Pharmacogn. Res., 2025; 17(2):507-518.
https://www.phcogres.com

Original Article

# Standardization and GC-MS Analysis of Phytochemicals in *Coccinia indica* (Wight and Arn.) Fruits

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## ABSTRACT

**Background:** *Coccinia indica*, commonly known as the ivy gourd, is traditionally utilized for its diverse medicinal properties. **Objectives:** The objectives of the study were to standardize and evaluate the fruits of *Coccinia indica* by identifying the phytochemicals using GC-MS analysis. **Materials and Methods:** Standard methodologies were used for the standardization, including macroscopical, microscopical, physico-chemical evaluations and phytochemical screening. Additionally, GC-MS analysis was performed to identify key components in the plant extract and its fractions. **Results:** The T.S. of the fruits of *C. indica* showed a single epicarp, mesocarp and several fibro-vascular bundles, while the T.S. of the seed revealed the presence of a testa, tegmen, embryo, starch grains and several oil globules. The main findings were as follows: total ash 16.52±0.13% w/w, water-soluble ash 11.35±0.12% w/w, acid-insoluble ash 1.45±0.06% w/w, water-soluble extractive value 18.45±0.2% w/w, alcohol-soluble extractive value 8.80±0.11% w/w, moisture content 7.33±0.08% w/w and the pH of the aqueous extract was 5.63±0.07 (1% w/v) and 5.79±0.09 (10% w/v). Phytochemical investigation revealed the presence of alkaloids, steroids, flavonoids, saponins, tannins, phenolics and terpenoids. GC-MS analysis identified more than forty notable chemicals in the methanol extract and fractions of chloroform and hexane. **Conclusion:** This study provides key insights into the standardization of *C. indica* fruits and identifies 43 major secondary metabolites through GC-MS analysis. Notable compounds include taraxerone, squalene, lupeol and piperine, all with potential therapeutic benefits. Moreover, the choice of solvent for extraction and fractionation significantly influenced the phytochemical profile and concentrations.

**Keywords:** *Coccinia indica*, Evaluation, GC-MS analysis, Phytochemical, Standardization.

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**Received:** 19-09-2024;
**Revised:** 22-11-2024;
**Accepted:** 07-01-2025.

## INTRODUCTION

Plants and plant products have been used for medicinal purposes since the early days of the human civilization. Plants with medicinal properties are an asset of significant revenue for economies around the world. Nature gifted upon us a vast botanical wealth, with a wide range of plant species growing around the many regions across the country.[1] Herbal medicines are still used by around 75% to 80% of entire population and the majority of traditional medication includes the use of herbs extract and their phytoconstituents. Herbal medicine went through an obstacle after the emergence of modern medicine, but in the past twenty or thirty years, developments in photochemistry and the knowledge of plant chemicals that are useful against certain diseases have reignited interest in the use of herbs.[2] The secondary metabolites of herbal materials, such as alkaloids, flavonoids, steroids, glycosides, tannins, phenol compounds, resins, fatty acids and gums which can have specific physiological effects on the body that are responsible for the therapeutic benefits.[3]

*Coccinia indica*, a member of the Cucurbitaceae family, is also referred to as bimba, kanduri and kundru. It is well-known in the Ayurvedic medical system for having hypoglycemic and anti-diabetic effects. *C. indica* is widely distributed in the oriental countries, India, Australia, Fiji and Tropical Africa. It can also be called *Cephalandra indica*.[4-7] *C. indica* is a climber, heavily branching perennial plant that grows wild across the country. Fruit is pepo; ovoid, glabrous that is 3.5-4.5 cm in length and 1.5-2 cm broad, brownish green to yellow-brown with some

**DOI:** 10.5530/pres.20251942

white mark and has no smell or taste. Seed is slightly obovoid, 0.7 cm in length around 0.2-0.3 cm in width, rounding at the top, compressed, grayish-yellow.[8]

The methenolic extract of *C. indica* fruits comprises alkaloids, glycosides, saponins, steroids, tannins, ellagic acid, phenols, lignans and triterpenoids.[9] Traditionally, the entire plant has been employed for a variety of therapeutic uses. Indian traditional medicine uses the leaves of this plant to cure a variety of conditions, such as diabetes, ulcers, cuts, inflammatory conditions, fever, allergies, asthma and coughing. Previous studies on *C. indica* revealed that extracts possessed analgesic, hepatoprotective, anti-diabetic, hypolipidemic, antipyretic, anti-bacterial, anthelmintic, wound healing and anti-inflammatory activities.[10-18]

Standardization of medicinal plants is the method used of establishing a group of specifications or innate features, constant values and definite qualitative and quantitative measures that ensure quality, purity, efficacy and reproducibility. It is a method of creating and agreeing on technical standards. Particular standards are developed by experimentation followed by observing, which leads to a way of presenting a series of qualities demonstrated by the specific herbal drug. As a result, standardization is a vital tool for the quality control technique.[19]

## MATERIALS AND METHODS

### Chemicals

All the chemicals and reagents used in this study were procured from SD Fine Chemical Limited, India and Qualigens Pharma Pvt. Ltd., India. These chemicals and reagents were of highest purity (analytical reagent grade), for accuracy and reliability in all experimental procedures.

### Collection and authentication of plant materials

*C. indica* fruits were purchased from the Khadra Sabji Mandi, Lucknow, Uttar Pradesh and were thoroughly dried under sun and shade. Authentication of the plant was done by Dr. Muhammad Arif, Department of Pharmacognosy, Faculty of Pharmacy at Integral University, Lucknow, Uttar Pradesh, India (IU/PHAR/HRB/01/23).

