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RESEARCH ARTICLE

Preliminary Phytochemical Analysis, *In-vitro* Evaluation of Antibacterial and Anticancer Activity of *Cleome aspera* Aerial Part Extracts

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Abstract

Various species of the *Cleome* genus belong to the circle of relatives Cleomaceae family, and order Brassicales, constitute flowering flora with a long record of use in conventional remedies, and have been related to the prevention of many illnesses. *Cleome aspera* has not been thoroughly investigated in the medicinal area; however, it may offer a therapeutic choice in favour of the management of various disorders. In this recent study, the aerial parts of *C. aspera* were extracted with petroleum ether, ethyl acetate, and ethanol sequentially in ascending polarity order. Ethanol extract exhibited a rich phytochemical profile and antibacterial and anti-cancer action among the solvent extracts. The phytochemical analysis of three extracts exhibited the existence of many secondary metabolites like flavonoids, alkaloids tannins, polyphenols, steroids, glycosides, and terpenoids. The antibacterial activity against two Gram (+) bacteria, *Staphylococcus aureus* (ATCC 6538), and *Bacillus subtilis* (ATCC 6633), as well as two Gram (-) bacteria, *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 8739) was assessed at three concentrations (50, 100, 200 µg/ml) of each extract. The broth dilution method tested the extract's minimum inhibitory concentration against four bacterial strains. Using the MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) assay, the three extracts (petroleum ether, ethyl acetate, and ethanol) of *C. aspera* were evaluated for *in-vitro* cytotoxic activity. The extracts were detected to have substantial antibacterial activity against Gram-positive as well as Gram-negative bacteria in a dose-dependent manner. The ethanol extract of *C. aspera* showed notable anticancer efficacy after comparing it with petroleum ether, and ethyl acetate extracts. This study proved that the plant *C. aspera* has potential as a source of plant-based drugs and has therapeutic qualities like antibacterial and anticancer activities. It may provide a healing choice for the control of several diseases. A further extensive study on the isolation of active ingredients, purification, characterization, and elucidation of viable mechanisms can be executed.

Keywords: Anti-bacterial Activity, Anti-cancer Activity, *Cleome aspera*, MTT Assay, Phytochemical Screening

1. Introduction

Nature has been the origin of medicine for hundreds of years, and plant-related systems continue to play a significant role in elementary health care in 75-80% of developing countries[1]. In India, more than 3,500 plant species are used to prepare natural medicines[2]. As per WHO more than eighty percent of the global population depend on traditional medicine for fundamental care. There are between 215,000 and 500,000 species of huger and higher plants on earth[3]. However, only 6% of these plants are used for medicinal use. Almost one hundred twenty-two (122) compounds were extracted from ninety-four plant

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species and 81% of these compounds were employed for similar, related uses[4,5]. Plants can produce specific compounds which are actively engaged in combating a variety of harmful bacteria. Most phytochemicals belong to various chemical classes with the ability to inhibit all types of microorganisms *in-vitro*[6]. However, there is a problem in ensuring bacterial pathogen resistance using currently accessible medicines, so the evaluation of antibacterial effectiveness is an urgent task for drug development for various bacterial diseases.

Cancer is an incurable disease. This has become a global burden in recent years and is having a huge impact on how it affects communities across the globe. It is a complicated illness characterized by aberrant cell proliferation that quickly spreads to other body parts[7]. The most prevalent cancers worldwide are lung, prostate, pancreatic, breast and colorectal cancer. The main reasons for cancer are radiation, environmental pollution, tobacco, obesity, alcohol, bad eating habits, lack of exercise, and a variety of viral and bacterial diseases. These illnesses are currently a significant health issue in developing nations. There is an exceptionally high demand for new medications to prevent or treat certain disorders. Since ancient times, plants have served as one of the primary and crucial sources of a continual supply of medicines to keep humans alive. Incurable disorders are better treated with herbal therapies because they have fewer adverse effects. Several plant and herbal ingredients have shown potential roles in cancer treatment. So, scientists are researching natural resources to discover anticancer drugs for the treatment of various types of cancer.

