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Phytochemical characterization and antimicrobial activity of *Senna alata* leaf and flower extracts with cytotoxicity against osteosarcoma cell line

Nidhi Singh, V. Vinothini and Jayanthi Abraham*

School Of BioScience and Technology, Vellore Institute of Technology, Vellore - 632 014, Tamil Nadu, India

ABSTRACT

The present work was aimed at screening for bioactive compounds with pharmaceutical properties from *Senna alata* flowers and leaves extract. The dried samples were powdered and extracted with ethanol (leaves) and methanol (flower) using Soxhlet apparatus. Both antibacterial and antifungal activities of the extracts were checked against different clinical pathogens. Thin layer chromatography (TLC) revealed the number of compounds present in each extract. The extracts were subjected to GC-MS analysis and phytochemical analysis for phenol, flavonoids, carotenoids etc. were performed to evaluate the biochemical nature of the extracts. Furthermore, cytotoxicity assay was done against osteosarcoma (MG-63) cell lines. The results suggest that they possess anticancer property and antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella* sp., *Penicillium* sp., and *Aspergillus* sp. They were also able to moderately inhibit cell growth of MG 63 cell lines carcinoma.

Keywords: Bioactive compounds, Soxhlet, TLC, GC-MS, MG-63 cell lines.

INTRODUCTION

Searching for a novel drug to deal with the problems of common diseases faced by the humans nowadays has become a major focus of research by scientists around the world today. Naturally procured drugs are less toxic and more acceptable by the people, thus worldwide there has been increasing interest on the therapeutic values of natural products. Plants have been a source of many medicinal products from historic times and there are many more unexplored areas to be dealt with, moreover they have capacity to produce large amounts of secondary metabolites [1].

Senna alata is an ornamental plant which is known for its effectiveness against skin diseases as an antimicrobial agent. It is also used to reduce fever and sore throat pain in Ivory Coast as well as to treat otitis in Congo. Emmanuel et al. [2] demonstrated the healing effect of ointment made of ethanolic extract of *S. alata* leaves on chronic crusty or acute lesions of bovine dermatophilosis. The leaf extract also showed marked antifungal effects against *Trichophyton* sp. and *Dermatophyton* sp. with a minimum inhibitory concentration (MIC) of 5 mg/ml [3]. It was observed that this antimicrobial activity may be accounted for the presence of phenols, tannins, alkaloids, flavonoids, aldehydes and carbohydrates in the extracts. It also contains anthraquinones such as emodin, aloemodin and rhein [4] and also possesses laxative activity [5].

Senna alata also known as *Cassia alata* is often called as ringworm bush due to its fungicidal activity to treat ringworms. It is a tropical herb which belongs to family Fabacea being a native of South America. Its several parts have been used as traditional medicine due to its diverse pharmacological properties. According to Timothy et al. [6], leaf extract of the plant showed better antifungal activity against *Candida albicans*, *Microsporium canis* and *Trichophyton mentagrophyte* than ketoconazole (200 mg). Similarly selective effectiveness of *Senna alata* extract against bacterial and fungal pathogens was reported by Ogunjobi and Abiala [7].

As considerable work has been explored regarding antimicrobial activity and no reports have been stated regarding anticancer activity. In the present work anticancer properties against bone cancer cell lines was evaluated along with antimicrobial activity.

MATERIALS AND METHODS

Plant Material and processing

The leaves and flowers of *Senna alata* were collected from local area of Thanjavur, India and were identified physically. The leaves and flowers were sun dried for 5-6 h and further reduced to coarse powder using a grinder. The powder was stored in air-tight container for processing.

Preparation of extracts

The dried samples were extracted with ethanol for leaves [3] and methanol for flowers [8] using a Soxhlet apparatus. 20 g of the powdered samples were placed in the extraction tube separately and extracted with their respective solvents for 4-5 h. The extracts were collected in the flask and vacuum dried. The dried extracts were stored at 4°C for further experiments.

