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Research Article

Comparative Analysis of Phytochemicals and Antioxidant Efficacy in *Coccinia indica* Leaf and Fruit Extracts

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ABSTRACT

Introduction: Naturally present small plants have an enormous amount of free radical neutralising chemicals that protect them from various lifestyle diseases. **Aim:** This study aims to evaluate the antioxidant potential, phytochemical composition, and LC-MS profiles of ethanol extracts obtained from the leaves and fruits of *Coccinia indica*. **Materials and Methods:** Leaves and fruits of *C. indica* were subjected to Soxhlet extraction using ethanol as the solvent. The antioxidant activity of the crude extracts was measured using the free radical reactive chemical DPPH, with ascorbic acid employed as a reference standard. Preliminary phytochemical screening was conducted to identify the bioactive constituents. **Results:** The IC_{50} values for the ethanolic extracts of *C. indica* leaves and fruits were 495.4 and 506.6 µg/mL. The leaf extract exhibited more potent antioxidant activity than the fruit extract. Plant chemical identification confirmed that the leaf extract has good antioxidant compounds. The fruit extract was found to contain alkaloids, terpenoids, tannins, phenols, amino acids, carbohydrates, and steroids. **Conclusion:** Both leaf and fruit extracts of *C. indica* demonstrated notable antioxidant activity, likely due to various phytochemicals including phenols, alkaloids, tannins, and saponins. These findings suggest that *C. indica* is a good source of bioactive antioxidants and warrants further investigation for its pharmacological applications.

Keywords: *Coccinia indica*, Antioxidant activities, Soxhlet extraction, LC-MS profiling, Phytochemical screening.

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INTRODUCTION

C. indica (syn. *Coccinia grandis*), which comes under the family Cucurbitaceae, is a fast-growing perennial climber commonly found in tropical and subtropical regions. The plant thrives in well-drained, sandy soils and sunny, sheltered environments. It exhibits vigorous vegetative growth, with herbaceous stems that produce adventitious roots when in contact with the ground. Climbing is facilitated by long, coiled tendrils, which support the plant's spread across surrounding vegetation or structures. The leaves are palmately lobed, typically with five segments, and vary in shape from heart-like to pentagonal, measuring between 5-10 cm in length and width. The flowers are white, tubular, and approximately 4 cm in diameter. The fruit is a smooth, ovate berry, green when unripe and turning bright red upon maturation. The plant also features tuberous roots with fibrous extensions.[1]

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C. indica, commonly known as ivy gourd, is widely used in traditional Indian cuisine. Both its fruit and leaves are consumed as vegetables. The tender green fruits are cooked in curries, stir-fries, and pickles, offering a mildly bitter taste and high nutritional value. Leaves are often sautéed or added to soups and dals, contributing to dietary fiber, vitamins, and minerals. Easy to grow and harvest, *C. indica* serves as a valuable and sustainable food source in many Asian households.

Traditionally, *C. indica* has been used in various systems of medicine, particularly for managing conditions such as diabetes, inflammation, and oxidative stress-related disorders.[2] These therapeutic effects are thought to stem from the plant's rich phytochemical content, including phenolics, alkaloids, tannins, and saponins compounds well-known for their antioxidant properties.[3,4] Antioxidants are critical in neutralizing free radicals, which are responsible for causing various diseases.[5-7] In light of the increasing interest in natural antioxidants, scientific validation of *C. indica*'s bioactivity is essential. While conventional assays like DPPH provide insight into antioxidant potential, advanced analytical techniques are required to accurately identify and characterize the bioactive constituents accountable for therapeutic importance effects.[8] LC-MS is a powerful device for comprehensive phytochemical reporting, offering high sensitivity and specificity for the detection of complex secondary metabolites in plant extracts.[9,10]

