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RESEARCH ARTICLE

FTIR Functional Group Analysis, Proximate Composition, Mineral Profile, and *In Vitro* Screening of *Cleome aspera* for Anthelmintic Activity

Anuradha Khuntia and M. K. Kathiravan*

Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Chengalpattu, Tamil Nadu, India; drmkkathir@gmail.com

Abstract

Background: The *Cleome* genus comprises flowering plants that have been extensively used in traditional medicinal practices for centuries. **Aim:** The current investigation aims to determine the FTIR profile, proximate composition, profile of minerals, qualitative phytochemical components, and study the *in vitro* anthelmintic activity of *Cleome aspera*. **Methods:** We investigated the aerial parts of *C. aspera* extracts, extracted in a Soxhlet apparatus using petroleum ether, ethyl acetate, and ethanol. The AOAC procedures were used for the determination of moisture, ash, fat, protein, and crude fiber content. The Fourier Transform Infrared Spectrophotometer (FTIR) instrument was used for detecting the presence of chemical bonds and functional groups. The anthelmintic properties against *Ascaridia galli* and *Pheretima posthuma* were conducted using a standard procedure. In the bioassay, various doses (25-100 mg/ml) of each extract were assessed, which included the determination of the worms' paralysis along with death times. **Results:** Qualitative phytochemical screening showed the presence of several important phytoconstituents. FTIR analysis of several extracts of *C. aspera* revealed prominent peaks for the substances amide, alcohols, alkanes, alkenes, alkyl halides, carboxylic acids, aldehydes, esters, ethers, ketones, phenols, primary amines, and aromatics. Results of the proximate analysis showed that *C. aspera* contained 10.04±0.02% moisture, 5.67±0.03% ash, 4.95±0.04% crude fibre, 17.7±0.01% crude protein, 3.43±0.03% fat, 34.5±0.05% carbohydrate and calorific value of 279 kcal/100g along with 38.5±0.05 sodium, 445±7 potassium, 72.6±0.2 magnesium, 16.7±0.5 manganese 229±0.4 phosphorus, 567±3 calcium, 16.2 ±0.6 iron, 9.5±0.5 zinc, 5.38±0.3 copper while lead was absent. A significant dose-dependent anthelmintic action was only observed with the ethanolic extract. **Conclusion:** The study's findings demonstrated that *C. aspera* was high in microminerals and may be a rich source of phytoconstituents. Significant amounts of proteins and carbohydrates were also present in the plants. As a result, the plant could be a useful dietary supplement and could be used to prepare drugs.

Major Findings: The plant *C. aspera* was found to contain several phytocomponents, primary metabolites, secondary metabolites, minerals, and demonstrated significant anthelmintic properties against *A. galli* and *P. posthuma.*

Keywords: Anthelmintic Activity, *C. aspera,* FTIR, Mineral Profile, Proximate Composition

Abbreviations: FT-IR; Fourier Transform Infrared Spectroscopy, HPLC; High-Performance Liquid Chromatography, UV; Ultraviolet, DAD; Diode Array Detector, GC-MS, Gas Chromatography-Mass Spectrometry, ELSD;Evaporative Light Scattering Detector. HPTLC; High-Performance Thin Layer Chromatography, NIR; Near Infra-Red, NMR; Nuclear Magnetic Resonance

1. Introduction

Cleome (family- *Capparidaceae*) has 12 species in India. Of these, 7 species were found in different states in South India. Roadside weeds include many species of *Cleome*, including *C. viscosa*, *C. gynandra*, and *C. chelidonii*. Additionally, some species have been used in conventional medical systems[1,2]. In the *Ayurvedic* medicine system, the plantsthatbelong to the *Cleome* genus are thought to possess a number of therapeutic qualities, including anthelmintic, laxative, diuretic, and stomachic effects. They are also thought

*Author for correspondence

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to be beneficial for skin conditions, ulcers, itching, leprosy, and malaria. In *Unani* medicine, the species of *Cleome* has been assigned a number of medicinal properties, including antibacterial, anthelmintic, and carminative properties[3]. Several parts of the plantare administered for diarrhea and fevers as well[4]. The plant species of the genus *Cleome* have been shown to have antipyretic, anticonvulsant, antidiarrheal, antimicrobial, antimalarial, anti-inflammatory, immunomodulatory, analgesic, wound healing, and local anesthetic properties. *C. aspera*is a prevalent annual weed that grows throughout the world's tropical regions, including the Indian plains[5-7].

