerated with ethyl acetate (200 ml) for 3 h. This process was repeated three times, and the mixture was filtered to remove marc. The filtrate was concentrated to 2.5%. Similarly, powdered leaves were extracted with methanol (200 ml) and chloroform (200 ml) under the same conditions, yielding 3.0% and 5.0%, respectively. The column loading required extract was 100–200 g, and pilot-scale extraction was calculated as a percentage yield.

Purification: Dried aerial parts of C. indica (200 g) were extracted four times with methanol (500 ml) in a Soxhlet apparatus. The combined extract was filtered and the solvent was evaporated under reduced pressure, yielding 6 g of crude residue. The whole residue was extracted with chloroform (200 ml) four times using a Soxhlet apparatus, filtered, and concentrated to yield 4 g of crude residue. Because these compounds were more soluble in chloroform, column packing of the methanolic extract was not performed directly.

Column Chromatography: Column chromatography on a silica gel (60–200 mesh) stationary phase was performed on the chloroform extract. A glass column 120 cm in length and 10 cm in diameter was wet-packed with hexane. Dry hexane extract (4 g) was added to the column and elution was performed using a gradient of increasing polarity (hexane, benzene, chloroform, and methanol). The fractions were pooled and analysed using TLC. Fraction (58–61) gave a pale yellow crystalline solid (470 mg), which was further purified using HPTLC.

Thin Layer Chromatography (TLC): TLC was carried out using various solvent systems. The chloroform extract was analysed with a mobile phase of chloroform: methanol: ammonia (13.5:2.7:0.3), and spots were detected at 366 nm using an anisaldehyde-sulfuric acid spray reagent.

High-Performance Thin-Layer Chromatography (HPTLC): HPTLC analysis of the C. indica extract was performed on a CAMAG HPTLC system with a pre-coated silica plate (silica gel 60 F254, 10 × 10 cm, Merck). The three sample preparations were methanol-extracted, filtered, concentrated, and spotted. A mobile phase consisting of Chloroform: Methanol: Ammonia (13.5:2.7:0.3) was prepared and the chromatographic tank was saturated for 30 min. Samples were applied using a Linomat applicator, and the plate was left to evaporate the solvent prior to development. The plate was removed after ascending to 1.5 cm below the top, dried, and detected under UV 254 nm and anisaldehyde-sulfuric acid. (Figure 1 A and Figure 1 B)



A B

Fig. 1: A. Detection of HPTLC by UV at 245nm; B. Detection of HPTLC by anisaldehyde sulphuric acid spraying reagent

The synthesised compounds were identified and characterised as follows.

Melting Point Determination: Conducted via Thiel's tube method to assess purity.

Thin Layer Chromatography (TLC): Used for compound identification and reaction monitoring. Pre-coated silica plates with a Chloroform:Methanol:Ammonia (13.5:2.7:0.3) mobile phase were analysed under UV light.

Infrared (IR) Spectroscopy: Determined functional groups based on molecular vibrations. The spectra were recorded using a SHIMADZU FTIR 8400S spectrometer. (Figure 2)

Nuclear Magnetic Resonance (NMR) Spectroscopy: Structural analysis was performed using a Bruker Spectrospin-400 NMR spectrometer with chloroform and DMSO as the solvents.

Mass Spectroscopy: This analysis provided molecular weight, structural, and fragmentation insights through

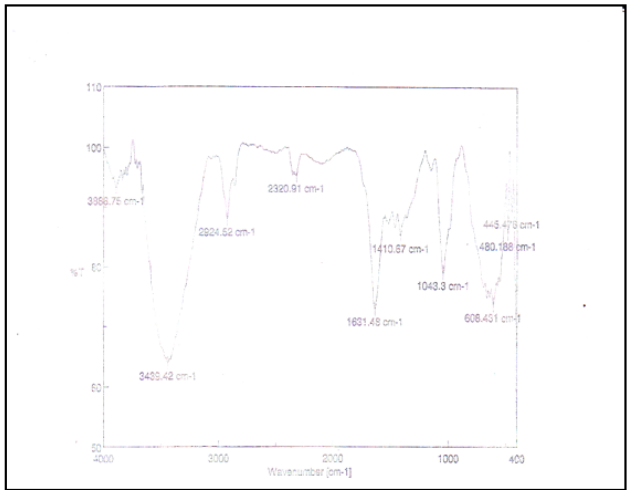


Fig. 2: IR graph of isolated compound of C. indica

electron bombardment ionisation. (Figure 3)

RESULTS

The isolated compound was found to have a molecular formula of $C_{29}H_{50}O$ with a molecular weight of 414.71 g/mol. It was a pale yellow crystalline solid that melted at 145°C and was soluble in both alcohol and chloroform. The Rf value was 0.55, and the yield was 470 mg.