### Organoleptic/macroscopic evaluation of *C. indica* fruits

The *C. indica* fruits and seeds were evaluated for its morphological and sensory features like size, shape, colour, odour, taste, texture and fracture.[20]

### Microscopic evaluation using qualitative and powder microscopy

The Transverse Sections (T.S.) and powdered samples of the fruits of *C. indica* fruits were used for this study by using standard procedure. The thin transverse sections of the fruits and seeds were cut. The sections and powders were placed on a clean slide, stained with Phloroglucinol+concentrated hydrochloric acid, mounted on a slide by using glycerine and coated with a new cover slip. The slide was examined underneath a compound microscope.[21]

### Physicochemical evaluation

#### *Ash values determination*

Ash values include total ash, water soluble ash and acid insoluble ash. For determining total ash, 2 g of powdered of *C. indica* fruits was transferred into a silica crucible that was previously dried and weighed. The ground drug was evenly distributed and precisely weighed. The raw materials were ignited at a temperature never moving above 450°C unless remove all carbon, after that cooled within a desiccator, weighed, after that percent ash was estimated by excluding empty mass of crucible from the mass of the crucible and total ash. For estimation of water-soluble ash, heated above obtained ash over 5 min using 25 mL water; gathered insoluble matter in Whatman filter paper, rinsed by heated water, The filter paper containing the insoluble material was transferred to a crucible, incinerated around 15 min to a certain temperature not above 450°C and then cooled inside desiccator, weighed and calculated the water-soluble ash. To determine the acid-insoluble ash, in place of water 25 mL of diluted hydrochloric acid added drop wise to crucible containing total ash, followed by above mention procedure for water soluble ash. Estimated the acid-insoluble ash in relation to the air-dried drug.[22]

### Extractive values determination

To determine extractive values such as water and alcohol soluble extractive value, 5 g of coarse ground air-drying *C. indica* fruits being agitated using 100 mL of water and alcohol individually within a sealed flask around 24 hr, agitating regularly for 6 hr, allowed to stand about 18 hr. After then, this was immediately filtered. In a shallow dish with a flat bottom 25 mL of the filtrate was evaporating to dryness at a temperature of 105°C and then weighed. The percentage of water and alcohol soluble extractive values were estimated with respect to the air-dried drug and is expressed as a percentage (w/w).[23]

### Moisture content determination by loss on drying

Weighed about 2 g of the drug, shifted it to a china plate and dried it in the oven at 105°C for 5 hr. Weighed the drug frequently every 1 hr, till the two repeated weights were no more than 0.01 g.[24]

### pH determination

Auto digital pH meter (Labtronics, L-11) was used to record the pH of the drug sample at concentrations of 1% and 10% w/v of water-soluble portion.[25]

## Extraction and fractionation of *C. indica* fruits for phytochemical analysis

The coarsely powdered sample of *C. indica* fruits (50 g) was packed in soxhlet and extracted with methanol (400 mL) around 72 hr for an exhaustive extraction. Methanol solvent was removed with the help of a rotary evaporator at reduced pressure to get the extract. The aqueous suspension of methanolic extract transferred to a separating funnel and thereafter it was extracted sequentially via various organic solvents, namely hexane, chloroform and butanol. To get rid of fine particles, each crude extract was filtered individually with Whatman No. 41 filter paper. Twice, the methanolic residue was extracted using the same solvents in same polarity order and filtered. Individually blended fractions of the same solvent and evaporated entirely using a rotary evaporator to produce dry crude extracts and calculated percentage yield (w/w).[26,27]

## Preliminary phytochemical screening

Secondary metabolites are usually responsible for the pharmacological activities of the crude drug. Methanolic extract, chloroform and hexane fractions of the *C. indica* fruits tested regarding the presence or absence of secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, saponins, steroids and phenolics compounds.[28,29]

**Table 1: Physicochemical parameters of *C. indica* fruits.**

| Sl. No. | Parameters | Results |
|---|---|---|
| 1 | Total ash value | 16.52±.13 (w/w) |
| 2 | Water soluble ash value | 11.35±.12 (w/w) |
| 3 | Acid insoluble ash value | 1.45±.06 (w/w) |
| 4 | Water soluble extractive | 18.45±0.2 (w/w) |
| 5 | Alcohol soluble extractive | 8.80±0.11 (w/w) |
| 6 | Moisture content | 7.33±.08 (w/w) |
| 7 | pH of 1% aqueous extract | 5.63±.07 |
| 8 | pH of 10% aqueous extract | 5.79±.09 |

Each value is expressed as mean%±S.D, except pH ($n$=3)

## GC-MS analysis

The extract/fractions from *C. indica* fruits were tested to GC-MS analysis using the GCMS-QP2010 model (Shimadzu®), attached with Column, GC, SH-I-5Sil MS Capillary (30 m x 0.25 mm x0.25 $\mu$m). Analysis was performed by split less mode. The GC-MS operational conditions during testing were set as follows: oven temperature 45°C for 2 min followed by 140°C at 5°C/min lastly this moved to 280°C and kept isothermally around 10 min. The amount of sample for injection was 2 µL, while the carrier gas was helium at 1 mL/min. The sample materials were ionized at a voltage of 70 eV. The compounds structures were then checked against those stored in the NIST database using a search through the NIST14 library (2020). Following that, molecules were identified using mass spectra and retention times in comparison to already identified compounds in the NIST library.[30,31]

# RESULTS

## Macroscopic Evaluation

The fruits were macroscopically evaluated and found to be yellowish green to red in colour, with a characteristic taste, odourless, an ovoid-oblong shape, all over length 2.5-5.8 cm and 1-1.6 cm diameter (Figure 1). The seeds were yellowish-grey in colour, had a characteristic taste, odourless and had an oblong form, measuring 0.5 cm-0.7 cm in length and 2-3 mm in width (Figure 2).

