

Screening the Therapeutic Potential of Methanolic Stem Extract of *Cissus arnottiana*

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Modern lifestyle, pollution, food habit, and stress have intensively enhanced the evolution of several diseases on human being. Medicinal plants are used from the ancient times for the therapeutic needs to cure various diseases as well as less toxic in nature. One of such plant is *Cissus arnottiana* which is used from the olden days which has been identified as an important medicine plant by many researchers all over the world. *Cissus arnottiana* contains phytochemicals such as alkaloids, tannins, terpenoids, flavonoids, phenolic compounds etc. In this present work methanolic stem extract of *Cissus arnottiana* is used to evaluate the antibacterial activity of gram negative and gram positive human pathogenic bacteria. DPPH assay is used to investigate the antioxidant properties of the methanolic extract. The anti-inflammatory activity of the extract has been studied using anti proteinase assay. The MTT cell proliferation assay is carried out against HeLa cell line in which 44% of cells are viable for the concentration of 100 µg/mL. Interestingly, the methanolic stem extract can be used as a potential candidate for new therapeutic applications.

Keywords: *Cissus arnottiana*; Cell Lines; Plants Extract; Phytochemicals.

From the ancient traditional time, medicinal plants had played a vital role in medication field. The best antidote for curing various diseases is done using plant kingdom^{1,2}. Interestingly, it is found that three quarters of world population depends on plant extracts for most of the healthcare issues³. According to WHO, it is reported in the recent years 80% of world population uses traditional medicinal plant extract for health coverage. Interestingly it is found that due to the nuisance in using drugs and its side

effects 38% of Americans uses traditional plant extracts for medicinal issues⁴. When compared with the allopathic medicine, plant and its extracts give better relief due to the discomfort caused by the synthetic drugs. The pharmaceutical industries that discover drugs also depend on the natural plant and its extract equally due to its aftermath caused by the drugs used in the medicines, which leads to ample loss in the field of pharmaceutical². Therefore, plants and its extracts-based health care products is trending in both developed and

developing countries. By using herbal plants for the treatment such as allergic, cardiovascular diseases, diabetes and metabolic diseases will alleviate that by using the drugs that causes side effects^{2,3}. In comparison with other countries, India is said to have thousands of natural herbal plants, were different parts such as leaves, stem, roots, fruit, seed and bark of the herbal plant is used to cure specific ailments as been vogue^{1,5,6}. Globally, due to the over population, continuous exploitation of herbal plants, urbanization, traditional knowledge about the natural herbals are getting depleting day by day^{2,6}.

In recent years, the medicinal plants have attracted many researchers with its potential antioxidant properties, which had received significant attention for an enhanced alarm for safe and non-toxic^{1,7}. Phytochemicals are defined as medicinal value of plant which activates the chemical compounds to produce the physiological action on the human being. It can be classified into two groups such as primary and secondary constituents according to the plant metabolism. Primary constituents consist of amino acids, common sugar, protein, and chlorophyll while the secondary constituents consist of alkaloids, tannins, terpenoids, flavonoids, phenolic compounds etc^{1,8}. In recent years, many researchers are encouraged in scientific authentication and explanation for the activity of the herbal plants, which are used as medicine around the world⁷. One such plant that is used in all continents and implemented to treat different ailments is the plant belonging to the genus "*Cissus*" among various plants evaluated in the therapeutic efficacies⁴. *Cissus arottiana* commonly known as "Nanaminukki" in tamil and "Nelagummadi" in telugu. This *Cissus arottiana* belongs to Vitaceae family. *Cissus arottiana* is an erect woody shrub with bruised root. Medicinal benefits of the bruised roots for diseases such as rheumatic swellings, possess antibacterial, antioxidant, anti-inflammatory, and anti-viral activity are reported earlier^{9,10}. Phytochemical screening of *Cissus arottiana* is found that most of the biologically active phytochemicals are present in the ethanolic extract of leaves and fruits extract contain alkaloids, flavonoids phenols, tannins, terpenoids, quinines, saponins, glycosides, and carbohydrates.