The genus *Cleome*, containing nearly 200 species of annual and perennial herbaceous plants, is the largest in the family Cleomaceae[8]. *C. aspera* is a medicinal plant of Cleomaceae family[9]. It is an annual creeping plant. They are usually found in sandy areas, moorlands, and crevices of rocks. It grows like a weed in most tropical countries. It is not cultivated in India and grows wild everywhere[10]. Different types of *Cleome* can be found in all states of India. It is a yellow herb with long, cylindrical capsules containing seeds. The colour of *C. aspera* seeds is rough brown[11,12]. Locals call it *Nela vominta*, *Nakka dhoruvu*[13]. Minorities in Tamil Nadu use leaf paste to treat eczema and other skin conditions. Several studies have reported that plants of the *Cleome* genus leaf can be used as anticancer agents as they exhibit cytotoxic activity against several cancer cells[14]. Fruit, fruit peel, and seeds from the *Cleome* genus of plants have all been linked to cytotoxic action[15,16]. However, the cytotoxic properties of this plant's aerial portions are not well documented.

In the literature, there are no reports available on *C. aspera* (Figure 1) to determine the biological, toxicological, and phytochemical activity[17,18]. To prepare

Figure 1. Plant *Cleome aspera* (Source- Google images).

different polarity of aerial extracts and determine their cytotoxic as well as antibacterial properties is the goal of the current investigation by the available agar well diffusion, and MTT (3-(4,5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide) assay[5].

2. Materials and Methods

2.1 Collection, Identification and Authentication of Plant Material

Nature fresh aerial *C. aspera* parts have been collected from the Sri Venkateswara National Park, Tirumala hills of Tirupati district, Andhra Pradesh. The taxonomy of the collected plant material was determined and validated by a taxonomist of the Botany Department, Shri Venkateswara University, Tirupati, Andhra Pradesh, India, and voucher specimen (0506) deposited in the herbarium. To get rid of any dust particles, the collected plant components (aerial portions excluding roots) were thoroughly washed with running tap water. Following washing, the plant material was dried in the shade. After drying was complete, a mechanical mixer was used to properly grind the plant material into a fine powder, and placed in an appropriately labelled and sealed container for future use.

2.2 Powder Analysis

Fresh *C. aspera* plants were gathered and cleaned under running water to get rid of stuck-on dirt. Following a distilled water rinse, the plants were blotted and dried in the shade. After being pounded into a powder in a mixer, the samples were filtered through a fine mesh sieve and used for physicochemical characterization, fluorescence analysis, organoleptic investigation, and behaviour of the ground aerial portions of *C. aspera* with various chemical reagents[19-21].

2.1.1 Organoleptic Study

Color, odour, taste, and other aspects of the plant powder were assessed for the plant.

2.1.2 Fluorescence Analysis

An important factor in the initial standardization of crude drugs is fluorescence analysis. For the analysis, eight distinct reagents or solvents were treated to the crude drug powder, and it was left for ten minutes. Each solution was put onto a slide with a small coating of activated gel and the fluorescence under normal light, long UV (320nm), and short UV (100nm) was observed[22].

2.1.3 Physico-chemical Characterization

According to the official procedures and recommendations for quality control of medicinal plant materials, many physicochemical characteristics were assessed[23,24].

2.1.4 Behavior of the Powder with Different Chemical Reagents

The behaviour of the powder with various chemical reagents was examined to identify the phytoconstituents that change colour during the daytime.

2.2 Preparation of Extracts

In a Soxhlet apparatus, 150 grams of the powdered, dried aerial parts of *C. aspera* were weighed, loaded, and extracted using 1L portions of petroleum ether, ethyl acetate, and ethanol successively following the hierarchy of polarity of solvents. 72 hours of extraction were carried out, or until the solvent that came out of the siphoning tube was colorless[25]. The determination of phytochemical screening, antibacterial, and anticancer properties was done using the prepared extracts.