## Microscopic evaluation

The transverse section of the *C. indica* fruits highlighted a one-layered epicarp, a mesocarp that consisted of a vast area of parenchymatous cells distinguished into two zones, the outer 6-7 strata rectangular to polygonal, lesser in dimensions, followed by the inner area, which is composed of larger-sized oval-polygonal cells; several fibro-vascular bundles were observed throughout this zone (Figure 3). The transverse section of the *C. indica* seeds revealed a testa made up of oval to polygonal, thin-walled parenchymatous cells; the tegmen is made up of a single layer of thin-walled, lignified cells and then follows a layer of parenchymatous cells that have collapsed. The embryo made up of thin walled hexagonal-polygonal cells, with several oil globules (Figure 4). The powder examination of *C. indica* fruits pointed


**Figure 1:** *Coccinia indica* fresh and dried fruits.

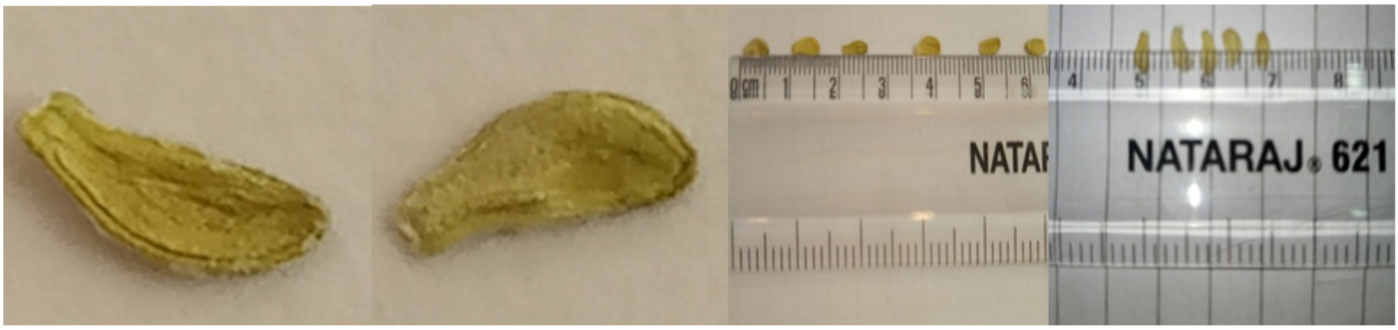



**Figure 2:** *Coccinia indica* seeds.

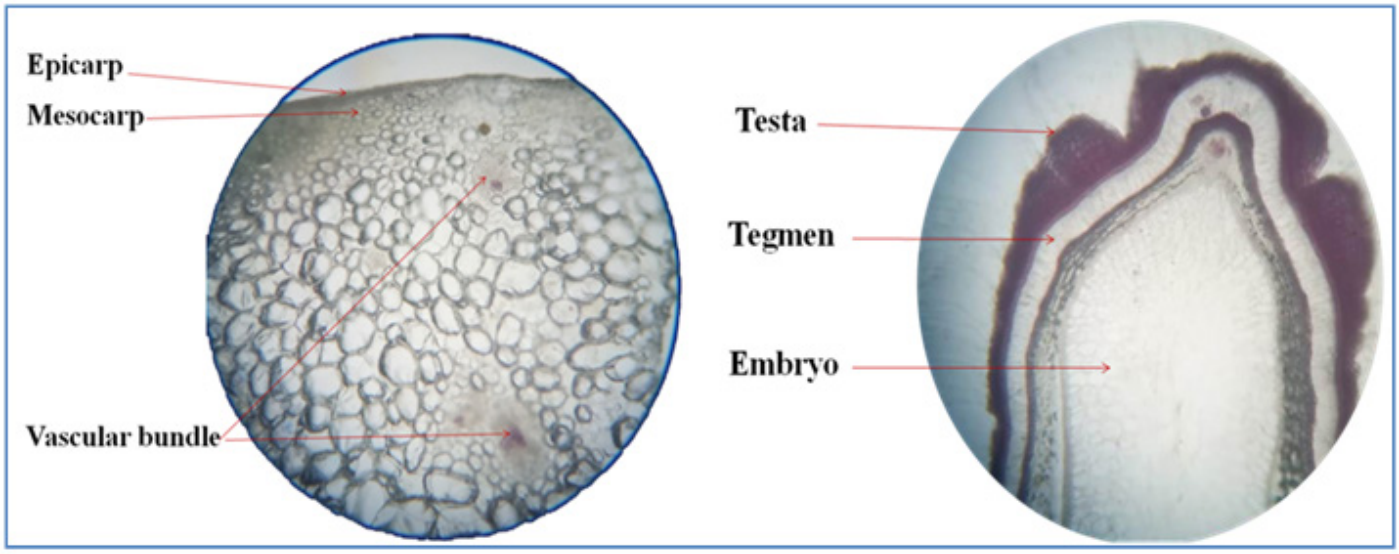



**Figure 3 and 4:** T.S. of *C. indica* fruit. T.S. of *C. indica* seed.

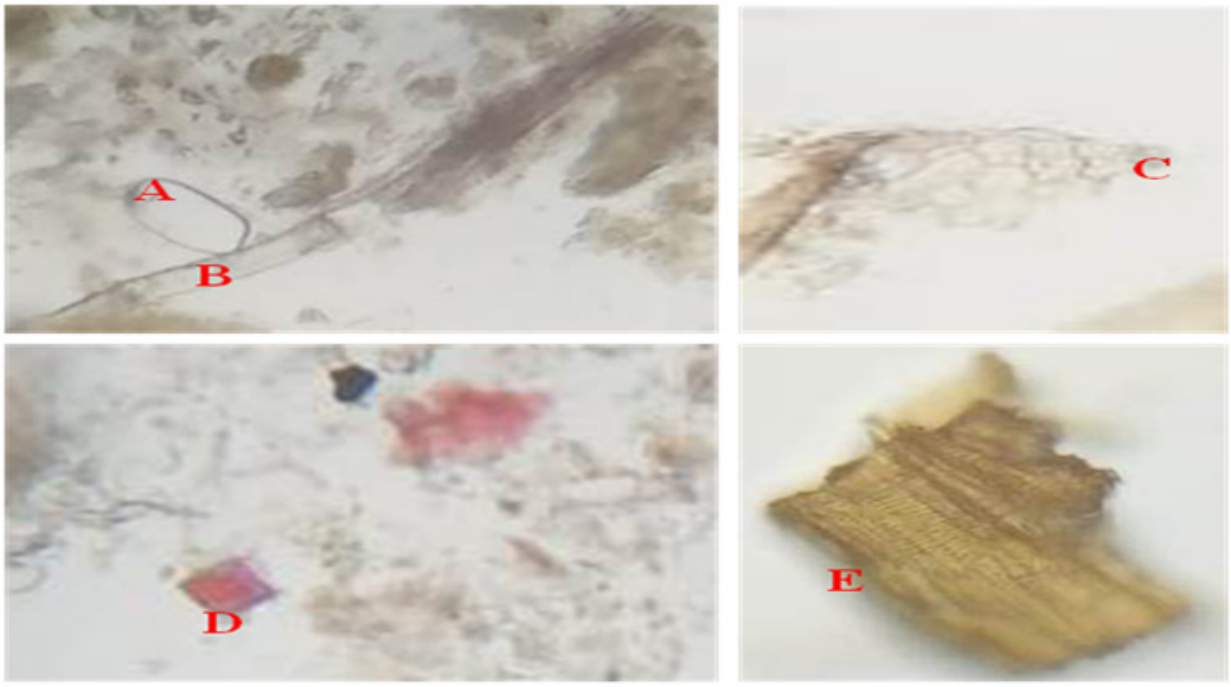



**Figure 5:** Powder characteristics of *C. indica* fruits: (A) Starch grain (B) Aseptate fibre (C) Parenchymatous cells (D) Stone cell (E) Reticulate, spiral and pitted vessels.

a round-polygonal structure of parenchymatous cells; the xylem vessels had pits, spirals and reticulations; aseptate fibres, starch grains and epidermal fragments with straight walled cells (Figure 5).

## Physicochemical evaluation

Frequently, physicochemical parameters were applied to determine the drug's identity, potency and purity. Such characteristics were also used to identify possible adulterants. To determine the purity of *C. indica* fruits, numerous characteristics were investigated, including ash values, extractive values, moisture content and determination of pH as shown in Table 1.

## Percentage yield (w/w) of extract and fractions of *C. indica* fruits

The methanol extract was fractionated using the separation funnel method with several solvents including n-hexane, chloroform and n-butanol. Table 2 shows the obtained percentage yield (w/w).

## Preliminary phytochemical screening

The preliminary phytochemical screening associated with methanol extract, chloroform and hexane fractions suggests detection of alkaloids, flavonoids, tannins, terpenoids, saponins, steroids and phenolics. The findings are presented in Table 3.

