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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *CLEOME GYNANDRA* L. AND ASSESS ITS ANTICANCER ACTIVITY AGAINST *IN VITRO* CANCER MODEL

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ABSTRACT

Cleome gynandra Linn. is the native herb distributed at tropical and sub-tropical temperature. In this study, the antioxidant, antimicrobial and anticancer properties of the ethanolic extract of *Cleome gynandra* leaves were investigated also the anti-cancer activity was assessed against A549 lung cancer cell lines. The antioxidant activities were evaluated by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity. The IC_{50} value of DPPH was ranged from 65.67 µg/ml for the leaf extract. The anti-microbial activity was showed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Salomonella typhi*. The cytotoxic study was conducted and the results exhibited the strongest inhibitory activity against A549 lung cancer cell line as concentration dependent manner. The cytotoxic activity of the leaf extract was supported by the induction of apoptosis cell. The other assays to evaluate anticancer activity are DAPI (4,6 – diamminodino-2-phenylindole), PI (propidium iodide) and EtBr/AO (Ethidium bromide/acridine orange) staining methods. All the study results revealed the efficient anticancer activity of the ethanolic leaf extract of *Cleome gynandra* leaves**.** Thus, the herbal drug treatment might highly be recommended to treat effectively lung cancer cell line as an ideal choice or combinational therapy.

Keywords: *Cleome gynandra,* antioxidant, propidium iodide, 4, 6 – diamminodino-2-phenylindole, anticancer activity

1. INTRODUCTION

Cancer is an anomalous condition, in which a group of cells do not respond to the normal cell cycle signaling as they have a degree of self-sufficiency, which gives rise to the uncontrolled growth and proliferation that leads to transformed cells. Cancer is the multifactorial disorder involving complex modifications in the genome affected by the interactions between host and environment [1].

Cleome is a genus of the family *Cleomaceae* (formerly Capparaceae). It is a major group of angiosperms comprising many species found in tropical and sub-tropical areas of the globe. *Cleomaceae* including more than 764 species belonging to 12 genera of which *Cleome* is the largest genus with about 601 species of ecological, ethnobotanical and of course medicinal importance [2, 3].

Antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. This can decrease oxidative stress-induced carcinogenesis by direct scavenging of ROS [4]. The medicinal plants are useful for healing as well as for curing of human disease because of the presence of phytochemical constituents [5].

Bacterial infections continue to be a major cause of morbidity and mortality and therefore a major public health problem. This is compounded by the increasing reports of anti-microbial resistance and the emergence of resistance mechanisms. The use of herbal medicine predates the introduction of

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antibiotics and cuts across socio-economic and religious barriers [6].

The present study is focused to evaluate the *in vitro* anti-oxidant, anti-microbial and anti- cancer activity of ethanolic extract of *Cleome gynandra* leaves was carried out in a systematic manner.

2. MATERIALS AND METHODS

2.1. Plant collection and identification

The fresh plant leaves of *Cleome gynandra* Linn. was collected from Palani, Dindigul district, Tamil Nadu, India. The plant samples were authenticated at Botanical Survey of India, South Circle, Coimbatore and specimen copy (BSI/SRC/5/23/2022/Tech./446) was deposited for reference purpose.

2.2. Preparation of Plant extract

The plant leaves were washed using clean water, shade dried at room temperature. Once dried, the leaves were grinded into fine powder by using mechanical grinder. Solvent extraction was carried out using the soxhlet apparatus. To 25 g of dried powder of plant were separately extracted with soxhlet extractor using 250 ml of ethanol. The extract solution was then evaporated by using rotary evaporator to remove the solvent from the extract solution and dried. The final product was stored at 4°C for further analysis [7].

2.3. Phytochemical Screening

2.3.1. Qualitative Analysis

Phytochemical screening of the ethanol extracts of herbal plant leaves were carried out using standard procedures to identify the secondary metabolites present in the plant extracts. Small quantities of the extracts were used in the analysis and the major secondary metabolites analyzed were alkaloids, flavonoids, saponins, phenols, glycosides, tannins and steroids [8].

2.4. Antioxidant activity

Free radical scavenging activity of plant extracts were measured in terms of hydrogen donating or radical scavenging ability using stable radical DPPH as described by [9]. Various concentrations of the sample (4.0 ml) were mixed with 1.0 ml of solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture were shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control. The percentage of inhibition in DPPH radical scavenging activity was calculated as follows;

% Inhibition = A0- A1/A0 X 100.