2.3 Percentage Yield of Different Solvent Extracts of *Cleome aspera*

It is the entire amount of extract obtained from the calculated mass of plant material. The extraction procedure was carried out three times, and the total yield of extracts was recorded and tabulated as follows.

Yield = (Weight of residue obtained/Weight of the plant material taken) × 100

2.4 Phytochemical Screening

Using standard techniques, the aerial parts of *C. aspera* were screened for the presence of phytochemicals[23,24].

2.5 *In-vitro* Antimicrobial Activity

2.5.1 Microorganisms

To evaluate the antibacterial activity of the plant extracts, four different strains of bacteria were tested, which were obtained from the Microbiology Department of

Dr. B.V. Raju College, Bhimavaram. *S. aureus* (ATCC 6538), and *B. subtilis* (ATCC 6633) two Gram-positive bacteria, *E. coli* (ATCC 8739), and *P. aeruginosa* (ATCC 9027) two Gram-negative bacteria, were obtained.

2.5.2 Media Preparation

Using the agar well diffusion method, different extracts were tested for antibacterial activity[26,27]. 1000mL of distilled water was used to dissolve about 25 g of nutritional agar. The combination underwent a 15-minute autoclave at 121°C. It was also allowed to cool at room temperature. It was then put into Petri plates after cooling (to about 45°C). Each Petri plate was allowed to completely cool for around 30 – 35 minutes.

2.5.3 Agar Well Diffusion Test

Sterilized nutrient agar medium was poured into sterile Petri plates and bacterial strains were spread on it. On the agar plate, wells were cut, into which the extracts could be added once they had been dissolved in DMSO. The wells were filled with 50,100, and 200µg/ml of the extracts. Petroleum ether, ethyl acetate along with ethanol extracts were added against *B. subtilis, S. aureus*, *E. coli*, and *P. aeruginosa* in the respective wells. Streptomycin (30 µg) was utilized as a reference control, and as a negative control, DMSO was employed. The zone of inhibition on the plates was noted after incubating at 37°C for 24 hrs[28].

By using the broth dilution method, the extract's Minimum Inhibitory Concentration (MIC) was tested against all of the test microorganisms at doses ranging from 25 g/ml to 500 g/ml in DMSO.

2.6 *In-vitro* Anticancer Activity

In vitro anticancer activity was performed at Maratha Mandal's Central Research Laboratory, Belgaum, Karnataka, India. The MDA MB 231(M.D. Anderson - Metastatic Breast 231) breast cancer cell line was utilized for *in vitro* cancer study[29-32]. The MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) assay method was utilized to determine the *in vitro* cytotoxic behaviour[33,34]. The cells were seeded in a 96-well flat-bottom microplate and maintained overnight at 37°C in 95% humidity and 5% CO_2. A range of concentrations (6.25, 12.5, 25, 50, and 100µg/ml) of samples were taken and treated. Then the cells were allowed incubated for an additional forty-eight (48) hours. After washing all wells two times with PBS, 20 µl of the MTT staining solution was poured into every well and the plate was left to incubate at 37°C. Following 4h, each well was administered 100 µl of DMSO for dissolving the formazan crystals. Then absorbance was measured at 570 nm by a micro plate reader. The cell viability percentage was estimated employing the formula such as:

Percentage of Cell Viability = A570 of treated cells/A570 of control cells × 100%.

3. Results

3.1 Powder Analysis

3.1.1 Organoleptic Study of Powder

The organoleptic characteristics like colour, odour, taste, and nature of *C. aspera* were determined and summarized in Table 1 below.

3.1.2 Fluorescence Analysis

Fluorescence analysis of dry powder of *C. aspera* was performed using eight solvents/reagents in normal light, long UV, and short UV which is depicted in Table 2 below.