Recent studies on *C. indica* have highlighted its promising role in antidiabetic therapy because of its bioactive compounds, such as flavonoids, terpenoids, and alkaloids. Investigations confirmed significant α-amylase and α-glucosidase inhibitory activities, aiding in glucose regulation. Advanced techniques such as LC–MS and GC–MS have identified novel phytoconstituents with antioxidant and anti-inflammatory potential. Additionally, nanoparticle formulations of Coccinia extracts show increased bioavailability and efficacy. Recent genetic and molecular studies have explored the pathways involved in insulin mimetic action, providing new insights into its therapeutic mechanisms.[11,12]

Current research evaluates the antioxidant activity of ethanolic extracts from the leaves and fruits of *C. indica*, performs preliminary phytochemical screening, and carries out LC-MS profiling to identify key bioactive compounds contributing to the plant's pharmacological potential.

MATERIALS AND METHODS

Plant Collection and Authentication

C. indica specimens were collected and their taxonomic identification was confirmed at the Institute of Forest Genetics and Tree Breeding (IFGTB), Indian Council of Forestry Research and Education, Coimbatore, India. The plant was identified as *C. indica* with the voucher specimen number 612/FRC/ID/FECC/IFGTB/2024, Accession No. 27315, dated 03/03/2025. Collected samples were rinsed with distilled water to remove surface debris, dried at 27°C, and then coarsely powdered. The powdered material was stored in airtight containers for further extraction procedures.

Preparation of Plant Extracts

The powdered leaves and fruits (25 g each) were subjected to Soxhlet extraction using 500 mL of ethanol. The samples were soaked overnight and then extracted continuously for 72 hr. The solvent was evaporated on a water bath to yield the crude extract. The extraction yields were 6.3% w/w for leaves and 2.4% w/w for fruits. Moisture-free extracts preserved at desiccator until further analysis.

Plant bioactive chemical analysis

Ethanol extracts of the plant material were subjected to confirmation of bioactive phytochemicals, following standard protocols described by Harborne (1998) and Trease and Evans (2002).[11,12] Alkaloids were identified through the appearance of red or orange precipitates upon reaction with Dragendorff's reagent and iodine solution. The presence of flavonoids was confirmed by the development of a scarlet to pink coloration following treatment with magnesium ribbon and hydrochloric acid. Reducing sugars were detected by the formation of a red precipitate after heating with Fehling's A and B solutions. Proteins were indicated by a yellow coloration when treated with mercuric chloride. A red ring at the interface of chloroform and sulfuric acid signified the presence of steroids. Quinones were identified through the appearance of red, blue, or green coloration upon addition of sodium hydroxide. Terpenoids were indicated by a greyish-yellow coloration following treatment with sulfuric acid. Carbohydrates were confirmed by the formation of a purple ring upon the sequential addition of Molisch's reagent and sulfuric acid. Tannins produced a yellow precipitate with 10% lead acetate and a blue-green color with 5% ferric chloride. Amino acids were detected by the formation of a white precipitate using Millon's reagent. Saponins were identified by persistent frothing upon vigorous shaking with water. The presence of phenols was confirmed by a deep blue or black coloration upon reaction with ferric chloride. Finally, glycosides were indicated by a yellow to orange coloration following treatment with sodium hydroxide and distilled water.

Antioxidant Activity by DPPH

The antioxidant potential of the extracts was assessed by determining their free radical scavenging ability using the DPPH assay according to the procedure outlined by Blois (1958).[13] Various concentrations of the plant extracts (200, 400, 600, 800, and 1000 µL) were prepared in ethanol, and each was adjusted to a final volume of 3 mL with methanol in test tubes. A 0.004% DPPH solution was made by dissolving 4 mg of DPPH in 100 mL of methanol, and 1 mL of this solution was added to each sample. The mixtures were incubated in the dark at room temperature for 30 min to prevent light-induced degradation of DPPH. Following incubation, the absorbance was recorded at 517 nm using a UV-visible spectrophotometer, with ethanol as the blank and ascorbic acid as the standard reference. The percentage of radical scavenging activity was calculated using the formula:

$$\text{Radical Scavenging Activity (\%)} = [(\text{Absorbance of control} - \text{Absorbance of sample})/\text{Absorbance of control}] \times 100$$