In the present investigation, we evaluate

the antimicrobial, antioxidant, anti-inflammatory, and anticancer activity of *Cissus arottiana* stem extract. To the best of our knowledge, no studies have been reported, for the investigation of the antimicrobial properties against a selection of gram-positive and gram-negative pathogenic bacteria. The study also evaluates antioxidant activity based on DPPH assay, anti-inflammatory, and anticancer properties of the methanolic stem extract of *Cissus arottiana*.

MATERIALS AND METHODS

Collection and preparation of plant materials

The stems of *Cissus arottiana* were freshly collected from Kanjamalai hills, Salem district, Tamil Nadu, India in the month of March 2016. The plant specimen was identified as authenticated by Botanical survey of India [ref no: BSI/SRC/23/2016/Tech/1071], Coimbatore, India. The stem samples were cleaned thoroughly using air dried technique to remove soil and unwanted particles attached to it. The stems were immediately washed with double distilled water, cut into pieces, and dried under shade conditions. The dried stem was grounded into fine particles using a blender and stored in an air-tight container for further use.

Extraction of *Cissus arottiana*

10g of finely grounded stem powder were independently macerated in 100mL of methanol for 24 hrs. The above soaked solution was filtered using Whatmann filter paper (No. 1). The filtered methanol solution is transferred to micro tubes and stored in the refrigerator at 4°C until for further use¹¹.

Characterization Techniques

UV-Visible spectroscopic analyses were carried out to confirm the presences of phytochemicals in the extract. The absorbance spectra were recorded using UV-Visible spectrophotometer (T-90 UV-Visible Spectrophotometer, PG instruments, Lab India Pvt). Fourier Transform Infrared spectroscopy (FTIR) carried out to identify the active functional groups present in the stem extract. IR spectrum is obtained using Bruker 1 FS 66U. FTIR operated under transmission mode with wavelength from 400-4000 cm^{-1} at a resolution of 4 cm^{-1} .

Antibacterial Activity

In this study, six different human

pathogenic bacteria (3 gram positive, 3 gram negative) *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cerus*, *Klebsiella planticola*, *Escherichia coli* and *Klebsiella pneumonia*. The antimicrobial activity of the plant extract was performed using well diffusion assay. The methanol (1mg/mL) is used as a control. The wells of 6mm were impregnated with serial concentration of methanolic stem extract (20, 40, 60 and 80 μ L). After inoculation, the plates were incubated at 37°C for 24 hrs and the zone of inhibition (ZOI) around the well was measured^{12,13}. The ZOI was measured in mm and the assays were repeated for triplicate.

Antioxidant Activity

The antioxidant activity of the methanolic stem extract of *Cissus arnottiana* against DPPH substrate was performed by the method reported by Gunjan Guha et. al¹⁴. A stem extract of (20, 40, 60, 80 and 100 μ L) were added with 1 mL of DPPH solution and the mixture is mixed well. The mixture was kept for 40 mins at 20°C in a dark condition^{9,15}. The absorbance is measured at 517nm using UV-Visible spectrophotometer. All analyses were carried out I triplicate and average values were reported.

In-Vitro anti-inflammatory activity by anti-proteinase action

The anti-inflammatory activity was performed according to the modified method by Oyedepo et al¹⁶ and sakat et al¹⁷. The mixture of 2 mL consists of 0.06 mg trypsin, 1 mL 20mM Tris HCl buffer (pH=7.4) and 1 mL of stem extract with different concentration (50), 100 and 150 μ g/mL). The above mixture was incubated at 37°C for 5 mins and 1 ml of 0.8% (W/N) casein was added again the mixture was incubated for 20 mins

additionally. Then, 2 mL of 70% per chloric acid was added to stop the reaction. The above cloudy suspension was centrifuged and then absorbance is measured at 660 nm against buffer as blank¹⁸. The experiment was performed in triplicate.