2.5. Antimicrobial activity

2.5.1 Test microorganisms

The test organisms used were clinical isolates viz., *E. coli*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Klebsiella Pneumoniae* which were obtained from Department of Microbiology, Coimbatore medical college and hospital (CMHC), Coimbatore, Tamil Nadu, India.

2.5.2. Growth and maintenance of Test microorganism for Antimicrobial studies

The bacterial cultures were maintained on nutrient broth (NB) at 37º C.

2.5.3. Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20 – 100 µl) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [10].

2.6. Anticancer activity

2.6.1. Cell culture

The Human Lung (A549) cancer cell lines were procured from the National Center for Cell Sciences (NCCS), Pune, India. The cancer cells were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 2mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to 1 mL/L. The cells were maintained at 37◦C with 5% CO_2 in a humidified CO_2 incubator.

2.6.2. Evaluation of cytotoxicity

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cancer cells were grown (1×10^4 cells/well) in a 96-well

plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted samples, and the cells were further incubated for 24 h. The culture medium was removed, and 100 µL of the MTT [3-(4, 5-dimethylthiozol-2-yl)-3, 5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37 ◦C for 4 h. After removal of the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

% of viability = OD value of experimental sample/OD value of experimental control ×100

2.6.3. Fluorescence microscopic analysis of apoptotic cell death

Approximately 1µL of a dye mixture (100 mg/mL acridine orange (AO) and 100 mg/mL ethidium bromide (EtBr) in distilled water) was mixed with 90 µL of cell suspension (1×10^5 cells/mL) on clean microscope cover slips. The selected cancer cells were collected, washed with phosphate buffered saline (PBS) (pH 7.2) and stained with 10 µL of AO/EtBr. After incubation for 2 min, the cells were washed twice with PBS (5 min each) and visualized under a fluorescence microscope (Nikon Eclipse, Inc, Japan) at 20× magnification with an excitation filter at 480 nm.

Likewise, the cells were seeded on glass coverslip in a 24-well plate and treated with compound for 24h. Then the cells were fixed with 70% ethanol, for 10 min at room temperature and incubated for 3 min with 10 µl of propidium iodide by placing a coverslip over the cells to enable uniform spreading of the stain. The cells were observed under (Nikon Eclipse, Inc, Japan) fluorescent microscope.

3. RESULTS

The phytochemical analysis was performed to evaluate the presence bioactive compound in the sample and support for qualitative separation [11]. The Table 1 shows that the results revealed extract contained flavonoids, glycosides, steroids, phenol, tannin, and saponins and absence of alkaloid.

Table 1. Phytochemical analysis of ethanolic extract of *Cleome gynandra* leaves

Test	Observation
Alkaloids	-
Saponins	+
Glycosides	+
Tannins	+
Phenols	+
Flavonoids	+
Steroids	+

Chemical compounds contained in the *Cleome gynandra* might be used for formulating new drugs, particularly for cancer treatment [12, 13]. Alkaloid and flavonoids can act as antioxidant that led to the invention of anti-analgesic, anti-inflammatory, and cardio–tonic activities, respectively [14, 15]. Saponins are characterized by a wide spectrum of anti-tumor activity: they can inhibit proliferation and induce the apoptosis of tumor cells, reduce their invasive activity; saponins show the multidirectional effects of saponins on various processes related to the promotion and progression of cancer, taking into account the main assumptions related to hallmarks of cancer with particular emphasis on the papers from recent years (2010–2015)[16]. Phenols have antioxidant, anti-carcinogenic, or anti-mutagenic and anti-inflammatory effects and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression. Glycosides isolated from various plants possessed marked anticancer activity against a variety of cancer cell lines. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane [17].