Secondary metabolites of so many plants have also been found to serve as drug precursors or pharmaceutical intermediates that could be used to create stronger synthetic medications. The chemistry of plant natural products has actively contributed to the development of a sizable number of potential molecules for use in drug discovery. However, the natural ability of medicinal plants to create secondary metabolites may be contingent upon environmental variables as well as other ecological factors, necessitating constant monitoring to preserve the plant medications efficacy. To assess any herbal product utilizing metabolomic fingerprinting, suitable techniques such as HPTLC, FTIR, GC-MS, ELSD, NIR, NMR, and HPLC with UV (DAD), or a combination of these methodologies can be employed[8,9]. These methods also offer helpful details regarding the identification of chemometric patterns and the qualitative and quantitative composition of herbal medicines. A quick, non-invasive, physicochemical analytical method that saves time, Fourier Transform Infrared (FT-IR) spectroscopy may offer a glimpse of a tissues metabolic makeup at a particular moment. Still, it cannot identify the concentration of specific metabolites[10]. The actual structures of particular plant secondary metabolites can now be ascertained using FT-IR, which is particularly useful in phytochemistry. It also provides a quick and non-destructive method of fingerprinting of herbal extracts or herbal powders. According to a review of the literature, *C. aspera* has not yet been used for the FTIR analysis of functional groups. Therefore, the current study aims to characterize the functional groups of phytoactive chemicals found in extracts of the plant's aerial parts (in various solvents including ethanol, ethyl acetate, and petroleum ether).

Plants play crucial roles in human health. Plants of the genus *Cleome* provide proteins, fats, and other nutrients and carbohydrates. The majority of these *Cleome* plant species are packedwith several nutrients, including vitamins and minerals[11]. *C. gynandra* contains a wealth of micronutrients, including calcium, iron, vitamin A, and vitamin C. Some species seed oils have important properties such as high iodine number, refractive index, Reichert-Meissl number, Hehner value, iodine number, and saponification value, making them edible. *C. gynandra* is grown by contemporary Hopi people for its nutritional value and food security. People frequently use these plant species in the Central Lowveld Savanna Region and South Africa, because of its nutritional advantages, *C. gynandra* as well as *C. viscosa* are also consumed in several parts of Benin. The *C. serrulata* Pursh is frequently eaten boiled in India[12,13]. They are essential to the human body. Many cultures employ boiled leaves from various species as medicine. Other cultures use leaves after marinating in soured milk as a medical food becausethey believe it relieves marasmus, a disorder associated with a protein-energy deficit, and improves vision and vitality. To cure an array of maladies, practitioners of traditional medicine use numerous types of medicinal plants[14-17]. The ethnopharmacological qualities of these plants, which include bioactive components, enable them to treat illnesses in both humans and animals. A diet rich in fruits, vegetables, and phytochemicals may lower the risk of heart disease and some types of cancer, according to research. A lot of plants are not used enough. The use of these therapeutic plants to treat serious human ailments is underexplored. The phytochemical and proximate component amounts of *C. aspera* that can be used to treat specific illnesses were measured in this study[18-20].

Traditional remedies show enormous promiseas suppliers of easily obtainable, highly effective human anthelmintic medications, especially in tropical developing countries like India. Various plants or compounds derived from plants are used in this context to treat helmintic disorders[21,22]. In the current work, we assessed the anthelmintic properties of petroleum ether and ethyl acetate, along with ethanol extracts of *C. aspera* aerial parts. Following a huge number

of claims regarding the several plants of the *Cleome* genus and the vast spectrum of traditional medicinal characteristics, by using pharmacological studies, considerable attempts have been made to confirm its efficacy as a healing agent.

2. Materials and Methods

2.1 Collection, Identification, and Authentication of *C. aspera* Plant Material

Fresh aerial sections of *C. aspera* were collected from the Sri Venkateswara National Park in the Tirumala hills of Andhra Pradesh, Tirupati region. A botanist from the Department of Botany at Shri Venkateswara University in Tirupati, Andhra Pradesh, India, identified and authenticated the taxonomy of the collected plant material. The voucher specimen (0506) was also deposited in the herbarium. The gathered plant parts (aerial sections without roots) were thoroughly washed with running tap water to eliminate any dust particles. Following the cleaning process, the plant material was shade-dried. Once the drying process was complete, the plant material was finely ground to a coarse powder using a mechanical mixer and subsequently stored in a properly labelled and well-sealed container for future use.