Anticancer Activity

Cancer cell proliferation test was carried out against methanolic stem extract of *Cissus arnottiana* using MTT assay. HeLa cells were cultured in 96 well plates with a density of 2.4×10^4 per well and incubated at 37°C for 24 hrs. The 100 μ L of methanolic stem extract were added in each well with different concentrations (20, 50 and 100 μ g/mL) were incubated at a temperature 37°C for 48 hrs. After incubation, medium was discarded ad a new medium of 150 μ L containing 0.5mg/mL MTT solution concentrated in DMEM were added to each well. Then, the plates were incubated at 37°C for 4 hrs until a formazan crystal is formed. Using an inverted microscope, the cells were imaged. Absorbance of the MTT assay is measured at 560 nm using UV-Visible spectrophotometer¹⁹. The well without the cells is used as the control. The effect of the methanolic stem extract on the HeLa cells was expressed as % cell viability, using the formula,

$$\% \text{ cell viability} = \frac{\text{Absorption of treated cell}}{\text{Absorption of control cell}} \times 100$$

Statistical Analysis

All experiments were done in triplicate. The results were expressed as mean \pm Standard deviation. P < 0.05 is considered statistically significant for all analysis.

Table 1. Antibacterial activity of methanolic stem extract of *Cissus arnottiana* against pathogenic bacteria. ZOI was measured as millimeter \pm standard deviation for three independent experiments

Bacterial Isolates	Zone of Inhibition (mm)			
	20 μ l	40 μ l	60 μ l	80 μ l
<i>Bacillus subtilis</i>	6.13 \pm 0.07	7.17 \pm 0.12	9.33 \pm 0.20	10.27 \pm 0.15
<i>Staphylococcus aureus</i>	6.17 \pm 0.09	7.45 \pm 0.18	9.13 \pm 0.09	12.37 \pm 0.19
<i>Bacillus cerus</i>	6.00 \pm 0.00	6.13 \pm 0.13	7.1 \pm 0.15	7.85 \pm 0.11
<i>Klebsiella planticola</i>	6.22 \pm 0.07	8.18 \pm 0.21	10.33 \pm 0.88	13.20 \pm 0.21
<i>Escherichia coli</i>	6.32 \pm 0.09	8.33 \pm 0.21	10.13 \pm 0.12	12.71 \pm 0.24
<i>Klebsiella pneumoniae</i>	7.52 \pm 0.21	7.93 \pm 0.13	9.1 \pm 0.21	11.65 \pm 0.98

RESULT AND DISCUSSION

Figure 1, shows the UV-Visible spectra of the methanolic stem extract of *Cissus arnottiana* typically consist of a broad absorption maxima in the range of 230-260 nm and 290-360 nm. The UV-Visible spectra confirm the presence

of phytochemicals such as alkanoids, phenols, flavonoids, steroids and tannins in the extract²⁰. The precise position and relative absorbance of these maxima give useful information on the nature of the phytochemicals present in the extract.

Figure 2, shows the FTIR spectrum is used to identify the functional group of the active