**Table 2: %yield (w/w) of extract and fractions of *C. indica* fruits.**

| Extract /Fractions | % yield (w/w) |
|---|---|
| Methanol extract | 18.30 |
| n-Hexane fraction | 7.42 |
| Chloroform fraction | 12.86 |
| n-Butanol fraction | 2.58 |

## GC-MS analysis

GC-MS profiling associated with methanol extract, n-hexane fraction and chloroform fraction revealed the occurrence of many peaks among them various chemical compounds were identified. All of these compounds fall under many chemical classes and the majority of them have been shown to have significant biological roles. Figure 6 (a-c) represents a particular chromatogram of methanolic extract, chloroform and hexane fractions of *C. indica* fruits. The major recognized phytochemicals with their mass spectra (Figure 7), retention time (RT) and peak area (%) are presented in Table 4. Table 5 shows major identified phytoconstituents, molecular weight, molecular formula, structure, nature of compound and reported biological activities.

# DISCUSSION

Herbal drug standardization is the process of defining a set of specifications to ensure quality, efficacy, safety and purity. Strong quality control procedures are required in light of the increasing demand for herbal treatments in order to ensure their safe and efficient use. The WHO points out that evaluating herbal drug under both macroscopical and microscopical parameters is the first step towards plant standardization. A complete standardization of *C. indica* fruits were carried out using macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening as well as GC-MS analysis for identification of active constituents. Macroscopic examination of *C. indica* fruits revealed a pepo, ovoid, glabrous, greenish brown-yellowish brown additionally white lines; there was no smell and taste. Microscopic evaluation showed one layered epicarp, mesocarp that consisted of a wide space of thin-walled parenchymatous cells and several fibro-vascular bundles. The physicochemical values for example ash values, extractive values, moisture content, pH etc. were evaluated for the determination of quality and purity of *C. indica* fruits. Ash values are crucial parameter to establish the purity and quality of crude drugs. Total ash is the remained materials