2.2 Preparation of Extracts

150 grams of the dried and powderedplant material of *C. aspera (*aerial parts) were first weighed, then loaded. Then the plant powder was extracted in a Soxhlet apparatus using 1 L volumes of petroleum ether, ethyl acetate, and ethanol successively in that order, according to the solvent hierarchy of polarity. The solvent that emerged from the siphoning tube was colourless after seventy-two (72) hours of extraction. Using the produced extracts, phytochemical screening, Fourier Transform Infrared (FTIR) functional group analysis, and anthelmintic activities were determined.

2.3 Determination of Phytoconstituents

Various phytoconstituents were screened for preliminary phytochemicals using a standardized technique[23].

2.4 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

The Fourier Transform Infrared spectrophotometer (FTIR) stands out as a crucial instrument for detecting the presence of chemical bonds, also known as functional groups, in various compounds. The spectrum, when annotated, reveals that the absorbed lights wavelength corresponds to specific chemical bonds. By examining the infrared absorption spectrum, one can determine the chemical bonds within a molecule. For FTIR analysis, researchers utilized dried powders obtained from different plant solvent extracts. To create transparent sample discs, 10 mg of the dried extract powder was mixed with 100 mg of potassium bromide (KBr) pellet. The powdered plant specimen was analyzed using an FTIR spectroscope, which operated with a 4 cm^{-1} resolution and scanned a range from 400 cm^{-1} to 4000 cm^{-1}.

2.5 Proximate Composition

The AOAC procedures were used for the determination of moisture, ash, fat, protein, and crude fiber content[24].

2.5.1 Determination of Moisture Content

To ascertain the amount of moisture present, a dry clean crucible was weighed, and recorded as W1. After weighing the sample of 3g, it was added to the Crucible, and recorded as W2. For 3 hours, the crucible containing the sample was dried constantly in an oven set at 105°C. After the crucible had been transferred to the desiccator to cool, the weight was noted. Until a constant weight (W3) was reached, the procedure was repeated. The % of weight loss while drying was used to calculate the moisture content percentage.

% of moisture = (weight loss /weight of the sample) ×100

2.5.2 Determination of Ash Content

One gram (1g) of the dried plant sample was weighed to ascertain its ash content (W1) using a crucible with a cover that had been cleaned, dried, and previously weighed. The crucible was placed in a muffle furnace (without a lid) and heated to 550°C. After that, the lid was removed, and the sample was burned to char the organic material. Soon after, the crucible was put

straight into a desiccator, left to cool, and weighed whenever the ash turned light grey or white (W2).

Ash= [(W1-W2)/weight of the sample] ×100

2.5.3 Determination of Fat Content

The procedure of Soxhlet extraction was used to estimate the amount of crude fat content. On a filter paper, two grams (2 g) of the sample were put. The filter paper carrying the plant sample was folded with care and then placed inside a thimble that had been dried out. The sample was put in a thimble and put inside the Soxhlet flask. A boiling flask was filled with diethyl ether, once it had been weighed (W1) and then thoroughly dried. A condenser, Soxhlet flask with the sample, and diethyl ether boiling flask were put together. For around 6 hours, the reflux-induced extraction was performed. The thimble was taken carefully out of the extraction barrel and left to dry after extraction. Once the solvent was properly removed by the distillation process, the boiling flask containing fat was then dried in an oven ata low temperature. The weight of the flask plus oil was measured and noted (W2). The percentage of crude fat content was then measured using the fat that was extracted from a predefined volume of the sample.

% of fat = [(W1-W2)/ sample weight] ×100

2.5.4 Determination of Nitrogen and Protein Content

We used the micro Kjeldahl method for thedetermination ofthe total nitrogen concentration of the plant sample. The sample (2 g) was boiled thoroughly with 20 ml of concentrated H_2SO_4 along with a Kjeldahl digestion tabletuntil the mixture in a Kjeldahl flask became clear. Distilled water was added to a 250 ml volumetric flask to fill it after the digest was filtered into it, readying it for distillation. After adding 50 mL of NaOH (45%) solution to the digest, the ammonia was recovered using steam distillation.150 ml of the obtained distillate and 100 ml of 0.1 N HCl were collected into a conical flask, and the indicator, methyl orange, was utilized. Back titration against 2 M NaOH was utilized for the estimation of the amount of Nitrogen (N) in the receiving flask after the acid reaction.