3.1.3 Physico-chemical Characterization

The following physicochemical parameters were evaluated in *C. aspera,* and the results are detailed in the following Table 3.

3.1.4 Behavior of the Powder of Aerial Parts of C. Aspera with Different Chemical Reagent

The powder of aerial parts of *C. aspera* was treated with several chemical reagents such as conc. H_2SO_4; $AqAgNO_3$, $AqFeCl_3$, 5%Aq KOH, I_2 solution and, picric acid, and the outcoming results are detailed in Table 4 down below.

Table 1. Organoleptic character of *C. aspera*

Characters	*Cleome aspera*
Colour	Yellowish green
Odour	Pungent
Taste	Bitter
Nature	Granular

Table 2. Fluorescence characteristics of powder of aerial parts of *C. aspera*

Powder (P) + reagent	Ordinary Light	Long UV	Short UV
Powder as such	Yellowish green	Dark green	Yellowish green
Powder+ H_2O	Parsley green	Uranium green	Fern green
Powder+1N HCl	Blackish brown	Black	Greenish Black
Powder+ 50%HNO_3	Yellow	Black	Greenish yellow
Powder + 50%H_2SO_4	Reddish brown	Blackish brown	Greenish black
Powder+ 1N NaOH in methanol	Yellowish green	Dark Yellowish green	Yellowish green
Powder+1N NaOH in water	Greenish yellow	Greenish black	Green
Powder+ Acetic acid	Green	Red	Green

Table 3. Physicochemical character of *C. aspera*

Parameters	*Cleome aspera* (%)
Loss on drying	8.94
Total ash	5.64
Acid soluble ash	3.87
Acid insoluble ash	1.69
Water insoluble ash	2.57
Water soluble ash	2.36
Sulphated ash	6.78
Alcohol soluble extractive	3
Water soluble extractive	8
Swelling index	7.2 ml
Fineness	Coarsely fine
Foreign matter	2.3

3.2 Extractive Values

The study's objective was to quantify the yield of bioactive components, present in *C. aspera* using various solvent extractions. The colour of the extracts ranged from brownish red to dark green and their consistency was a powder (petroleum ether extract) to paste (ethanol as well as ethyl acetate extract). The ethanol extract had the highest yield (4.85 ± 0.18 %) followed by ethyl acetate (3.92 ± 0.12 %), and petroleum ether (1.76 ± 0.23 %). Because ethanol may easily enter the cellular membrane and solubilise the intracellular components from the plant cells, there may be a larger yield of bioactive metabolites from ethanolic extract. The findings also showed that certain plants had more polar compounds than others. The yield of crude extracts of *C. aspera* is given in Table 5.

3.3 Phytochemical Screening

The following chemical constituents were detected in different extracts of *C. aspera* given in Table 6.

3.4 *In-vitro* Antimicrobial Activity

The agar well diffusion method was utilized to examine the extracts' antibacterial activity at concentrations of 50, 100, and 200µg/ml. Streptomycin was employed in this investigation as a control and zone of inhibitions is shown in Table 7 and Minimum inhibitory Concentration (MIC) of the extracts is shown in Table 8.

3.5 *In-vitro* Anticancer Activity

Moderate anticancer activity was seen against the MDA MB 231 cell line after screening of *C. aspera* ethanolic extract. In the present study, the maximum concentration (µg/ml) used was 100µg/ml. The cytotoxicity of the petroleum ether, ethyl acetate, and ethanol extracts was assessed *in-vitro* against MDA MB 231 human breast cancer cell line varying concentrations like 6.2, 12.5, 25, 50, and 100 µg/ml (Figure 2). A direct dose-response association between cytotoxicity and concentration was evident in the samples' cytotoxicity study; cytotoxicity rose with increasing concentrations. Against the cancer cell lines (MDA MB 231), the ethanol extract exhibits significant cytotoxicity. The cytotoxic effect of the *C. aspera* ethanol extract increased as the concentration increased from 6.25 to 100 µg/mL, and the IC_{50} value was then determined at the concentration of 50µg/ml for the MDA MB 231cell lines. When tested using the MTT assay, the ethanol extract exhibited anti-cancer activity against the MDA MB 231 cancer cell line significantly (Figure 3). Doxorubicin had more cytotoxic effects than the extract at the same dose, it was discovered. For instance, the vitality of MDA MB 231 cells was just 3.2% at a dose of 100µg/ml of doxorubicin, compared to 21.79% for the extract. By calculating the IC_{50} value, it is possible to demonstrate the extract's potency as a cytotoxic agent more clearly.