The compound was characterised using melting point determination, thin-layer chromatography (TLC), infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectroscopy. The melting point was obtained using the Thiel melting point tube method to ensure purity. TLC analysis was conducted using chloroform and methanol (7:3) as the mobile phase to ensure the presence of the compound.

The IR spectral scan, which was done with KBr pellets, showed characteristic absorption bands at 3439.6 cm^{-1} (O-H stretching), 2924.7 cm^{-1} and 2867.9 cm^{-1} (C-H stretching), 1631.6 cm^{-1} (C=C absorption), 1410.3 cm^{-1} (CH_2 bending), 1043.7 cm^{-1} (cycloalkane), and 881.6 cm^{-1}, verifying the presence of functional groups in accordance with the expected structure.

The ^{1}H-NMR spectrum, which was obtained in $CDCl_3$, exhibited signals at δ 5.26 (H-6), 5.19 (H-23), 4.68 (H-22), 3.63 (H-3), and other typical peaks, confirming the suggested molecular structure. Other signals appeared between δ 0.69-2.00 ppm due to alkyl groups.

Mass spectroscopy scanning by Electron Spray Ionization (ESI) also validated the molecular ion peak at m/z 414 according to the molecular formula $C_{29}H_{50}O$. The fragmentation ion peaks at m/z 367, 271, 255, 229, 189, 175, 161, 133, 121, 105, 95, 81, 69, 55, and 41 provided additional structural confirmation.

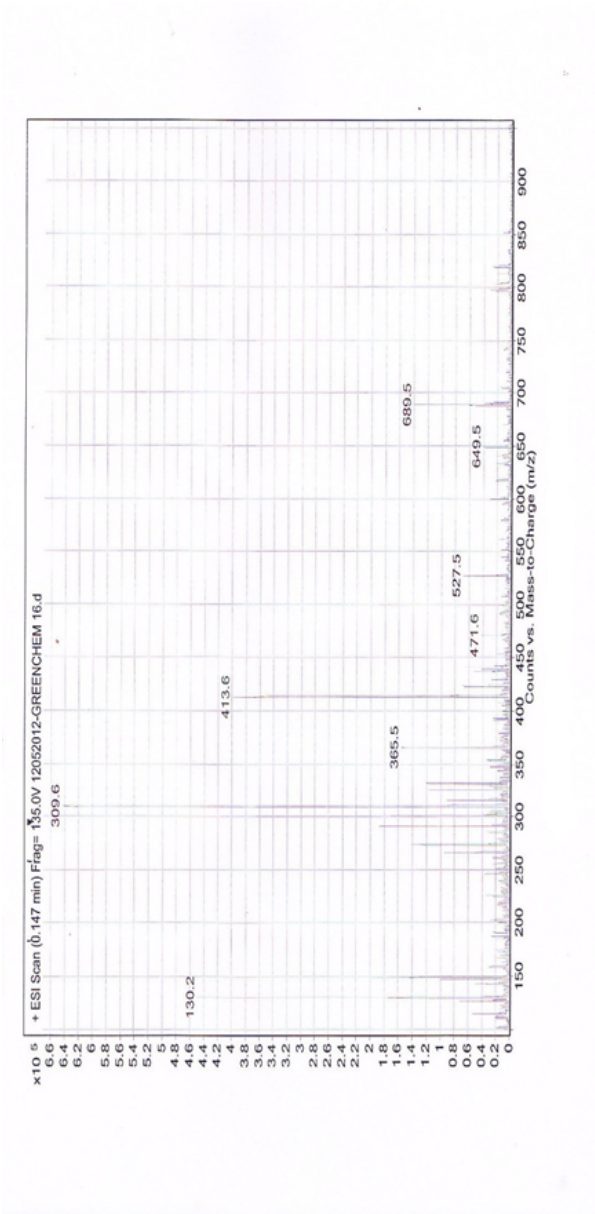


Fig. 3: Mass spectroscopy of isolated compound of C. indica

Phytochemical screening of the C. indica extract showed the presence of carbohydrates, amino acids, phytosterols, triterpenoids, alkaloids, fats and oils, and flavonoids; however, tannins, phenols, vitamin C, and saponins were not detected.

Characterisation confirmed the successful isolation of the compound and provided clues to its structure and composition.