The equation used to compute the nitrogen content was as below

Nitrogen = {(A-B) ×1.4007/weight of sample} ×100

A = volume (ml) of standard HCl x normality of standard HCl

B = volume (ml) of standard NaOH x normality of standard NaOH

To get the crude protein percentage, the nitrogen amount was multiplied by 6.25%.

2.5.5 Determination of Crude fibre and Carbohydrate Content

To measure the amount of crude fibre, using a Soxhlet apparatus, a sample weighing 2.5 g was extracted using diethyl ether. After being air dried, the extracted sample was heated for precisely 30 minutes in a 1L conical flask that was equipped with a water-cooled reflux condenser containing 1.25 percent sulfuric acid. After letting the mixture cool, it was filtered through a fresh andclean white linen cloth. After that, hot water was used to wash it until the mixture was no longer acidic to litmus paper. The leftover residue was then further boiled using a 1.25% NaOH solution. The flask boiled for around 30 minutes after being instantly connected to the reflux condenser. Using the filtering cloth, the mixture in the flask was filtered. The sample residue was placed in a Gooch crucible that had been previously prepared with a thin, dense layer of burned asbestos after thorough cleaning with hot water and the application of approximately 15 ml of ethyl alcohol. The Gooch crucible, along with its contents, was then dried in an oven at 105±2°C until a stable weight was achieved. After cooling; the contents of the dried crucible were weighed. In a muffle furnace, the contents of the Gooch crucible were entirely burned until only carbonaceous material was left. After being dried in a desiccator, the resultant ash was cooled and weighed. The estimated amount of crude fibre was:

Crude fibre% by weight = (W1-W2/ W) × 100

Where, W1 = Gooch crucible along with its contents weight before ashing(in grams)

W2 = weight of asbestos along with ash-filled Gooch crucible (in grams)

W = weight of the dried test sample (in grams)

Carbohydrate content was determined by calculating the difference as the formula given below

{100 - (ash + moisture + crude protein + crude fibre + crude fat contents)}.

By multiplying the crude protein content by 4, the crude carbohydrate content by 4, and the crude fat

value by 9, the given sample's calorific value (kcal/100g) was determined.

2.6 Mineral Content Determination

The mineral ingredients of plant *C. aspera* were identified by Omotoso and Adedire using the Oshodi technique[25]. A muffle furnace was used to ash the plant *C. aspera* for several hours at around 550°C. After the ash sample was completely dissolved in 10% HCl, distilled water was added to a standard flask to bring the volume approximately up to 100 ml. The amounts of calcium, iron, magnesium, manganese, zinc, copper, and lead in the sample were measured using an atomic absorption spectrophotometer. While the Vanado molybdate technique was used to determine phosphorus concentrations, a flame photometer was used to evaluate potassium and sodium concentrations.

2.7 Anthelmintic Assay

2.7.1 Worm Collection and Authentication

Earthworms of the species *P. posthuma* L. Vaill (belonging to the *Annelida* family) were collected from moist soil, while *A. galli* Schrank worms (from the *Nematode* family) were obtained from recently killed chickens of the *Gallus gallus* Spadiceus species (*Phasianidae* family). The 2 worm species were identified by the Zoology Department at B.V. Raju College in Bhimavaram.

2.7.2 Procedure

With the appropriate adjustments, the anthelmintic assay was conducted using the Ajaiyeoba *et al.*, method[26]. 6 worms of the same type were put in 50 mL of formulations that had varying concentrations of different crude extracts (25, 50, and 100 mg/mL in distilled water). This was carried out for both types of worms. When there was no movement at all, except in cases following intense agitation, the period of immobility exhibited by the worms was documented. They subsequently confirmed the absence of any movement from the worms when subjected to either violent shaking or submersion in 50°C water. The time required for the worms to perish was then recorded. Distilled water was employed as the control, while albendazole (20 mg/mL) and piperazine citrate (15 mg/mL) were utilized as the reference standard.

3. Results and Discussion

3.1 Preliminary Qualitative Phytochemical Analysis

Table 1 outlines the chemical components identified in the analyzed extracts of *C. aspera*. The initial phytochemical screening results indicated the presence of several significant constituents, including steroids, alkaloids, flavonoids, phenols, glycosides, terpenoids, saponins, and tannins.

Primary metabolites, which mostly include proteins, carbohydrates, and lipids, as well as secondary metabolites, which comprise alkaloids, flavonoids, phenols, sterols, terpenes, essential oils, and tannins, are produced by plant cells. A review of the literature revealed that natural substances are mostly used to treat several types of illnesses. The results of the initial phytochemical screening prompted an in-depth investigation to determine biological activity, as different disorders are treated with these naturally distinct groups[27,28].