LC-MS Analysis of Bioactive Compounds

The bioactive compounds present in the ethanol extracts were characterized using LC-MS. Analysis was performed on a Mariner Bio spectrometry system equipped with a binary pump and connected to a Q-TOF mass spectrometer set to operate in positive electrospray ionization mode, following the method described by Hossain *et al.*, (2010).[14] Chromatographic separation was achieved using a Phenomenex C8 column (5 µm, 150 × 2 mm i.d.). The mobile phase consisted of methanol with 0.3% formic acid and was delivered isocratically at a flow rate of 0.1 mL/min. The ESI source was kept at 140°C, and the mass spectra were acquired in full-scan mode over an m/z range of 100 to 1200. Detected peaks were identified and interpreted by referencing established compound databases and scientific literature (Tables 1 and 2).

RESULTS AND DISCUSSION

Phytochemical Characterization

The phytochemical characterization of *Coccinia indica* leaf and fruit extracts reveals a rich and diverse profile of bioactive compounds, underscoring the plant's therapeutic potential. Both leaf and fruit extracts tested positive for a broad spectrum of phytoconstituents, including alkaloids, carbohydrates, glycosides, and flavonoids, indicating their abundance in these plant parts. Notably, alkaloids, carbohydrates, glycosides, and flavonoids were strongly present, suggesting significant roles in the pharmacological activities attributed to*C. indica*. The fruit extract demonstrated a higher intensity of reducing sugars, tannins, steroids, and phenols compared to the leaf, which may contribute to its distinct medicinal properties. The presence of phenols and flavonoids, known for their antioxidant activities, was prominent in both extracts, though phenols were more abundant in the leaf. Terpenoids, saponins, and amino acids were moderately present in both extracts, supporting their contribution to the plant's anti-inflammatory and immunomodulatory effects. The overall phytochemical profile highlights the complementary nature of the leaf and fruit, with each part offering a unique combination and concentration of secondary metabolites. These findings provide a scientific basis for the traditional use of*C. indica* in herbal medicine and warrant further investigation into its bioactive compounds for potential therapeutic applications.

Phytochemical Screening

The preliminary phytochemical analysis of *Coccinia indica* ethanol extracts revealed a rich diversity of bioactive compounds. Both leaf and fruit extracts tested positive for alkaloids, carbohydrates, amino acids, glycosides, terpenoids, flavonoids, and tannins. Notably, flavonoids, phenols, and glycosides showed significant presence in both extracts, consistent with previous reports indicating their abundance in *Coccinia* species. The leaf extract demonstrated a higher qualitative intensity for reducing sugars, phenols, and saponins, while the fruit extract showed a significant presence of steroids, which were absent in the leaves. The strong occurrence of phenolic and flavonoid compounds supports the plant's antioxidant potential, given these groups established free radical scavenging properties.[15]

Antioxidant Activity

The antioxidant potential was evaluated using the DPPH radical scavenging assay. The half-maximal inhibitory concentration (IC_{50}) of the ethanolic extract was 495.4 µg/mL for *C. indica* leaves and 506.6 µg/mL for fruits. While both extracts demonstrated significant antioxidant activity, the lower IC_{50} value of the leaf extract indicates superior radical scavenging efficiency. This may be attributed to the leaf sample's higher concentration of phenolic compounds and flavonoids (Figures 1 and 2). These findings align with previous studies highlighting the correlation between phenolic content and antioxidant activity in medicinal plants.[16,17] Ascorbic acid, used as the reference standard, demonstrated the highest scavenging ability, but the extracts showed noteworthy potential in comparison.