**Table 3: Phytochemical screening of extract and fractions of *C. indica* fruits.**

| Secondary metabolites/Tests | | Methanol extract | $CHCl_3$ fraction | Hexane fraction |
|---|---|---|---|---|
| Alkaloids | Mayer test | + | + | – |
| | Wagner test | + | + | – |
| | Dragendorff test | + | + | – |
| | Hager test | + | + | – |
| Flavonoids | Shinodha test | + | + | – |
| | Alkali reagent | + | + | – |
| Tannins | Ferric chloride test | + | – | – |
| | Lead acetate test | + | – | – |
| Terpenoids | Salkowski test | + | + | + |
| Saponins | Foam test | + | – | – |
| Steroids | Liberman Burchard test | + | – | + |
| Phenolics | FeCl3 Test | + | + | – |

**Table 4: Major Phytochemicals identified in extract and fractions of *C. indica* fruits by GC-MS.**

| Sl. No. | Compound name | RT (Min) | Peak area (%) |
|---|---|---|---|
| | **Methanol extract** | | |
| 1 | 2H-Pyran-2,6(3H)-dione | 10.127 | 0.52 |
| 2 | 2-Methoxy-4-vinylphenol | 19.146 | 0.40 |
| 3 | L-Proline, 5-oxo-, methyl ester | 22.361 | 1.20 |
| 4 | Tetradecanoic acid/myristic acid | 31.986 | 0.57 |
| 5 | 2,5-Pyrrolidinedione,1-(phenylmethyl) | 32.492 | 0.46 |
| 6 | Pentadecanoic acid | 33.345 | 0.78 |
| 7 | n-Hexadecanoic acid | 35.023 | 16.69 |
| 8 | 7-Hexadecenal, (Z) | 36.335 | 0.89 |
| 9 | Octadecanoic acid/stearic acid | 38.253 | 2.75 |
| 10 | 4-(5-Ethyl-1,2,4-oxadiazol-3-yl)benzoic acid | 38.993 | 1.50 |
| 11 | 6,9-Octadecadienoic acid, methyl ester | 41.122 | 1.82 |
| 12 | gamma-Sitosterol | 42.615 | 11.03 |
| 13 | D-Friedoolean-14-en-3-one/taraxerone | 43.422 | 8.15 |
| 14 | Lup-20(29)-en-3-one | 44.056 | 7.86 |
| 15 | Lupeol | 45.170 | 6.60 |
| 16 | 13-Docosenamide, (Z)- | 45.246 | 0.85 |
| 17 | gamma-Sitostenone | 46.288 | 0.74 |
| 18 | Cholesta-4,6-dien-3-ol, (3.beta.) | 49.278 | 1.01 |
| 19 | alpha-Tocopherol-beta-D-mannoside | 50.309 | 0.86 |
| | **Chloroform fraction** | | |
| 20 | 1,2-Cyclopentanedione | 8.121 | 6.37 |
| 21 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl | 14.483 | 1.53 |
| 22 | 2,4,5,6,7-Pentamethoxyheptanoic acid, methyl ester | 15.123 | 1.03 |
| 23 | 3-Allyl-6-methoxyphenol | 20.194 | 3.24 |
| 24 | L-Proline, 5-oxo-, methyl ester | 21.189 | 2.61 |
| 25 | Loliolide | 31.441 | 3.92 |
| 26 | Caffeine | 32.971 | 2.36 |
| 27 | n-Hexadecanoic acid | 34.491 | 14.17 |
| 28 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl | 42.647 | 2.87 |
| 29 | 13-Docosenamide, (Z) | 45.166 | 6.20 |
| 30 | Piperine | 46.941 | 2.19 |
| 31 | Cholesta-4,6-dien-3-ol, (3 beta) | 49.196 | 1.06 |
| | **Hexane fraction** | | |
| 32 | Tetradecanoic acid | 31.884 | 0.66 |
| 33 | Oleic Acid | 33.305 | 0.98 |
| 34 | 2-Methyltetracosane | 33.909 | 1.86 |
| 35 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 36.249 | 1.29 |
| 36 | Methyl stearate | 36.781 | 0.46 |
| 37 | Dotriacontane | 40.218 | 0.99 |
| 38 | Methyl 20-methyl-heneicosanoate | 42.819 | 0.57 |
| 39 | Tetrapentacontane | 44.327 | 0.62 |

| Sl. No. | Compound name | RT (Min) | Peak area (%) |
|---|---|---|---|
| 40 | Squalene | 45.524 | 1.00 |
| 41 | alpha-Tocospiro A | 45.886 | 0.62 |
| 42 | alpha-Tocospiro B | 46.126 | 0.81 |
| 43 | alpha-Tocopherol-beta-D-mannoside | 50.358 | 0.86 |