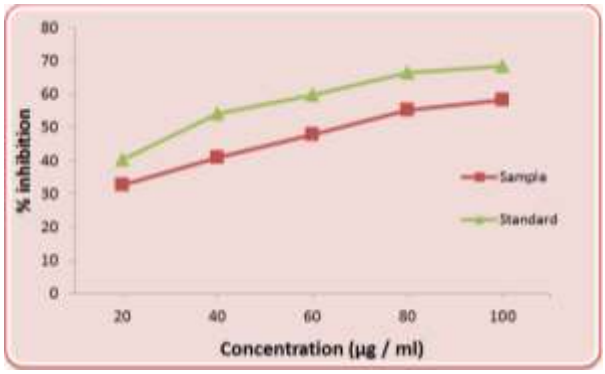
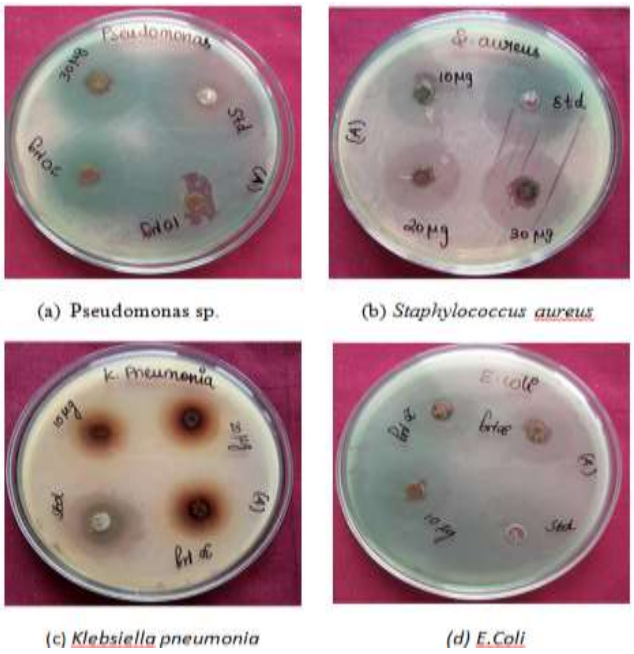


Fig. 1. DPPH radical scavenging assay of ethanolic extract of *Cleome gynandra* leaves

Free radical can cause degenerative diseases including cancer. Currently, researches have increased interest to discovery therapeutic medicinal plants that have high antioxidant activity to reduce oxidative stress tissue injury [18]. The selection of DPPH free radical scavenging method is simple and inexpensive methods [19]. The results revealed that the extract possess an IC_{50} value of 65.67 µg / ml. This value was higher than the commercial antioxidant (the IC_{50} of ascorbic acid was 32.66 µg /ml) (Figure 1). However, the result could be antioxidant capacity of different parts of *Cleome gynandra* that were extracted using ethanol solvents. The results exhibited that the leaf part had the moderate activity when compared to other plant parts [20].

In vitro systems are easier, faster and more cost-effective compared to traditional bioassays than *in vivo*. Infectious diseases caused by pathogenic and opportunistic microorganisms remain a major threat to public health, in-spite of tremendous progress in anti-microbial drug discovery. Indiscriminate use of antibiotics, have leads to the emergence of multidrug resistant pathogens that are progressing towards final line of antibiotic defense [21]. So this studies undertaken to evaluate the antimicrobial activity of *Cleome gynandra* leaves against various pathogenic bacteria.



(a) Pseudomonas sp. (b) *Staphylococcus aureus*

(c) *Klebsiella pneumonia* (d) *E.Coli*

Fig. 2. Antibacterial activity of ethanol extract of *Cleome gynandra* leaves

Table 2. Antibacterial activity of ethanol extract of *Cleome gynandra* leaves

S.No	Pathogenic bacteria	Zone of inhibition (mm)			Standard (Amoxicillin)
		10 ug	20 ug	30 ug	10 ug
1.	*Staphylococcus aureus*	12	14	22	24
2.	*Escherichia coli*	10	12	14	22
3.	*Klebsiella pneumonia*	10	14	18	20
4.	*Pseudomonas sp.*	08	12	16	24

The experiment was conducted in triplicates (n=3)

The cytotoxic effects of *Cleome gynandra* on A549 cells were evaluated via MTT assay. The MTT assay is used to measure the cellular metabolic activity as an indicator of the cell viability, proliferation and cytotoxicity. The cytotoxicity effect showed that *Cleome gynandra* leaves extract had potent cytotoxicity effect. The results of the MTT are shown in the Figure 3 and the cytotoxicity is given in the Table 3.