Table 4. Behavior of powder treated with different chemical reagents

Treatment	Color /Precipitate	Constituent
Powder as such	Yellowish green	--
Powder +Conc H_2SO_4	Reddish brown	Steroids/Triterpenoids present
Powder +Aq $AgNO_3$	No Precipitation	Proteins absent
Powder +Aq $FeCl_3$	Geenish Black	Tannins/Flavonoids Present
Powder +5%Aq KOH	No Change	Anthraquinone glycosides absent
Powder +I_2 solution	No Blue colour	Starch absent
Powder+ Picric acid	Precipitation	Alkaloids present

Table 5. Extractive value of *C. aspera*

Solvent	Dried Powder (g)	Color and consistency	Yield of extract (%)
Petroleum ether	100	Dark green powder	1.76±0.23
Ethyl acetate	100	Dark green paste	3.92±0.12
Ethanol	100	Brownish–yellow paste	4.85±0.18

Values are stated as mean ± standard deviation (n=3)

The IC_{50} values of the ethanol extract as well as Doxorubicin were calculated, and they were found to be, respectively 23.9 ± *0.35* µg/ml and 1.81 ± 0.03 µg/ml shown in Table 9. After comparing the extract with Doxorubicin from its IC_{50} value, Doxorubicin is found to be superior to the extract, it is concluded. Doxorubicin is categorized as having very strong anticancer capabilities with such an IC_{50} value, whilst the extract shows powerful anticancer activity against

Table 6. Qualitative phytochemical screening for aerial parts of *C. aspera*

Phytochemicals	Inference			
	Test	Petroleum ether	Ethyl acetate	Ethanol
Alkaloid	Dragondraffs	-	-	+
Steroid	Salkowski	+	+	+
Flavonoid	Alkaline reagent	-	+	+
Phenols	Ferric Chloride	+	+	+
Tannin	Ferric Chloride	+	+	+
Glycoside	Borntrager's	-	+	+
Terpenoid	Salkowski	+	+	+
Saponin	Froth	-	+	+

NOTE: '**+**' implies presence of phytochemical, '-' implies absence of phytochemical

Table 7. Antibacterial activity of *C. aspera* aerial parts extracts

Name of the Organism	Zone of Inhibition in mm										
	Petroleum ether extract(µg/ml)			Ethyl acetate extract(µg/ml)			Ethanol Extract(µg/ml)			Positive Control (Streptomycin)	Negative Control (DMSO)
	50	100	200	50	100	200	50	100	200		
Bacillus subtilis	4	6	8	8	8	10	7	9	13	14	0
Staphylococcus aureus	0	4	7	5	7	10	6	8	11	15	0
Pseudomonas aeruginosa	0	3	6	6	8	10	9	11	12	15	0
Escherichia coli	0	0	6	7	9	11	9	9	12	16	0

Table 8. MIC (µg/ml) values of extracts of *C. aspera*

Extract	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
Petroleum ether	125	125	100	100
Ethyl acetate	250	250	250	125
Ethanol	>500	>500	>500	250