3.2 FT-IR Spectroscopic Analysis

The FT-IR spectra (frequency range 4000-400 cm^{-1}) of *C. aspera* extracts were analyzed, focusing on specific wave numbers and intensities to identify different kinds of functional groups of active components in the three plant extracts. The results of FT-IR peak values and functional groups are presented in Tables 2, 3, and 4. The petroleum ether extract of *C. aspera* exhibited 12 primary peaks in its FT-IR spectra, spanning from 3677.04 to 953.57 cm^{-1}. The ethyl acetate extract displayed 21 main peaks, ranging from 3732.95 cm^{-1}

Table 1. Qualitative phytochemical screening for aerial parts of *C. aspera*

Phytochemicals	Ethanol	Ethyl acetate	Petroleum ether
Alkaloid	+	-	-
Flavonoid	+	+	-
Steroid	+	+	-
Phenols	+	+	+
Glycoside	+	+	-
Tannin	+	+	+
Terpenoid	+	+	-
Saponin	+	-	-

Note: '+' implies presence of phytochemical, '-' implies absence of phytochemical

Table 2. FT-IR spectral peak values and functional groups obtained for the petroleum ether extract of *C. aspera*

Peak	Bond	Functional group
3677.04	O-H stretch	alcohol
2914.30	C-H stretch	alkanes
2848.17	C-H stretch	carboxylic acid
2360.51	C-H stretch	
1836.29	C-H bend	aromatic compound
1736.43	C=O stretch/ C=O stretch	esters/ δ-lactone/aldehyde
1706.50	C-H bend/ C=O stretch	aromatic compound/ aliphatic ketone/ carboxylic acid/ conjugated aldehyde/ conjugated acid
1518.26	N-O stretch	Aromatic nitro compounds
1465.48	C-H bend	carbonate ion, alkane
1370.92	O-H bend/ S=O stretch	nitrate ion, alcohol, sulphonate
1168.46	C-F stretch/C-N stretch/C-O stretch	Fluoro compound/amines/ ester/tertiary alcohol
953.57	Aromatic C-H in-plane bend	aromatic ring

Table 3. FT-IR spectral peak values and functional groups obtained for the ethyl acetate extract of *C. aspera*

Peak	Bond	Functional group
3732.95	O-H stretch	alcohol
3677.07	O-H stretch	alcohol
3619.40	O-H stretch	alcohol
2996.18	C-H stretch	aldehydes
2915.33	C-H stretch	alkane
2848.94	O-H stretch/N-H stretch/C-H stretch	carboxylic acid, alcohol, amine, alcohol
2692.00	O-H stretch	carboxylic acid
2360.09		
1702.94	C-H bend/ C=O stretch	aromatic compound/ conjugated acid/ conjugated aldehyde
1600.71	C=C stretch/ N-H bend	conjugated alkene/ amine/ cyclic alkene
1511.42	N-O stretch	nitro compound
1458.73	C-H bend	alkane
1366.21	O-H bend/ C-F stretch/ S=O stretch	alcohol/ phenol/fluoro compound/ sulfonamide/ sulfonate
1167.47	C-F stretch/ C-N stretch/ C-O stretch	fluoro compound/ amine/ ester/ tertiary alcohol
1080.68	C-F stretch/ C-N stretch/ C-O stretch	fluoro compound/amine/ primary alcohol
1007.63	C-F stretch	fluoro compound
951.68	C=C bending	alkene
891.74	C=C bend/ C-H bend	alkene/1,2,4-trisubstituted/1,3-disubstituted
832.36	C-Cl stretch/ C=C bend	halo compound/ alkene
722.11	C-Cl stretch/ C=C bend	halo compound/ alkene
664.82	C-Cl stretch/ C-Br stretch	halo compound

to 664.82 cm^{-1}, and the ethanol extract showed 18 primary peaks, extending from 3828.37 to 632.16 cm^{-1}. The detected peaks primarily consisted of amides, alcohols, alkanes, alkenes, alkyl halides, carboxylic acids, aldehydes, esters, ethers, ketones, phenols, primary amines, and aromatics.