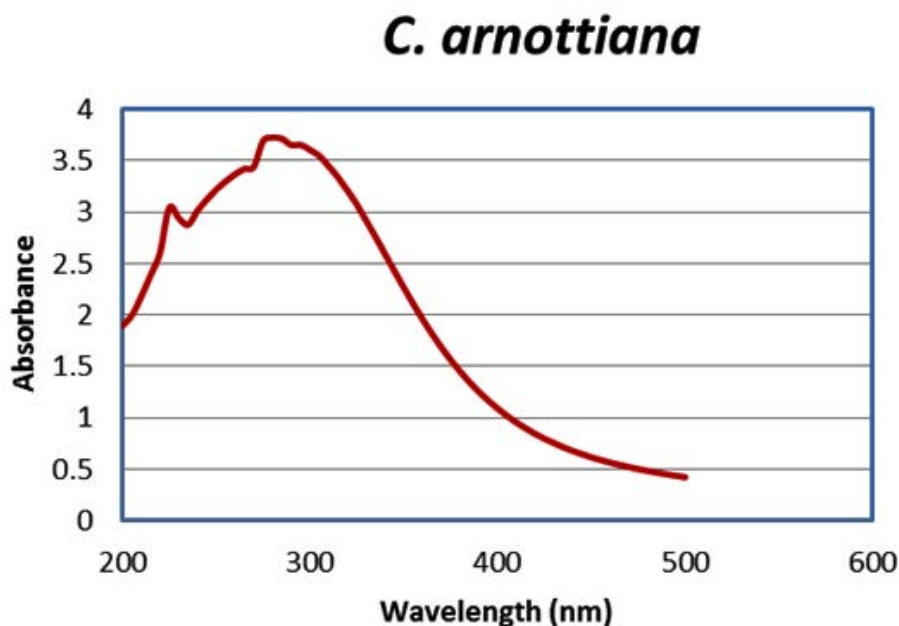


Fig. 1. UV-Visible spectra of the Methanolic stem Extract of *Cissus arnottiana*

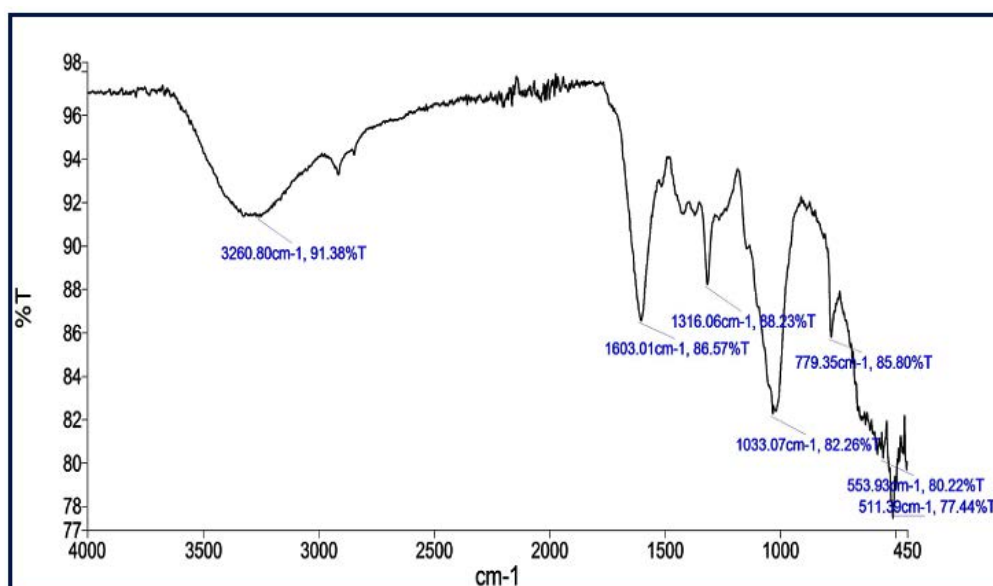


Fig. 2. FT-IR spectrum of the Methanolic stem Extract of *Cissus arnottiana*

components present in the extract. By interpreting the IR absorption spectrum, the chemical bonds present in the compound can be determined. The FTIR spectra of *Cissus arnottiana* wave peaks characteristic phenyl group, aromatic group, hydroxyl aromatic group, carboxylic and alkane group in the extract²¹. The peak at frequency of 3260, 1603, 1316, 1033 and 779 cm^{-1} were strong peaks as shown in the figure 2.