LC-MS Profiling of Bioactive Compounds *Coccinia indica* leaf extract

LC-MS analysis of *C. indica* ethanol extracts revealed a diverse range of bioactive phytochemicals in leaf and fruit samples. The leaf extract identified nineteen compounds, including major flavonoids such as apigenin, luteolin, kaempferol, quercetin, and rutin. The presence of these compounds is noteworthy, as they are well-known for their potent antioxidant and anti-inflammatory properties. Leaf and fruit extracts observed significant amounts of cucurbitacins B and E, bioactive triterpenoids with reported anticancer and anti-diabetic activities. Other essential

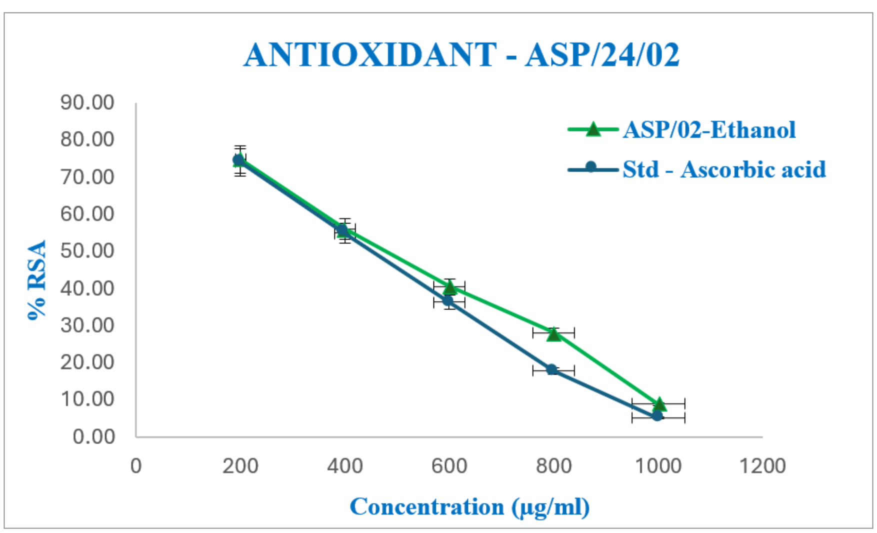

Figure 1: Antioxidant potential of the leaf extract compared with the Ascorbic acid standard.

phytochemicals detected in the leaf extract included β-sitosterol, stigmasterol, lupeol, caffeic acid, gallic acid, and ellagic acid, all of which are associated with a range of therapeutic properties such as cholesterol-lowering, anti-inflammatory, and antimicrobial effects Figures 3 and 4. Comparatively, the fruit extract showed a slightly less complex phytochemical profile, with thirteen compounds detected, most of which overlapped with the leaf extract. The consistent presence of flavonoids, cucurbitacins, phenolic acids, and phytosterols in both extracts underscores the medicinal potential of *C. indica*. Identifying secondary metabolites such as hexamethyl-cyclotrisiloxane and palmitic anhydride in the leaf further distinguishes its profile. These findings suggest that both leaf and fruit extracts of *C. indica* are rich sources of health-promoting constituents, thereby supporting their traditional use in herbal medicine and highlighting their potential for development as therapeutic agents.[18]