Phytochemical analysis of extracts

The phytochemical analysis was performed to check the presence of biomolecules such as anthraquinones, carbohydrates, flavonoids, phenols, saponins, tannins, alkaloids, terpenes and sterols using standard procedures [9,10].

Tannins

0.5g of powdered sample was boiled in 20ml of distilled water in a test tube and filtered. 0.1% FeCl₃ was added to the filtered samples and observed for brownish green or a blue black colouration which confirms the presence of tannins.

Saponins

To 2 ml of extract, 6 ml of distilled water was added in a test tube. The mixture was boiled, filtered and shaken vigorously to observe froth formation. The frothing was then mixed with 3 drops of olive oil and the formation of emulsion indicated the presence of saponins.

Phenols

The extract was dissolved in 5 ml distilled water and to this few drops of neutral 5% ferric chloride solution was added. Phenolic compounds were indicated by the presence of dark green coloration.

Alkaloids

A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and observed for the formation of reddish brown precipitate or coloration.

Terpenes

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Carbohydrates

2 ml of extracts were taken and few drops of Molisch's reagent were added followed by addition of 2 ml of conc. H₂SO₄ along the sides of the test tube. Formation of red or dull violet coloration at the interphase of two layers after 2-3 min. shows positive test results.

Sterols

1ml of extract was treated with drops of chloroform, acetic anhydride and conc. H₂SO₄ and observed for the formation of dark pink or red color.

Flavanoids

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.

Anthraquinones

0.5 g of extract was boiled with 1 ml of 10% sulphuric acid and filtered. Further 2.5 ml of benzene was added to the filtrate and shaken. The benzene layer was separated and half volume of 10% ammonia solution was added. The presence of pink or red-violet color in lower ammonia phase indicated presence of anthraquinones [3].

Thin Layer Chromatography (TLC)

For TLC determination of the extracts, silica gel plated glass slides were used with different solvent systems like hexane:ethylacetate:methanol (2:1:1), hexane:ethyl acetate (60:40) and hexane:ethylacetate:chloroform (2:1:1). Later the plates were observed under UV with 5% potassium hydroxide as the detection reagent.

Antimicrobial activity of extracts

Antibacterial activity

Activities of the extracts against 9 clinical pathogens were observed using Kirby Bauer's well diffusion method. Pathogens were plated on Muller Hinton agar (MHA) and 100 µl of the extracts were placed in the wells, incubated for 24-48 h at 32°C [11]. The plates were checked for zone of inhibition (diameter) after incubation is over to interpret the results. The clinical pathogens used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Serratia marcescens*, *Enterobacter* sp., *Shigella* sp. and *Salmonella* sp., obtained from Microbial Biotechnology Laboratory, VIT Vellore, India.

Antifungal activity

The antifungal activity was screened by well diffusion method. For inoculation of pathogens spore suspension was prepared in 1% tween 20 and then this spore suspension was plated on Potato dextrose agar (PDA) plates. The pathogens used were *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Fusarium* sp. and the plates were incubated for 72-96 h to obtain the results in the form of zone of inhibition.

Antioxidant assay

DPPH assay was performed to determine the antioxidant activity of the flower and leaf extracts. The effect of the alcoholic extracts on 1-diphenyl-2-picrylhydrazyl (DPPH) was estimated following the protocol mentioned by Chatterjee et al. [12]. 100 µl of extract was diluted to 2 ml with methanol followed by addition of 100 µl of DPPH reagent (0.1 mM), thoroughly mixed and left for 30 min in dark for the reaction to occur. After incubation period the absorbance was read at 515-517 nm using a UV-Vis spectrophotometer (Hitachi, Model U-2800 spectrophotometer). Ascorbic acid was taken as the standard (control) with methanolic DPPH and the percentage of DPPH radical scavenging activity was calculated using following equation:

$$\% \text{ scavenging} = [\text{Abs of control} - \text{Abs of sample}] / \text{Abs of control} \times 100$$

Cytotoxicity assay

MTT assay was performed to determine the cytotoxic property of the extracts against MG 63 (osteosarcoma cell line). It was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained and the cell suspension was diluted with medium containing 5% FBS to give final density of 1×10^5 cells/ml. Further cell line was seeded into 96-well titre plates and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. Different concentrations of the extracts were made and added to the wells already containing 100µl of the culture medium and incubated for 48 h at 37°C. Non-treated cells were taken as control and entire experiment was performed in triplicates.