The methanolic stem extract of *Cissus arnottiana* shows an excellent antibacterial activity against human pathogenic gram positive and gram negative bacteria. The antimicrobial assay were performed against *K. planticola*, *E. coli* and *K. pneumonia* gram negative bacteria

with different concentration of methanolic stem extract (20, 40, 60 and 80 μL) using well diffusion method. Figure 3, clearly shows that the ZOI increases as the concentration of the methanolic stem extract increases against the gram negative bacteria, whereas the ZOI of the control is very less compared with the extract. *K. planticola* shows a higher activity against the methanolic stem extract which is 13.20 mm at 80 μL . interestingly *E. coli* and *K. pneumonia* also exhibits a excellent activity with a ZOI of 12.11 and 11.65 mm at 80 μL which is comparably lesser than *K. planticola*^{12,13}.

Comparing the ZOI, gram positive bacteria also exhibits antimicrobial activity. *S. aureus* shows a good activity against methanolic

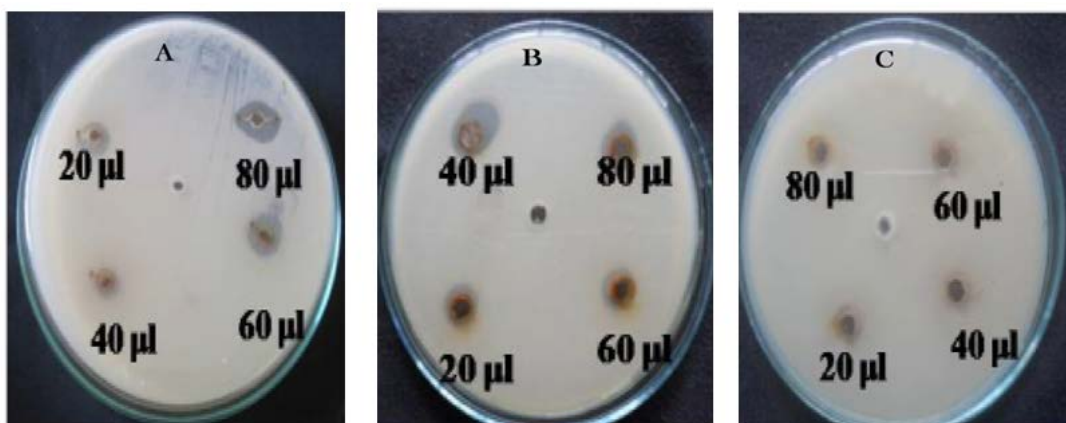


Fig. 3. Antimicrobial activity of methanolic stem extract of *Cissus arnottiana* against gram positive bacteria (A. *B. subtilis*, B. *S. aureus* and C. *B. cerus*)

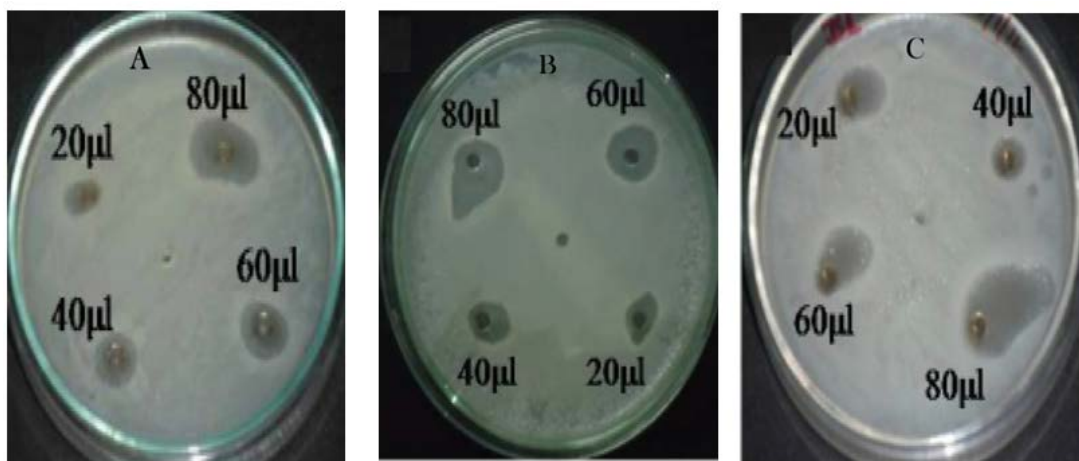


Fig. 4. Antimicrobial activity of methanolic stem extract of *Cissus arnottiana* against gram negative bacteria (A. *K. planticola*, B. *E. coli* and C. *K. pneumoniae*)