Table 3. MTT assay of ethanol extract of *Cleome gynandra* leaves

COMPLEX	MTT ASSAY
Plant Extract	30±0.2
Doxrubicin (standard)	18±0.5

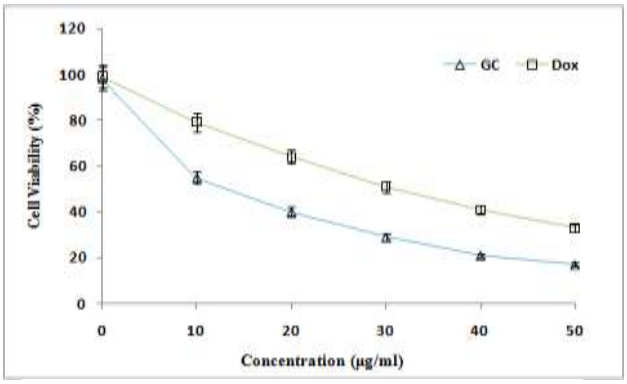


Fig. 3. Cytotoxic activity of ethanolic extract of *Cleome gynandra* leaves on A549 lung cancer cells lines

The morphological studies A549 lung cancer cell line treated with leaves extract of *Cleome gynandra* was shown in given Figure 4. Cells exposed to 20 µg/ml -60 µg/ml concentrations of *Cleome gynandra* for 24 hours reduced the normal morphology and cell adhesion capacity of A549 cells as compared to control. The morphology of A549 cells started to reduce the normal shape and cell adhesion capacity as compared to control as shown in figure 4. The growth of the cells inhibited respect to increase in concentration of plant extracts [22]. The morphological changes of the cells due to various concentrations are based on the reduced proliferation increased apoptosis involving in the cells. Treatment with plant extract possessing medicinal active compounds resulting the biological functions mentioned above as per the previous reports. The compounds are may be responsible for the same.

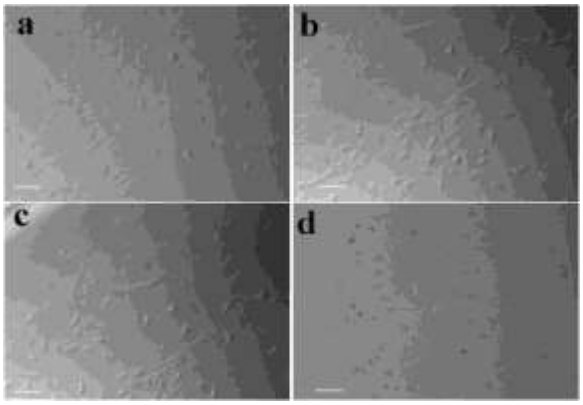


Fig. 4. Anticancer activity of ethanolic extract of *Cleome gynandra* leaves against A549 cell line (Human lung cancer)

Apoptosis is a programmed cell death process; it eliminates matured cells and infected cells. The apoptotic studies of A549, treated with ethanol leaf extract of *Cleome gynandra* were shown in Figure 5. The green colour cells are transformed into orange or red colour which is due to induction of apoptosis and the nuclear condensation effect on the cells. From the results, the control A549 cell lines showed the green colour, it indicates the down regulation of apoptosis. The A549 cell lines treated with *Cleome gynandra* plant extract at different concentration were showed the colour change from green colour to yellowish orange colour it indicates the mild apoptosis of cells. The maximum concentration of plant extract were showed the reddish orange colour it because of the vigorous apoptosis of the cells. The high intensity of the reddish orange colour was observed in A549 cell line, which treated with maximum concentration of *Cleome gynandra* it indicates maximum apoptosis.

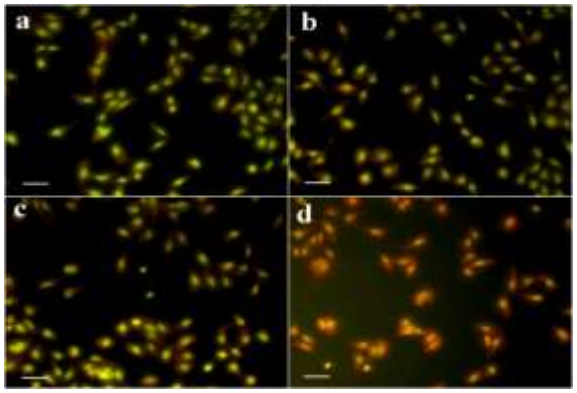


Fig. 5. Effect of ethanolic extract of *Cleome gynandra* leaves on AO/EtBr staining of A549 cells.