After 48 h, 15 µL of MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) (5 mg/ml) in PBS was added to each well and incubated at 37°C for 4 h [13]. The tetrazolium salt is used to determine cell viability in

assays of cell proliferation and cytotoxicity. The medium with MTT was then removed and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

$$\% \text{ Cell Inhibition} = 100 - \text{Abs (sample)}/\text{Abs (control)} \times 100.$$

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software [14].

RESULTS AND DISCUSSION

Phytochemical analysis

The results indicate the presence of tannins with the formation of blue-green coloration, formation of emulsion confirmed the presence of saponins, dark green color was indicative of phenolic groups, precipitate formation of reddish brown color showed presence of alkaloids and terpenes were confirmed with reddish brown color without precipitate. Furthermore, carbohydrate presence was depicted by dull violet color, sterols were confirmed by red color formation with colorless solution showing presence of flavonoids and pink color was formed due to anthraquinones present in the extracts.

Thin Layer Chromatography

The TLC revealed that the ethanolic extract of leaves of *Senna alata* contained anthraquinone and the methanolic extract of flower contained some polar compounds similar to anthraquinones.

Antimicrobial activity

Of all the pathogenic bacteria tested, the assay showed prominent activity against *Serratia marcescens* (18 mm) and *Staphylococcus aureus* (19 mm) for leaf extract and against *Staphylococcus aureus* (19 mm) and *Salmonella* sp. (15 mm) for flower extract as shown in table 1. The ethanolic extract of leaves of *Senna alata* was highly effective against *Penicillium* sp. with a zone of 13 mm and flower extract was active against *Penicillium* sp. and *Aspergillus* sp. with zones of 18 mm and 15 mm respectively (Table 2).

Table 1. Antibacterial activity of *Senna alata*

S. No.	Pathogen	Zone of Inhibition (in mm)	
		Ethanolic leaf extract	Methanolic flower extract
1	<i>Staphylococcus aureus</i>	19	19
2	<i>Pseudomonas aeruginosa</i>	-	-
3	<i>Klebsiella pneumonia</i>	-	11
4	<i>Serratiamarcescens</i>	18	-
5	<i>Proteus mirabilis</i>	-	-
6	<i>Escherichia coli</i>	-	-
7	<i>Shigella</i> sp.	-	-
8	<i>Salmonella</i> sp.	-	15
9	<i>Enterobacter</i> sp.	-	-

Table 2. Antifungal activity of *Senna alata* extracts

S. No.	Pathogen	Zone of Inhibition (in mm)	
		Ethanolic leaf extract	Methanolic flower extract
1	<i>Penicillium</i> sp.	13	18
2	<i>Aspergillus</i> sp.	13	-
3	<i>Penicillium</i> sp.	-	15
4	<i>Rhizopus</i> sp.	-	-
5	<i>Fusarium</i> sp.	-	-

Antioxidant assay

DPPH radical scavenging assay revealed that both the extracts showed substantial amount of scavenging activity (%) with respect to the standard, ascorbic acid, after the incubation period was over. The flower extract showed 54.93 % scavenging while leaves extract showed 45.64% of activity. The above results shows promising antioxidant activity of the extracts leading to a final speculation that they can be used as an antimicrobial as well as antioxidant agents.

Cytotoxicity assay

The anticancer results of both the extracts showed mild reduction in the growth of MG 63 (osteosarcoma) cell lines as shown in figure 1 and figure 2.