stem extract which is 12.37 mm at 80 μ L. *B. subtilis* and *B. cerus* also exhibits antimicrobial activity with a ZOI of 10.27 and 7.85 mm at 80 μ L respectively. Overall, from the antimicrobial activity of gram negative bacteria is higher compared with gram positive bacteria. Notably,

in gram negative bacteria the thickness of the peptidoglycan layer is less compared with the gram positive bacteria, where the major function of this layer is to protect the cells against antibacterial agents such as antibiotics, chemicals, enzymes and toxins^{11,22}. Thus, from the results it is reveals that

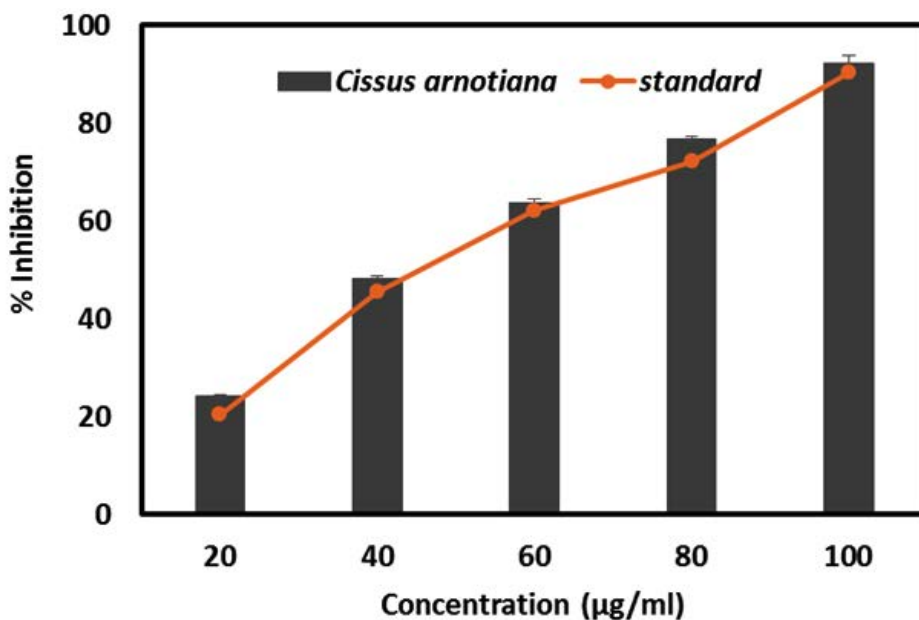


Fig. 5. DPPH assay of methanolic stem extract of *Cissus arnotiana*

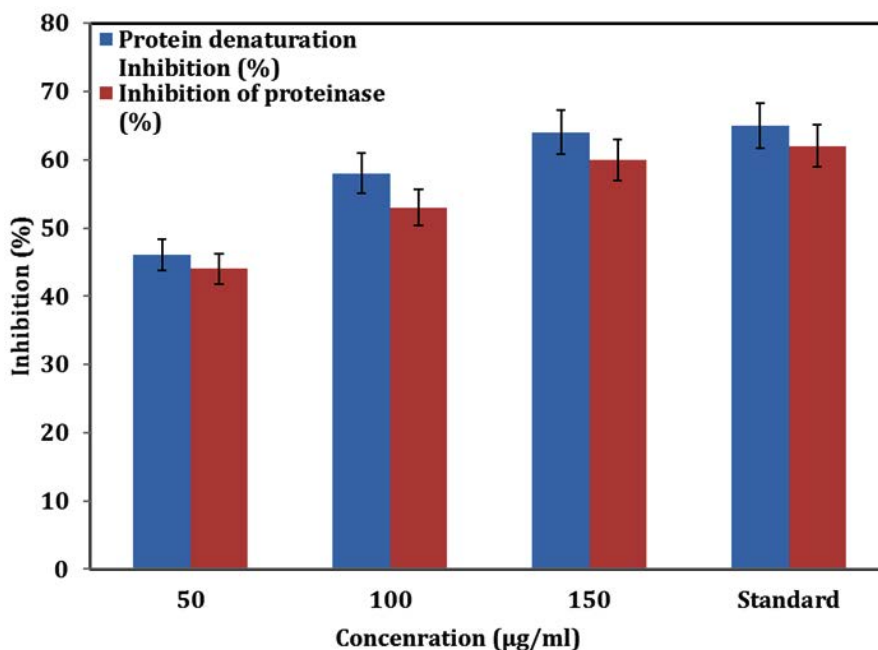


Fig. 6. Antiproteinase assay of methanolic stem extract of *Cissus arnotiana*

the methanolic stem extract shows a maximum antimicrobial activity against the gram negative bacteria than the gram positive bacteria (Table 1).

The DPPH assay is used to measure the free radical scavengers or hydrogen donor present in the methanolic stem extract. The antioxidant activity of the extract was evaluated using DPPH assay is shown in the figure 5. The oxidant inhibition of the molecules by inhibiting the oxidative chain reaction and formation of non-reactive radicals is known as antioxidant activity.

It is reported that the extract have a tendency to scavenge free radicals by either accepting or donating electrons based on the reaction, which results in the change of color from purple to yellow colored hydrazine molecule. Figure 5, shows the effect of the methanolic stem extract with different concentration of DPPH assay. The concentration of extract increases, with increase in the DPPH scavenging activity. From the figure 5, shows a strong radical scavenging potential of the methanolic stem extract. The increase in the