Apoptosis cells were detected with DAPI (4'-6'-diamidino-2-phenyl indole) nuclear technique. It is known fluorescent complexes with double standard DNA. A morphological change is typical of apoptosis, such as cell shrinkage, rounding and detachment of the cells from the plate, as observed by fluorescence microscopy (Figure 6). Cells were exposed to respective IC_{50} concentration after 24 hours' time exposure of ethanolic extract of *Cleome gynandra* and stained with nuclear dye DAPI. DAPI staining also revealed a significant level of nuclear fragmentation in the selected lung cancer (A549) cells after the exposure. It was observed that the total number of apoptotic cells and intensity of fluorescence increased when the cells were treated with different concentrations of plant extract. The previous research study shows that the apoptotic cell death in human hepatocellular carcinoma Hep3B cells treated with the ethanolic extracts of *Euchresta formosana* radix using DAPI Staining [23].

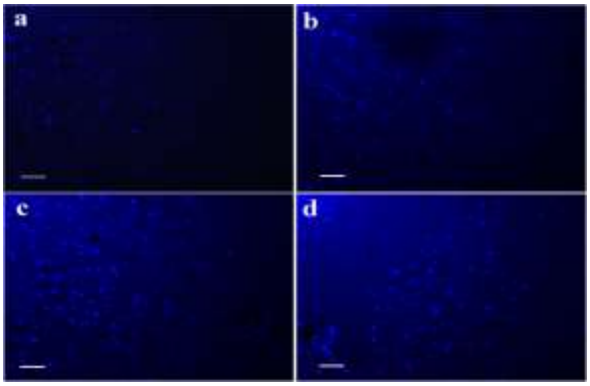


Fig. 6. Effect of ethanolic extract of *Cleome gynandra* leaves on DAPI staining of A549 Cells.

Propidium iodine (PI) is a fluorescent dye that is unable to penetrate intact membrane and therefore stains the nucleus of cells that have lost their membrane integrity due to apoptosis. The apoptotic cells were detected using the green filter of Fluorescence microscope at 40X magnification. Treatment showed a dose-dependent response directly proportional of apoptotic rates. The results confirm that the presumable mechanism of cell death is apoptosis (Figure 7). The apoptosis by disrupting the mitochondrial membrane potential and reducing AKT phosphorylation by PI staining in C6 glioma cell line by inducing Flavonoid, extracted from *Brosimum acutifolium* [24]. The results obtained showed that PI staining is a reliable method to quantify the effect of apoptosis.

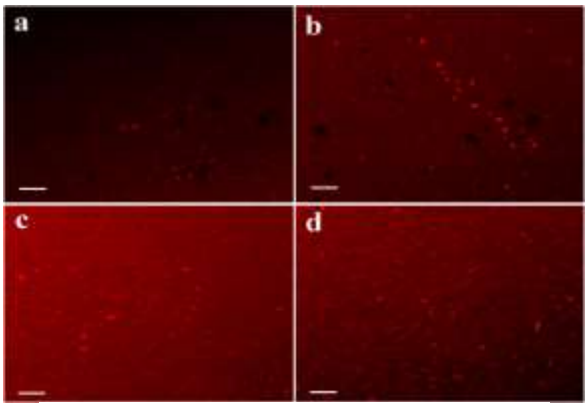


Fig. 7. Effect of ethanolic extract of *Cleome gynandra* leaves on PI staining of A549 cells.

5. CONCLUSION

Cleome gynandra is the medicinal plants that contain a fairly high amount of bioactive Compounds. The antioxidants have high free radical scavenging activities, thus inhibit oxidative stress. Inhibition of oxidative stress by these antioxidants can prevent inflammation and the onset of several chronic diseases. The extract showed strong antimicrobial activity. The bioactive compound from *Cleome gynandra* leaves possess antioxidant and anticancer effects. Our results demonstrate that ethanolic leaf extract of *Cleome gynandra* significantly reduced the cell viability of human lung cancer cell line, and suggested that our novel remedial extract *Cleome gynandra* androgynous is an effective remedial treatment to cure lung cancer.

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Conflict of interest

The author declares no conflict of interest.

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