All values are average of three determinations

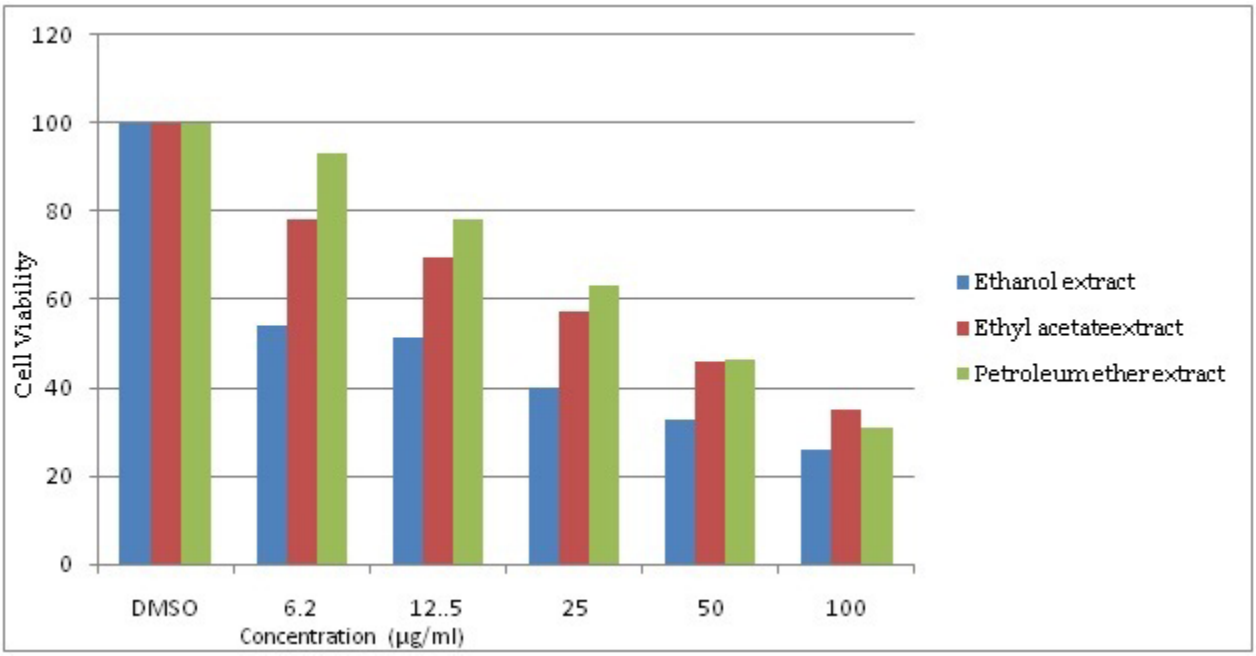

Figure 2. Cell viability of MDA MB 231 cell treated with aerial parts extracts of *C. aspera* (Values are expressed as mean ± standard deviation (n=3)).

Table 9. IC_{50} Value of aerial part extracts of *C. aspera*, and Doxorubicin

Extract	IC_{50} (µg/ml)
Ethanol	23.9± 0.35
Ethyl acetate	47.44± 0.26
Petroleum	48.68± 0.26
Doxorubicin	1.81± 0.03

Values are stated as mean±standard deviation (n=3)

the MDA MB 231 cell line. Therefore, the cytotoxic effects of the ethanolic extract of *C. aspera* against MDA MB 231 cells showed better activity.

4. Discussion

Today, many pharmaceutical industries concentrate on medicinal plants as a source of main bioactive substances to produce new drugs. Nowadays, 25 to 50 percent of medicines come from herbal sources[35]. Many medicinal plants have distinctive biological properties, but different tribes or cultures employ them to cure various diseases, demonstrating the wide range of healing properties that plants have as a result of their chemical makeup. The preliminary phytochemical screening revealed the presence of steroids, alkaloids, flavonoids, phenols, glycosides, terpenoids, saponins, and tannins. Preliminary phytochemical screening helps to comprehend herbal medicines and their manufacture, understanding of phytochemical ingredients is required to separate and characterize chemical components contained in plant extracts, ultimately to realize the true value of discovery therapies[36]. Different extracts contain popular phytochemicals with biological activity, including alkaloids, flavonoids, terpenoids, cardiac glycosides, phenols, and saponins, which can prevent the majority of chronic diseases[37].