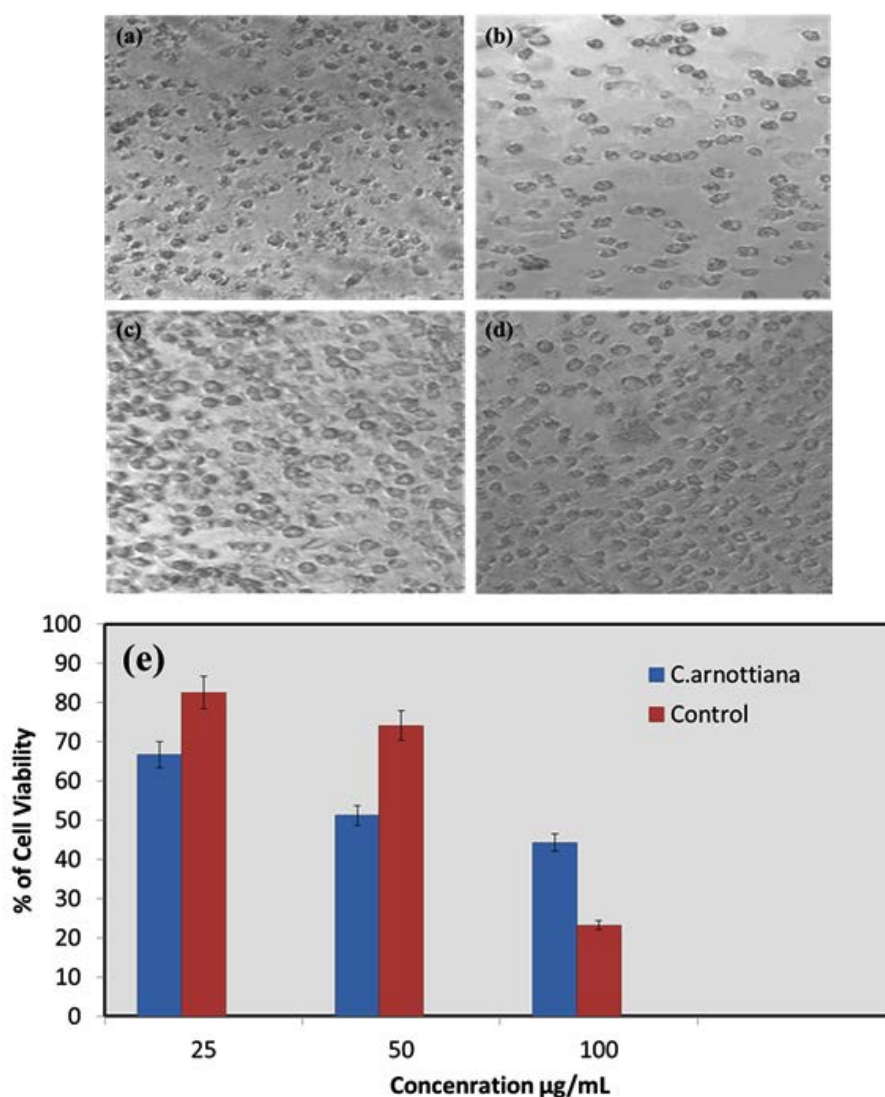


Fig. 7. In vitro MTT assay of methanolic stem extract of *Cissus arnottiana* in HeLa cell lines. (a) Control, (b) 25 µg/mL, (c) 50 µg/mL, (d) 100 µg/mL treatment and (e) percentage of cell viability at various concentration of Extract. Values represent mean \pm SD of three replications. Statistically significant at $p < 0.05$

scavenging potential of the extract is due to the phytochemicals present in the *Cissus arnottiana*. Therefore, the phytochemicals present in the methanolic stem extract is solely responsible for the enhancement of free radical scavenging activity^{9,15}. Additionally, the presence of phytochemicals such as polyphenols and flavonoids were confirmed from the FT-IR and UV-Visible studies. These phytochemicals are well known to possess persuasive antioxidant activity, which protects the cells against free radicals from oxidative stress.

Figure 6, shows the antiproteinase activity of the methanolic stem extract of *Cissus arnottiana*. The maximum antiproteinase inhibition was observed at 150 µg/mL which exhibits 64%, followed by the 100 and 50 µg/mL. The concentration of the methanolic stem extract of *Cissus arnottiana* increases as the anti-inflammatory activity also increases. The methanolic stem extract of *Cissus arnottiana* shows an excellent anti-inflammatory activity at higher concentration of the extract^{18,23}.