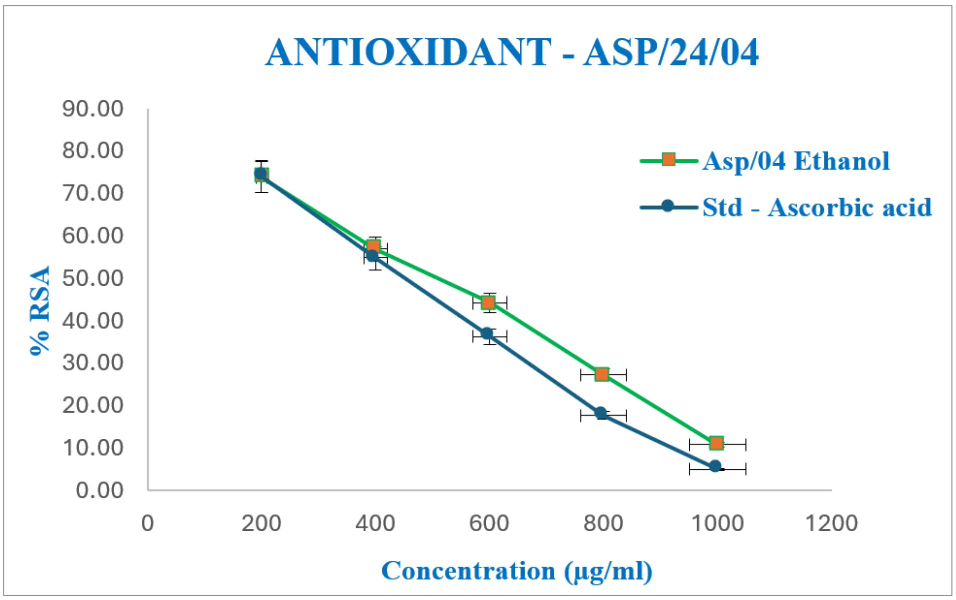

Figure 2: Antioxidant potential of the fruit extract compared with the Ascorbic acid standard.

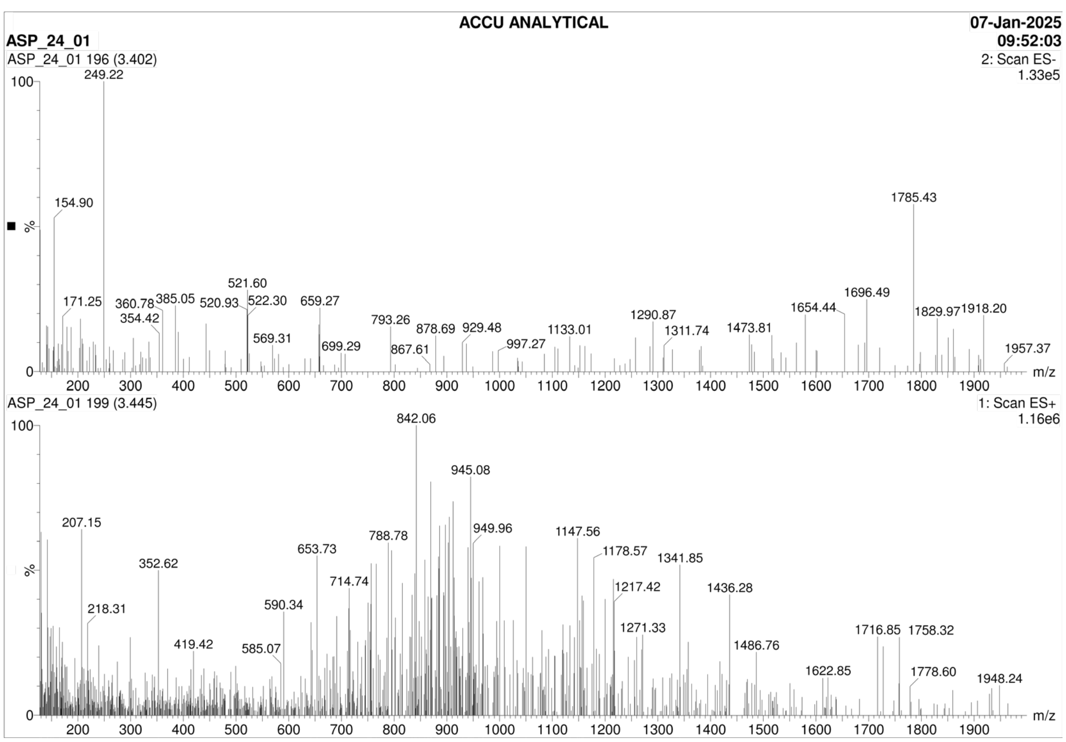

Figure 3: Phytochemical evaluation of ethanolic extract of *Coccinia indica* leaf extracts by LC-MS.