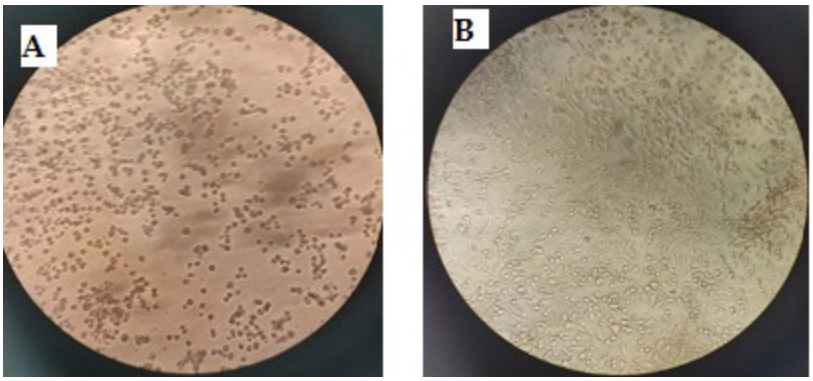

Figure 3. A- MDA MB 231 cell line treated with ethanol extract (100µg/ml) of *C. aspera,* B- MDA MB 231 cell lines for control.

According to traditional uses, parts of the plants of the genus *Cleome* such as leaves, seeds, and roots are used as analgesic, anti-arthritic, stimulants**,** anti-inflammatory, antipyretic, antibacterial, anticancer, anti-diabetic, antidepressant, and hepatoprotective[38]. The aerial parts of *C. aspera* were utilized to investigate their antibacterial and anticancer properties. We discovered that ethanol and ethyl acetate extracts of the aerial parts of *C. aspera* significantly prevented the growth of both Gram-positive and Gram-negative bacteria[39,40]. Several studies claimed that medicinal plants' powerful antibacterial capabilities were caused by phytochemicals such as alkaloids, flavonoids, tannins, and phenolic substances[41]. This study reports the anticancer potential of the *C. aspera* aerial parts extract in addition to its antibacterial properties. The results of the MIC study revealed three extracts' antibacterial activity against the tested strains of microorganisms. In this present investigation, the ethanol extract significantly reduced the viability of the cancer cell line MDA MB 231[42]. Many claimed that the potent antibacterial activities of medicinal plants were because of the existence of phytochemical components such as alkaloids, flavonoids, phenolic compounds, and tannins[41]. In addition to antibacterial activities, additionally, this study details the anticancer activity of aerial parts extracts of *C. aspera*. The ethanol extract showed profound cytotoxicity against the MDA MB 231 cancer cell line in the current research.

5. Conclusion

This study represents the initial report on the phytochemical analysis of *C. aspera* aerial part extracts. Various chemical constituents were identified from the ethyl acetate and ethanol extract. The existence of medicinally useful phytocomponents in the extracts implies the phytopharmaceutical importance of the plant. To determine the pharmaceutical action of the concerned compounds, more research is needed. Their isolation and characterization are in progress. In this work, we examined the antibacterial and anticancer characteristics of *C. aspera* aerial parts. We found that the ethanol extract of *C. aspera* aerial parts showed substantial antibacterial activity against *P. aeruginosa* followed by *E. coli, B. subtilis,* and *S. aureus.* Effective antibacterial characteristics may be a result of the phytochemical components found in medicinal plants, such as alkaloids, flavonoids, tannins, and phenolic compounds[43]. The anticancer properties of extracts from *C. aspera* aerial parts are also reported in this work. In the present investigation, the ethanol extract considerably reduced the viability of the cancer cell line MDA MB 231[44].

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