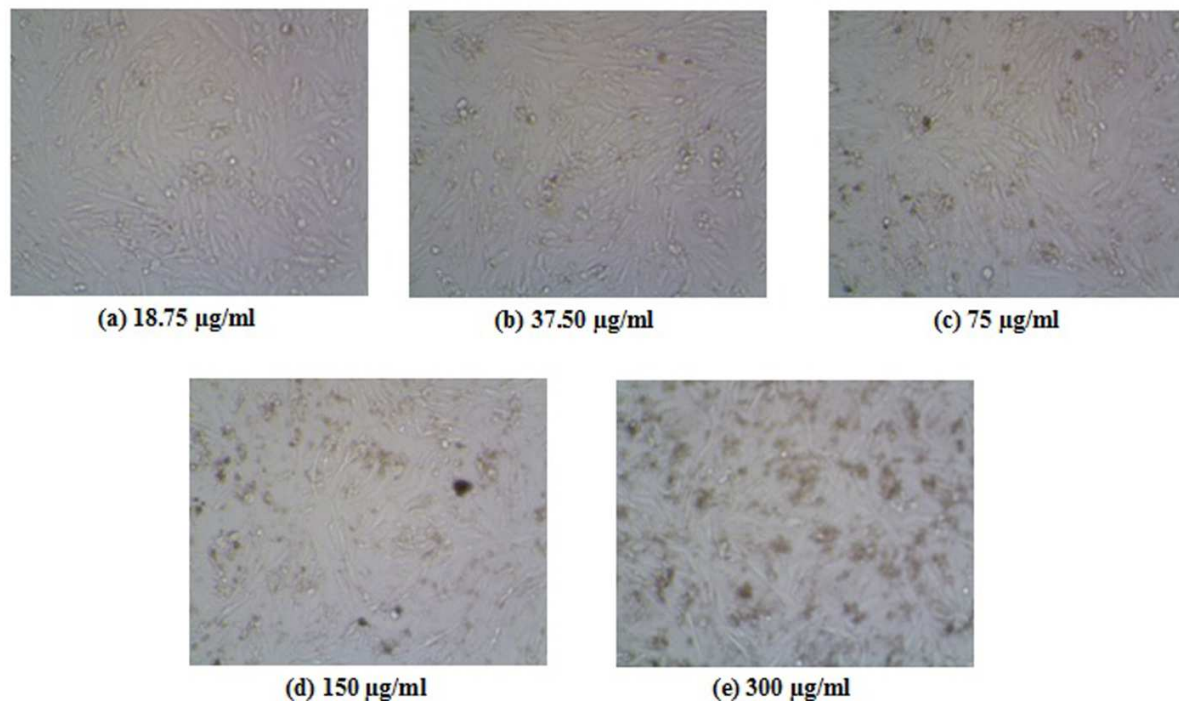


Fig. 1 Anticancer effect of *Senna alata* leaf extract.