Structural Elucidation

The IR absorption spectrum exhibited absorption peaks at 3439.6cm-1(O-H stretching.); 2924.7 cm-1and 2867.9 cm-1 (aliphatic C-H stretching); 1631.6 cm-1 (C=C absorption peak); additional absorption peaks are 1410.3 cm-1 (CH2);

1043.7 cm-1(cycloalkane) and 881.6 cm-1. (Figure 4)

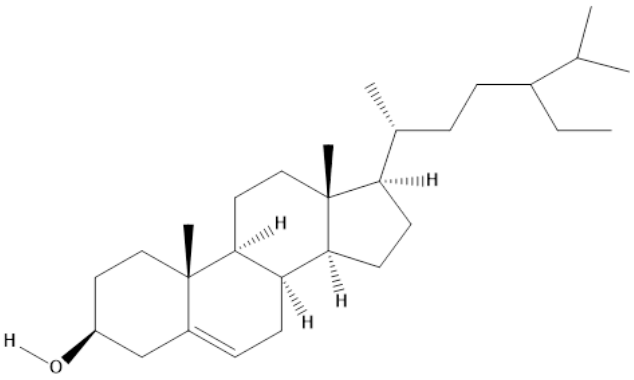


Fig. 4: Structure of isolated compound ofC. indica extract

1HNMR (CDCl3) of isolated compound: 1HNMR has given signals at δ3.2(1H, m, H-3), 5.26 (1H, m, H-6), 5.19(1H, m, H-23), 4.68(1H,m,H-22), 3.638(1H, m, H-3), 2.38(1H, m, H-20), 1.8-2.0 (5H, m) ppm. Other peaks are observed at δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H),1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H), 1.8-2.00 (m, 5H), 1.07-1.13 (m,3H), 1.35-1.6 (m, 9H) ppm.

^{1} **H-NMR:** 7.69-7.67 (2H, m, 2OH), 4.82-4.64 (1H, s, alkyl), 4.38 (1H, d, J=8 Hz), 3.86 (1H, m, alkyl), 3.74,61 (2H, m, alkyl), 3.52-3.11 (7H, m, alkyl), 2.75 (2H, t, J= 16 Hz), 2.51-1.55 (4H, m, alkyl), 1.22 (2H, s, alkyl), 1.11-0.75 (5H, m, alkyl).

FAB-MS showed molecular ion peaks at 414, corresponding to the molecular formula $C_{29}H_{50}O$. Ion peaks were also observed at m/z 367, 271, 255, 229,189, 175, 161, 133, 121, 105, 107, 95, 81, 69, 55, 41.

DISCUSSION

Phytochemical analyses confirmed the presence of saponins, flavonoids, glycosides, steroids, tannins, and alkaloids. The varied pharmacological activities of the plant are due to its bioactive components, which are found to exert a variety of beneficial effects, including blood sugar control, prevention of cancer, pain, fever reduction, parasite inhibition, reduction of inflammation, relaxation of muscles, healing of wounds, protection against stomach, seizure prevention, liver protection, and immunomodulation[26] . Traditional healers in Kerala, India, normally use plant leaves and fruits to treat various medical problems, ranging from skin conditions to muscle weakness, infertility issues, and diabetes. The residents commonly use a paste made of C. Indica fruits and leaves are combined with milk to treat diabetes and with sugar to treat jaundice[1].

Several clinical trials have been conducted to prove the efficacy of C. indica in humans, and most of these trials focused on its antidiabetic activity due to the extensive history of the plant as a natural antidiabetic agent. Venkateswaran et al.[6] had an experiment to identify the effect of C. indica on Blood glucose and insulin. Administration of C. indica leaf ethanol extract to diabetic animals for 45 days resulted in a remarkable reduction in blood glucose and glycosylated hemoglobin content, with concomitant increases in total hemoglobin and plasma insulin levels. Therefore, the initial phytochemical tests are useful in identifying chemical constituents in plant material that might lead to their quantitative estimation, as well as for identifying the site of pharmacologically active chemical compounds. Quantitative estimation of proteins, phenols, and flavonoids provides an insight into their chemical nature, which might provide rich data for understanding some elementary patterns of growth and metabolism. Phytochemical indicators of C. indica, such as proteins, phenols, and flavonoids, can also act as chemical markers in taxonomic studies. They were separated via column chromatography/HPLC and characterised using analytical data through MASS and NMR spectroscopy.

CONCLUSION

This study was able to isolate and identify the bioactive phytoconstituents of Coccinia indica using chromatographic and spectroscopic methods. Structural elucidation was performed to validate the molecular structures and functional groups. These results enrich the knowledge of Coccinia indica phytochemistry to further support its future pharmacological applications and potential drug development.

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