The current FT-IR data verified that alcohols, carboxylic acids, sulfur compounds, alkanes, aldehydes, alkyl halides, ketones, amines, aromatic compounds, nitro compounds, alkenes, phenols, ester and were present in the three extracts of *C. aspera* (Tables 1, 2 and 3) according to earlier research on other plants of genus *Cleome*. The cuticle, as well asthe epicuticular wax of numerous plant species, contains alkanes. They shield the plant from dehydration, stop rain from removing vital minerals, and guard against micro-hazardous insects and creatures. Alkenes play a verysignificant role in the production of plastics like polythene as well as fuel and lighting. They are used as raw materials to make aldehydes and alcohols[29]. They are employed in the production of toxic mustard gas, ethylene-oxygen flame, a general anesthetic, and the artificial ripening of fruits. The primary building blocks of protein synthesis are amides and amines. Carboxylic acids are potent antibacterial agents and play an importantand crucial biological role in the body's fat production. They are the primary pharmaceutical medicines used to treat rheumatic joint problems, ulcers, jaundice, headaches, fevers, liver pain, and cow wounds. Combining aldehydes with phenols results in the creation of resins.

Table 4. FT-IR spectral peak values and functional groups obtained for the ethanol extract of *C. aspera*

Peak	Bond	Functional group
3320.57	O-H stretch/ N-H stretch/ H stretch	alcohol/ aliphatic primary amine/ secondary amine/ alkyne
2921.88	O-H stretch/ N-H stretch/ C-H stretch	carboxylic acid/ alcohol/ amine salt/alkane
2361.99	O=C=O stretch	carbon dioxide
1695.70	C-H bend/ C=O stretch	aromatic compound/ conjugated acid/ conjugated aldehyde
1649.17	C=N stretch/ C=C stretch/ N-H bend	alkene/ conjugated alkene/ amine/ cyclic alkene
1609.28	C=C stretch/ N-H bend/ C=C stretch	conjugated alkene/ amine/ cyclic alkene
1512.46	N-O stretch	nitro compound
1443.65	C-H bend	alkane
1364.53	O-H bend/ C-F stretch/ S=O stretch	alcohol/ phenol/ fluoro compound/ sulfonamide/ sulfonate
1248.06	C-F stretch/ C-O stretch/ C-N stretch	fluoro compound/ alkyl aryl ether/ amine
1061.28	C-F stretch/ C-N stretch/ =O stretch C-O stretch	fluoro compound/ amine/ primary alcohol/ sulfoxide
1017.36	C-F stretch	fluoro compound
822.91	C-Cl stretch/ C=C bend/ C-H bend	halo compound/ alkene/1,4-disubstituted/1,2,3,4-tetrasubstituted
776.75	C-Cl stretch/C-H bend	halo compound/1,2,3-trisubstituted
729.40	C-Cl stretch/C=C bend	halo compound/alkene
662.17	C-Cl stretch/ C-Br stretch	halo compound
632.16	C-Cl stretch/ C-Br stretch	halo compound

Fruits have a pleasing scent when volatile oils and esters are combined. The plant contains sulphur compounds in 3 different forms: Sulphates, volatile oils, and protein amino acids. They served as dental creams and disinfectants. Chlorinated tryptophan is produced by halogen chemicals inside plant cells and subsequently transported into the metabolism of monoterpene indole alkaloids to produce alkaloids with chlorination. Chlorates serve as disinfectants, and eosinophils require bromide in order for their peroxidase to produce anti-parasitic brominating chemicals[30].

3.3 Proximate Composition of *C. aspera*

Table 5 shows that *C. aspera* contained 10.04±0.02% moisture, 5.67±0.03% ash, 4.95±0.04% crude fibre,17.7±0.01% crude protein, 3.42±0.03% fat, 34.5±0.05% carbohydrate, and 279 kcal/100g calorific value. Plants that have more than 15% moisture content are vulnerable to degradation from heat, mould growth, insect damage, and sprouting. Consequently, given its low moisture content, it is possible that *C. aspera* could be kept in storage for an extended period of time[31]. An indicator of the plants inorganic mineral content is the amount of ash. According to the findings of this investigation, *C. aspera* contained 4.95% crude fibre. A lower incidence of diverticulosis, heart disease, and several types of cancer has been associated with fibre consumption[32]. *C. aspera's* crude protein content is relatively higher than the range of values (14.67-21.28%). Since *C. aspera* can offer more than 12% of the calorific value from protein, it could be considered a potentially high-quality protein source. At 3.42%, *C. aspers's* crude fat content was somewhat comparable to that of *Amaranthus hybridus* (4.80%)[33]. Because it absorbs and retains flavour, dietary fat improves foods flavour. Though extra fat has been connected to certain cardiovascular issues, humans are thought to need a diet that contains 1.20 % fat. *C. asperac*ontainsa higher percentage of carbohydrates (34.5%). It is possible to extract a substantial amount of carbohydrates from *C. aspera*, which can help the body meet its energy needs. One important class of naturally occurring organic compounds that sustains and promotes life in both plants and animals is carbohydrates[34-38].