Anticancer activity of the methanolic stem extract was evaluated using an MTT *in-vitro* cell proliferation assay is shown in the figure 7. Based on the MTT assay the absorbance decreases with the increase in the concentration of the extract. The concentration of 100 µg/mL of the extract was found to be 44.28% of cells are viable. Therefore, it can be concluded that *Cissus arnottiana* can be used as a potential anticancer agent. Thus, the anticancer activity is mainly due to the phytochemicals present in the stem of the *Cissus arnottiana*^{11,19,24}. Then methanolic stem extract has decreases the percent of viability of HeLa cells. Figure 8, shows the microscopic images which reveal the morphological changes and shrinkage of the cells leading to the cell death induced by the extract.

CONCLUSIONS

The present study suggests that the methanolic stem extract of *Cissus arnottiana* shows an excellent antimicrobial activity against gram negative bacteria compared with gram positive bacteria. Based on the results of antioxidant, anti-inflammatory using anti proteinase assay and anti-cell proliferation shows an excellent activity at a concentration of 100 µg/mL of the

extract was found to be 44.28% of cells are viable against the methanolic stem extract of *Cissus arnottiana*. Additionally, it is observed that *Cissus arnottiana* constituents a wide variety of secondary metabolites that holds strong biological activity based on the experiments performed in this work. Thus, the methanolic stem extract can be used as a potential candidate for new therapeutic option as an anticancer drug. Therefore, it overlays to further research to identify the active phytochemicals responsible for the biological activity and its mechanism must studied further.

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