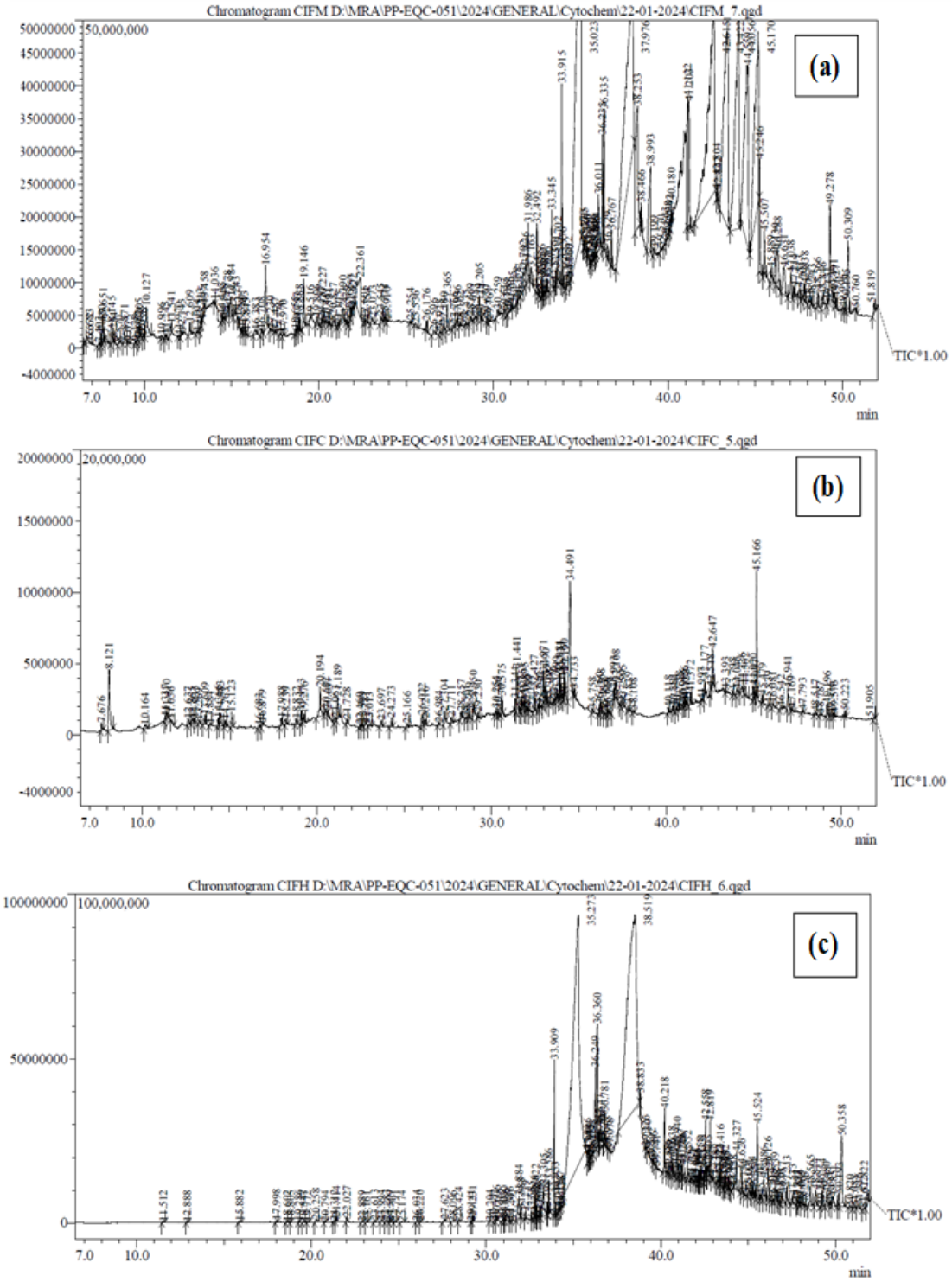
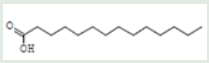
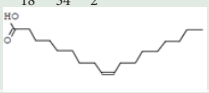
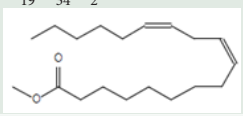
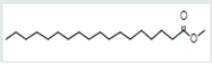
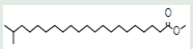
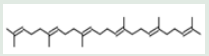
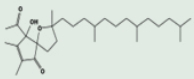
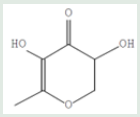
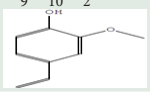
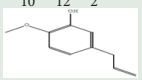
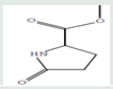
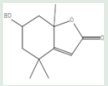
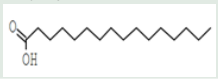
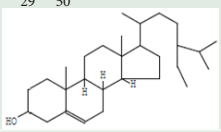
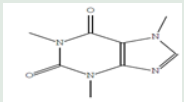
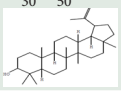
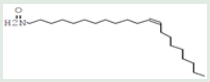
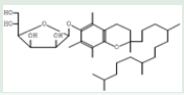
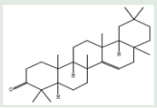
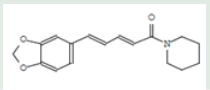



**Figure 6:** GC-MS chromatogram of *C. indica* fruits (a) methanol extract (b) chloroform fraction (c) hexane fraction.

**Table 5: Major Phytoconstituents identified in *C. indica* fruits by GC-MS along with their reported biological activities.**

| Sl. No. | Phytoconstituents | Molecular formula and structure | Mol. Wt. | Nature | Biological activity | References |
|---|---|---|---|---|---|---|
| 1 | Tetradecanoic acid | $C_{14}H_{28}O_2$  | 228 | Fatty Acid | Antidiabetic | [32] |
| 2 | Oleic Acid | $C_{18}H_{34}O_2$  | 282 | Fatty Acid | Anti-inflammaory | [33] |
| 3 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | $C_{19}H_{34}O_2$  | 294 | Fatty acid derivative | Analgesic, ulcerogenic | [33] |
| 4 | Methyl stearate | $C_{19}H_{38}O_2$  | 298 | Fatty acid derivative | Pharmaceutical aid | [34] |
| 5 | Dotriacontane | $C_{32}H_{66}$  | 450 | Alkane | Pharmaceutical aid | [34] |
| 6 | Methyl 20-methyl-heneicosanoate | $C_{23}H_{46}O_2$  | 354 | Fatty acid derivative | Fumigant and repellent | [35] |
| 7 | Squalene | $C_{30}H_{50}$  | 410 | Triterpene | Antioxidant and antitumor | [36] |
| 8 | alpha Tocospiro A | $C_{29}H_{50}O_4$  | 462 | Tocopheroid | Antioxidant | [37] |
| 9 | 4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6- methyl | $C_6H_8O_4$  | 144 | Heterocyclic compound | Antioxidant | [38] |
| 10 | 2-Methoxy-4-vinylphenol | $C_9H_{10}O_2$  | 150 | Phenolic compound | Anti-inflammaory | [39] |
| 11 | 3-Allyl-6-methoxyphenol | $C_{10}H_{12}O_2$  | 164 | Phenolic compound | Antiseptic | [40] |
| 12 | L-Proline, 5-oxo-, methyl ester | $C_6H_9NO_3$  | 143 | Amino acid derivative | Nutraceuticals | [41] |
| 13 | Loliolide | $C_{11}H_{16}O_3$  | 196 | Lactone derivative | Antidiabetic | [40] |