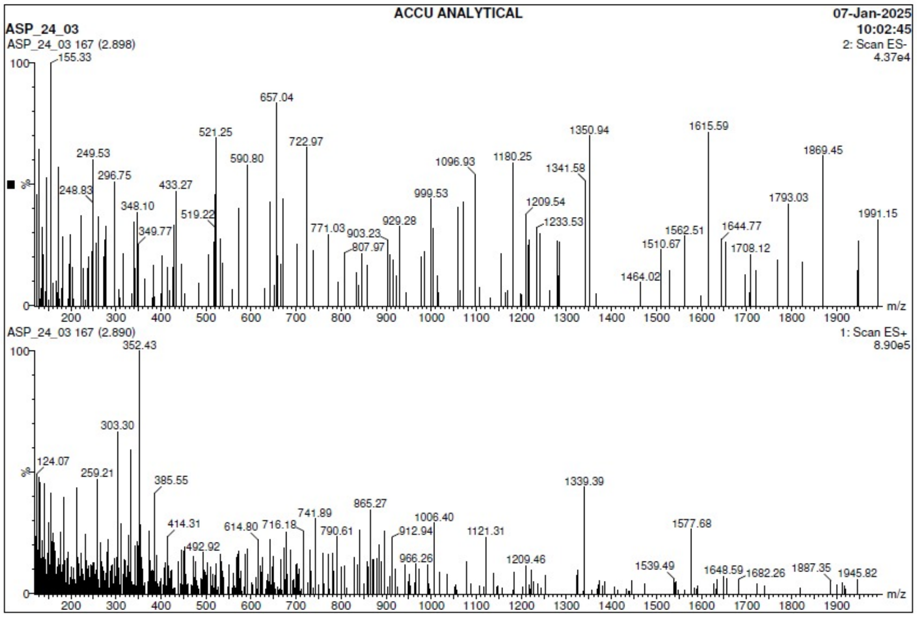

Figure 4: Phytochemical evaluation of ethanolic extract of *Coccinia indica* fruit extracts by LC-MS.

Coccinia indica fruit extract

Table 1: Phytochemical analysis of ethanol LC-MS extract of *Coccinia indica* leaf- LC-MS.

Sl. No.	Phytochemicals	m/z
1	Cis-7-Decen-1-al	154
2	1,1-dichloro-2-propanone	127
3	Hexamethyl-cyclotrisiloxane	222
4	Palmitic anhydride	494
5	Cucurbitacin B	558.60
6	Cucurbitacin E	576.60
7	Apigenin	270.24
8	Luteolin	286.24
9	Kaempferol	286.24
10	Quercetin	302.24
11	β sitosterol	414.70
12	Stigmasterol	412.69
13	Lupeol	426.70
14	Caffeic acid	180.16
15	Gallic acid	170.12
16	Ellagic acid	302.19
17	Rutin	610.52