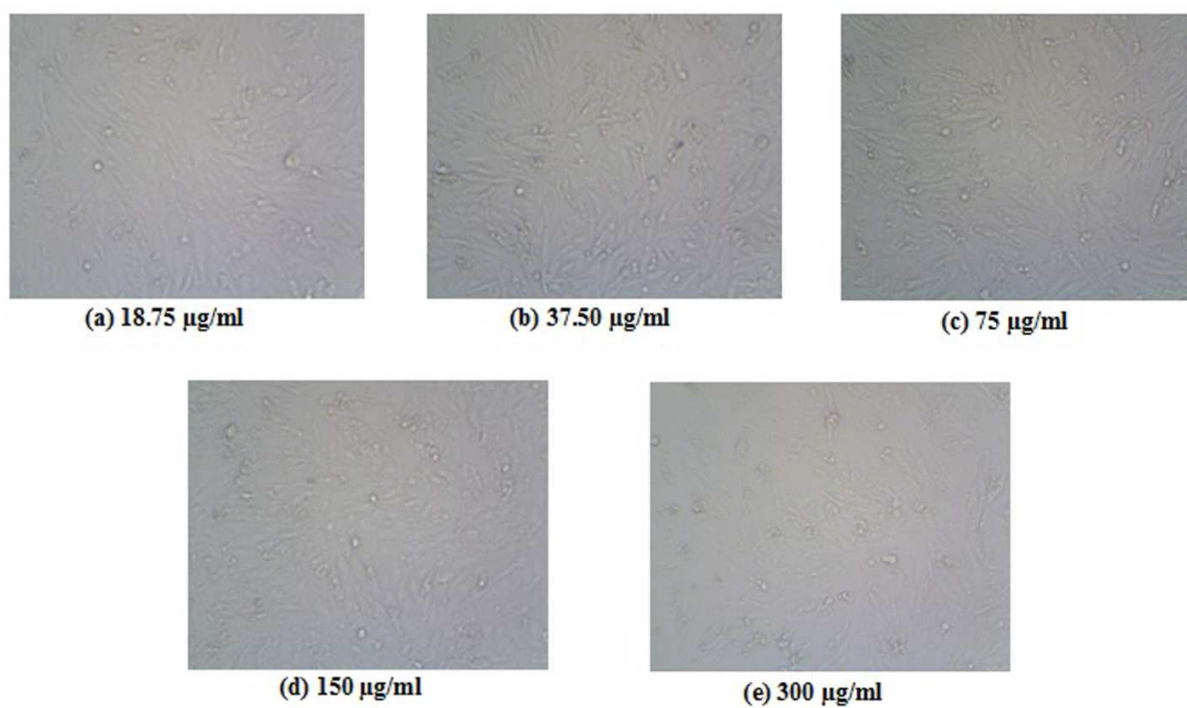


Fig. 2 Anticancer effect of *Senna alata* flower extract.

The graphical representation of the cytotoxicity shown in figure 3 clearly depicts the reduction in cell viability and cell count after treatment with the leaf and flower extracts. Thus these extracts can be used as a potent natural anticancer drug in preliminary stages of bone cancer.

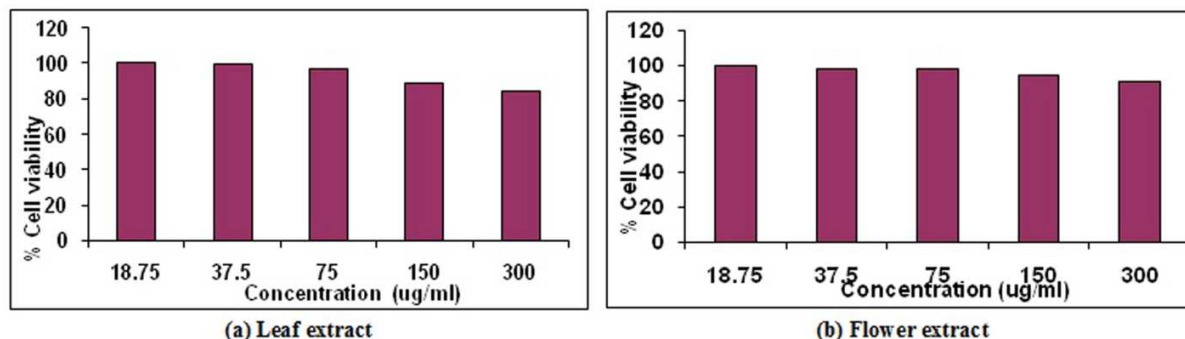


Fig. 3 Graphical reduction in Cell viability

Similar kind of work was done with hexane extract of leaves on A549 lung cancer cell lines by Levi and Lewis [15]. They reported cytotoxicity in parental and caspase 9 negative A549 cells. The hydrothermal extract of *Senna alata* was found to exhibit better antioxidant and DNA protection activity. Moreover, the extract was cytotoxic to K562 leukaemia cell line [16].

CONCLUSION

The above work establishes a ground for the screening of leaf and flower extracts of *Senna alata* as an antimicrobial as well as anticancer agent. It shows that the extracts could be a good natural solution to the problems of bacterial infections and increased rate of cancer emergence faced by the common people. The cytotoxicity assay results prove that it can be used as a prefatory drug for primary screening procedures of osteosarcoma.

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