| Sl. No. | Phytoconstituents | Molecular formula and structure | Mol. Wt. | Nature | Biological activity | References |
|---|---|---|---|---|---|---|
| 14 | n-Hexadecanoic acid/ palmitic acid | $C_{16}H_{32}O_2$  | 256 | Fatty acid | Anti-inflammatory, antioxidant | [33] |
| 15 | gamma-Sitosterol | $C_{29}H_{50}O$  | 414 | Phytosterol | Antidiabetic, anticancer | [41] |
| 16 | Caffeine | $C_8H_{10}N_4O_2$  | 194 | Alkaloid | Diuretic | [41] |
| 17 | Lupeol | $C_{30}H_{50}O$  | 426 | Diterpenoid | Antimicrobial, anti-inflammatory | [42] |
| 18 | 13-Docosenamide, (Z) | $C_{22}H_{43}NO$  | 337 | Fatty amide | Antimicrobial | [37] |
| 19 | alpha-Tocopherol-beta-D-mannoside | $C_{35}H_{60}O_7$  | 592 | Glycoside | Antioxidant | [43] |
| 20 | D-Friedoolean-14-en-3-one | $C_{30}H_{48}O$  | 424 | Terpenoid | Antiviral | [43] |
| 21 | Piperine | $C_{17}H_{19}NO_3$  | 285 | Alkaloid | anti-inflammatory | [42] |

after igniting at 450°C, whereas carbon is completely eliminated. Total ash consists primarily of carbonates, phosphates, silicates and silica. The total ash, water soluble ash and acid insoluble ash values were found to be 16.52±.13% w/w, 11.35±.12% w/w and 1.45±.06% w/w, respectively. These results are within the limits that confirm the crude drug's purity. Alcohol and water-soluble extractive values are used for quantifying chemical constituents in herbal drugs. Adding exhausted materials to the crude drugs may cause an alteration in extractive values. The water and alcohol soluble extractive values were found 18.45±0.2% w/w and 8.80±0.11% w/w respectively. A higher extractive value with water suggests the chemicals constituents in the fruits are more water soluble. Moisture content is established as a means of thermo gravimetric method that is by loss on drying, wherein the sample is exposed to heat and the weight loss because of the evaporation of moisture is noted. Drug quality is indicated by moisture content; efficacy is decreased by high moisture levels because they can cause the hydrolysis of active constituents. Minimum moisture is ideal for protecting the drugs from decay. The moisture content of *C. indica* fruits has been estimated to be 7.33±.08% w/w. A preliminary qualitative test confirmed the presence of secondary metabolites in the fruits of *C. indica*, including flavonoids, phytosterols, triterpenoids, polyphenols, alkaloids, tannins, saponins and proteins, indicating a varied range of phytoconstituents.[24]

GC-MS analysis associated with methanol extract, chloroform and hexane fractions of *C. indica* fruits was performed to provide a more complete quantitative study. The results highlighted the presence of the most prominent phytochemicals (% peak area) that includes piperine (2.19%) and caffeine (2.36%) are alkaloids, D-friedoolean-14-en-3-one/taraxerone (8.15%) and lupeol (6.60%) are terpenoids, n-hexadecanoic acid (16.69%) and oleic