DISCUSSION

The comprehensive phytochemical investigation of *C. indica* leaf and fruit extracts highlights the plant's rich composition of bioactive metabolites, supporting its traditional use in folk medicine. Qualitative screening of ethanolic extracts confirmed the presence of several key phytoconstituents, namely alkaloids, carbohydrates, glycosides, flavonoids, tannins, steroids, phenols, terpenoids, saponins, and amino acids in both plant parts, with variations in predominance between leaves and fruits. Notably, flavonoids, phenols, and glycosides were abundant in both extracts, reflecting their established role in imparting significant antioxidant and therapeutic effects. The leaf extract displayed enhanced levels of phenols, saponins, and reducing sugars, suggesting superior antioxidant capacity and additional health benefits. In contrast, the fruit extract exhibited a stronger presence of steroids and tannins, compounds recognized for their anti-inflammatory and protective properties. The antioxidant efficacy of both extracts was confirmed using the DPPH free radical scavenging assay. The leaf extract demonstrated a marginally greater antioxidant capacity, as reflected by a lower IC_{50} value (495.4 µg/mL for leaves versus 506.6 µg/mL for fruits), which may be attributed to its higher content of phenolic and flavonoid compounds. These results align with mounting evidence that phenolic-rich plant extracts possess potent free radical quenching ability, thereby protecting against oxidative damage and related pathologies. Although both leaf and fruit extracts exhibited marked antioxidant activity, ascorbic acid used as the reference remained the most effective radical scavenger; however, the sizable activity of the extracts highlights their therapeutic promise and supports their continued exploration as natural antioxidants. Further elucidation using LC-MS allowed for the in-depth profiling of individual phytochemicals within the extracts. The LC-MS analysis of the leaf extract identified a remarkably diverse set of nineteen compounds spanning several classes, including flavonoids (apigenin, luteolin, kaempferol, quercetin, and rutin), phenolic acids (caffeic, gallic, ellagic acid), triterpenoids (lupeol), sterols (β-sitosterol, stigmasterol), and the

Table 2: Phytochemical analysis of ethanol LC-MS extract of *Coccinia indica* fruit- LC-MS.

Sl. No.	Phytochemicals	m/z
1	Cucurbitacin B	558.60
2	Cucurbitacin E	576.60
3	Apigenin	270.24
4	Luteolin	286.24
5	Kaempferol	286.24
6	Quercetin	302.24
7	β sitosterol	414.70
8	Stigmasterol	412.69
9	Lupeol	426.70
10	Caffeic acid	180.16
11	Gallic acid	170.12
12	Ellagic acid	302.19
13	Rutin	610.52

prominent triterpenes cucurbitacin B and E. Such a spectrum points to the presence of molecules known for antioxidant, anti-inflammatory, and potential anti-cancer effects. The overlapping profile between leaf and fruit extracts, particularly for the main flavonoids and cucurbitacins, underscores shared pharmacological value among both parts of the plant. Leaf extract stood out by containing additional secondary metabolites, such as hexamethyl-cyclotrisiloxane and palmitic anhydride, further diversifying its bioactive potential.

The fruit extract presented a slightly reduced phytochemical complexity, with thirteen compounds detected, most corresponding to those found in the leaf extract. Still, the pronounced presence of compounds like rutin, gallic acid, and β-sitosterol in both extracts reaffirms their contribution to the antioxidant and medicinal profile of *C. indica*. The convergence of results from qualitative screening, antioxidant assays, and LC-MS profiling suggests a potential complementary effect of leaf and fruit extracts, harbouring unique and overlapping phytochemicals conducive to therapeutic synergy. The findings from this multi-tiered phytochemical and bioactivity assessment substantiate the medicinal basis for *C. indica*'s traditional use and encourage further investigation into its isolated compounds for development as novel therapeutic agents. The leaf and fruit extracts provide a valuable source of antioxidants and other health-promoting constituents, confirming *C. indica* as a promising candidate for natural product-based drug discovery and as an additive for functional foods and nutraceuticals.

CONCLUSION

This study demonstrates that *C. indica* is a promising source of diverse phytochemicals with significant antioxidant potential. The ethanolic leaf extract exhibited a higher concentration of phenolic and flavonoid compounds and showed superior DPPH radical scavenging activity compared to the fruit extract. LC-MS analysis confirmed the presence of numerous bioactive molecules, including cucurbitacins, flavonoids, sterols, triterpenoids, and phenolic acids, all of which have established pharmacological relevance. The results support the traditional use of *C. indica* in herbal medicine and highlight its promise as a source of natural antioxidant agents. To advance its pharmaceutical potential, additional research, such as *in vivo* studies and detailed investigations into the underlying mechanisms, is needed to thoroughly assess the therapeutic effectiveness and safety of these plant extracts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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