3.4 Mineral Composition of *C. aspera*

Cleome aspera (Table 6) contained 38.5±0.05 sodium, 445±7 potassium, 72.6±0.2 magnesium,16.7±0.5

Table 5. Proximate composition (%) and calorific value (kcal/100g) of *C. aspera*

Parameter	Results
Moisture	10.04±0.02
Ash	5.67±0.03
Crude fiber	4.95±0.04
Protein	17.7±0.01
Fat	3.42±0.03
Carbohydrate	34.5±0.05
Calorific value	279

Results were given as mean ± Standard Deviation (SD) of triplicate determinations.

Table 6. Mineral composition of *C. aspera*

Elements	Concentrations(mg/100g)
Sodium (Na)	38.5±**0.05**
Potassium(K)	445±**7**
Magnesium (Mg)	72.6±**0.3**
Manganese (Mn)	16.7±**0.5**
Phosphorous(P)	229±**0.4**
Calcium (Ca)	567±**3**
Iron (Fe)	16.2±**0.6**
Zinc (Zn)	9.5±**0.5**
Lead (Pb)	-
Copper (Cu)	5.38±**0.3**

Results were given as mean ± Standard Deviation (SD) of triplicate determinations.

manganese, 229±0.4 phosphorus, 567±3 calcium, 16.2±0.6 iron, 9.5±0.5 zinc, 5.38±0.3 copper, and lead was absent.

The main mineral in bone is calcium, which also helps regulate how the heart and skeletal muscles contract. Furthermore, calcium has a role in exocytosis, neurotransmitter release, and smooth muscle contraction, among other functions[39]. Potassium and salt are essential elements for preserving cellular balance. Phosphorus, found in the sample, plays a crucial role in sustaining life. It is a key element in DNA, RNA, ATP, and cellular membranes, contributes to bone and tooth formation, and acts as a cofactor for enzymes[40]. Magnesium is vital for maintaining the structure, metabolic processes, and replication of lipid membranes. Among the micronutrients, manganese was the most prevalent at 16.7 mg/100g, with iron at 16.2 mg/100g, zinc at 9.5 mg/100g, and copper at 5.38 mg/100g following in abundance.

A vital human vitamin that supports brain function and reproduction, as well as blood sugar management, manganese is found in *C. aspera*. Iron is necessary for several essential human functions, such asdevelopment, sexual maturation, and haemoglobin synthesis[41]. Zinc, the most crucial mineral, is necessary for both the catalysis of enzymes and the synthesis of proteins. The vital trace element (also known as a macronutrient) copper, which is found in *C. aspera* plants, is necessary for the health of humans, animals, and plants[42]. Many essential physiological functions in the bodies of animals that eat plants, including blood coagulation, skeletal development, neuromuscular irritability, and enzyme activity, may be significantly impacted by the minerals found in plants. Additionally, they are crucial as a cofactor for brain cells. Any one of the required minerals might cause long-term metabolic issues and have a detrimental effect on the health of the organism that lacks it. Consequently, the fact that *C. aspera* contains all of these elements suggests that it may be helpful in treating certain metabolic diseases and preserving overall health[43-46].

3.5 Anthelmintic Assay of *C. aspera*

Ethanolic extract of *C. aspera* demonstrated paralysis(P) and death(D) times of 6.35 and 23 minutes against the earthworm *P. posthuma*, while ethanol extract induced paralysis of 8 minutes and a time of death of 30 minutes.

Additionally, *Ascardia galli* worms showed sensitivity to *C. aspera*'s ethanol and ethyl acetate extracts. The ethanol extract caused paralysis for 7 minutes. Of the death of 13 minutes, the ethyl acetate extract showedparalysis(P) and death (D)times of 8 and 16 minutes, respectively. The same thing happens when piperazine is citrated in 10 and 40 minutes, and the resulting results are outlined in Table 7 below.