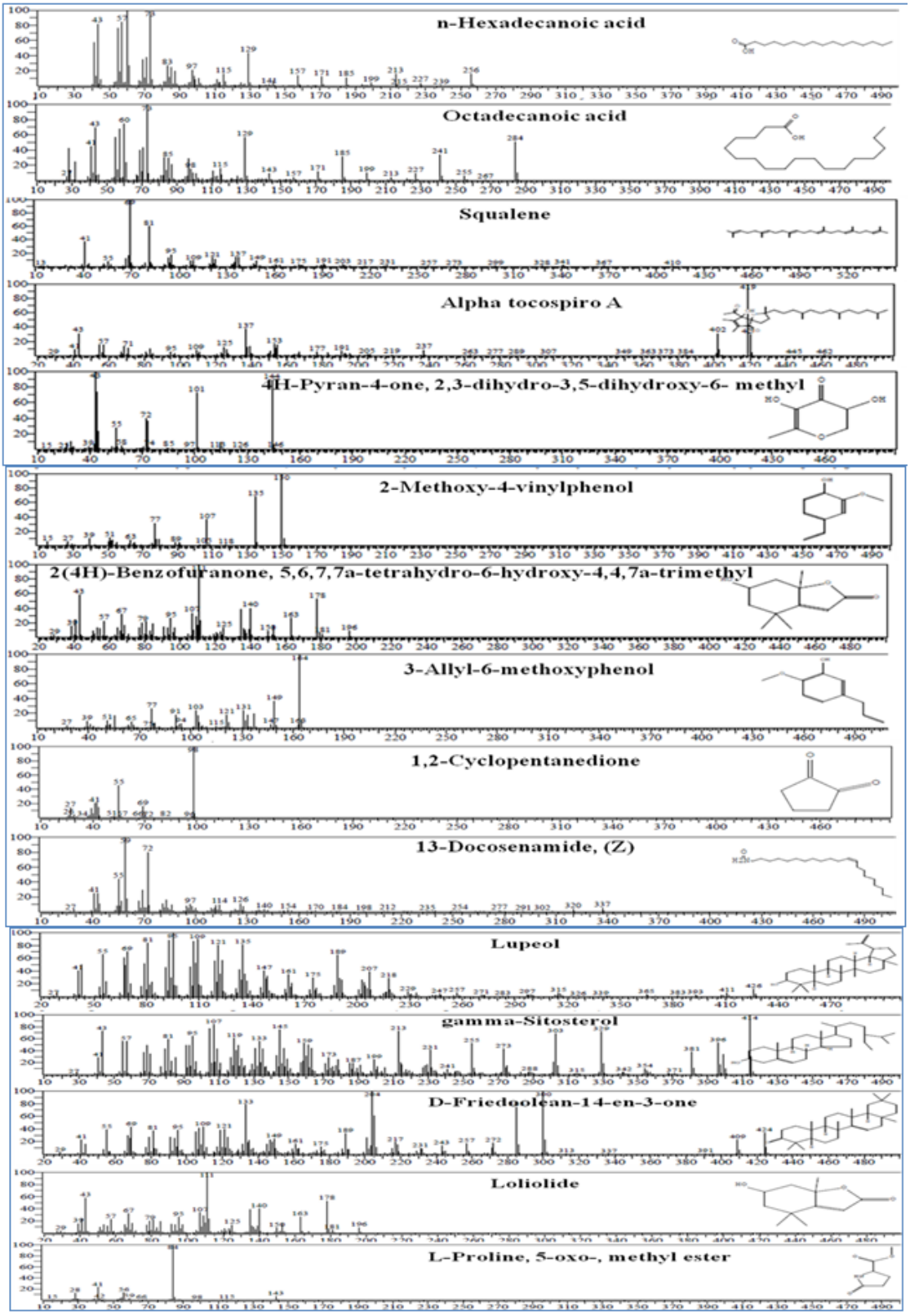



**Figure 7:** Mass spectra of chief compounds from fruits of *C. indica*.

acid (0.98%) are fatty acids, 2-methoxy-4-vinylphenol (0.40%) and 3-Allyl-6-methoxyphenol (3.24%) are phenolic compounds, L-proline, 5-oxo-, methyl ester (2.61%) is amino acid derivative, loliolide (3.92%) is lactone derivative, gamma-sitosterol (11.03%) is phytosterol. The molecular weight, molecular formula, molecular structure, nature and reported biological activities of major identified phytochemicals are outlined in Table 5.[23]

## LIMITATION OF THE STUDY

The study has the limitation that requires the isolation of phytoconstituents. This can be achieved through column chromatography and incorporating this method is essential to address the current gap in the research.

## CONCLUSION

The standardization of *C. indica* fruits will set important identifying standards for future investigations. Furthermore, the GC-MS analysis showed the presence of secondary metabolites like terpenoids (taraxerone, squalene), phenolics (2-methoxy-4-vinylphenol, 3-allyl-6-methoxyphenol), glycoside (alpha-tocopherol-beta-D-mannoside), alkaloids (piperine, caffeine) at higher concentration (peak area %) in chloroform fraction of *C. indica* fruits as compared to the hexane fraction. In conclusion, solvents used for extraction/fractionation altered phytochemical availability and concentrations (peak area). These secondary metabolites have various reported biological activities that include antibacterial, analgesic, antioxidant, anti-inflammatory, anticancer, antidiabetic and other activities, supporting the use of whole fruit for medicinal applications. However, additional efforts should be undertaken to isolate the identified phytoconstituents for the feasible development of novel herbal formulation.

## ACKNOWLEDGEMENT

The authors thank Hon. Chancellor Prof. Syed Waseem Akhtar and Vice-Chancellor Prof. Javed Mussarat of Integral University for their support and facilities. They also express gratitude to the Dean of Doctoral Studies for technical assistance and assigning the manuscript communication number (IU/RandD/2024-MCN0002962).

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**GC-MS:** Gas chromatography and mass spectrophotometry; **µL:** Microlitre; **WHO:** World health organization; **NIST:** National institute of standards and technology; **eV:** Electron volt.

## SUMMARY

*C. indica* is renowned plant in the Ayurveda for its hypoglycemic and antidiabetic properties. One of the most important requirements for verifying the efficacy, safety and quality of medicinal herbs includes their standardization. This study concentrated on the standardization and phytochemical investigation of *C. indica* fruits, with the purpose of identification and determination of the purity and quality of this plant for further study efforts. The research comprised of various standardization parameters like macroscopical, microscopical and physico-chemical evaluation, phytochemical screening, additionally GC-MS study to recognize main phytochemical of *C. indica* fruits. GC-MS analysis showed the presence of secondary metabolites like terpenoids, phenolics, glycosides, alkaloids at higher concentration (peak area %) in chloroform fraction of *C. indica* fruits. These secondary metabolites have various reported biological activities listed in tabular form. The produced research data is an important resource of chemical profiling of *C. indica* fruits.

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**Cite this article:** Alam S, Khan MI, Rizvi A, Badruddeen, Akhtar J, Ahmad M, *et al*. Standardization and GC-MS Analysis of Phytochemicals in *Coccinia indica* (Wight and Arn.) Fruits. Pharmacog Res. 2025;17(2):507-18.