Due to their wide availability, earthworms are frequently employed in initial *in vitro* evaluations of anthelmintic substances. The research utilized *P. posthuma*, the most commonly used adult Indian earthworm species, for conducting the assay procedure, due to their anatomical and physiological resemblance to human intestinal roundworm parasites[47]. Previously, *A. galli* earthworms from recently slaughtered poultry were suggested as a suitable model for anthelmintic drug screening due to their abundance and accessibility[48]. The anthelmintic activity assessment (Table 7) revealed that *C. aspera*'s ethyl acetate and ethanol extracts exhibited significant anthelmintic effects at increasing

Table 7. Anthelmintic activity of three extracts of the aerial parts of *C. aspera*

Test Substance	Concentrations (mg/mL)	Time taken for Paralysis (P) and Death (D) of worms in mins			
		P. posthuma		*A. galli*	
		P	D	P	D
Petroleum ether extract	25	-	-	-	-
	50	62.27± 0.62	-	41.52 ± 0.67	-
	100	40.00± 0.35	52.24±0.67	28.34 ± 0.45	46.30± 0.47
Ethyl acetate extract	25	28.53± 0.74	87.28±0.79	18.34 ± 0.56	55.64± 0.64
	50	15.34± 0.87	56.46±0.65	12.65 ± 0.43	33.67± 0.27
	100	8.00 ± 0.21	30.44±0.68	8.43 ± 0.68	16.17± 0.65
Ethanol extract	25	24.32±*0.21*	67.24±0.85	15.58 ± 0.67	42.54± 0.64
	50	13.67± 0.45	31.3±0.21	8.63 ± 0.44	20.65± 0.56
	100	6.35±0.52	23.24±0.47	7.35 ± 0.79	13.78 ± 0.34
Albendazole	20	34.66± 0.72	65.38±0.58	21.45 ± 0.25	36.46± 0.37
Piperazine Citrate	15	17.30± 0.25	57.35±0.35	10.45 ± 0.47	40.36± 0.35

Values are mean ±Standard Deviation (SD) of threeindependent readings; (-) indicates no activity.

concentrations. Both extracts demonstrated dose-dependent anthelmintic properties, with the shortest Paralysis (P) and Death (D) times for both worm species occurring at 100 mg/mL concentration. The reference drug, piperazine citrate, produced similar results after 20 and 59 minutes. The activity order was determined to be ethanolic extract > ethyl acetate extract > petroleum extract. Piperazine citrate, one of the standards used, induces flaccid paralysis in worms, leading to their expulsion through peristalsis[49]. Although the extracts paralyzed and killed the worms in a manner similar to albendazole, their anthelmintic effect was found to be comparable to commonly used medications. The study concludes that *C. aspera* plant extracts demonstrate strong antiparasitic effects against the examined helminth species, validating the traditional application of the plant as a deworming agent. Further research is necessary to elucidate the mechanism of action and isolate the phytochemical components responsible for the observed activity. Additionally, FTIR analysis of various *C. aspera* extracts revealed the presence of key functional groups associated with proteins, lipids, oils, phenolic compounds, flavonoids, saponins, tannins, and carbohydrates. The diverse functional groups found in the various extracts most likely suggest the existence of carbohydrates, glycogen, cellulose, phosphates, starch, amides, carbs, and carotenoids. Since OH groups can form hydrogenbonds, their presence might increase the capability of the ethanol extracts anti-parasitic activity. It has previously been shown that extracts of the different *Cleome* plants had a stronger anthelmintic activity[50].

4. Conclusion

The current investigations findings revealed new phytochemical markers for *C. aspera* detection, which were composed ofamides, alcohols, alkanes, alkenes, alkyl halides, primary amines, aromatics, carboxylic acids, aldehydes, ketones, phenols, esters, ethers, and aliphatic amines. Due to its high phytochemical content and potential for therapeutic physiological impact on the human body, *C. aspera* may one day be used to make essential medications. Furthermore, because of its high protein, carbohydrate, and other nutritional content, it may help meet human nutritional demands and combat disorders linked to malnutrition. Also, because the ethanol extract demonstrated anthelmintic qualities against the worms utilized in the study, the traditional usage of *Cleome* plants as an anthelmintic has been validated. Furthermore, identifying the potential mechanism or mechanisms of action and isolating the potential elements that contribute to anthelmintic activity would be intriguing. In conclusion, the plants might be abundant in phytoconstituents, which, based on their documented medicinal applications, can be separated and examined for various biological activities.

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6. Author Contribution

A.K, and M.K.: Conceptualization, methodology, formal analysis, investigation, resources, writing-original draft preparation, writing-review and editing, visualization, supervision, A.K.; data curation. All authors have read and agreed to the published version of the manuscript.

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