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Research Article

Phytochemical Screening, In Vitro Antioxidant, Antibacterial and Cytotoxic Activities of Methanol Extract of *L. Camara* (L.) Leaves

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ABSTRACT

The present study reports the antibacterial, in vitro antioxidant and cytotoxic properties of leaves of Lantana camara L., methanolic extract. The phytochemical screening of methanolic extract of L. camara revealed the presence of alkaloid, phytosterol, tannin, flavonoid and absence of carbohydrates. The antibacterial assay was studied using both well diffusion and MIC broth dilution method. The methanolic extract of L. camara recorded the maximum activity against S. aureus showing a zone of inhibition of 16 mm and a minimum inhibitory concentration (MIC) value of 31.25 µg/ml. The in vitro antioxidant property of the plants was tested using DPPH and ABTS assay methods. The methanolic extract of L. camara showed considerable antioxidant activity with an IC50 of 76.5 µg/ml and 70.68 µg/ml in DPPH and ABTS assays, respectively and also showed significant cytotoxic activity against HeLa cells with an IC50 value of 58.53 µg/ml.

Keyword: *L. camara;* Antibacterial; *in vitro* antioxidant; *in vitro* cytotoxic

INTRODUCTION

The bioactive products derived from various plants that helps in prevention and treatment of various diseases is still enormous, regardless the recent developments in the synthetic chemistry as a method for developing new chemical drugs and drug products [1]. Since many years, the plants are found to be beneficial in treating different types of diseases in humans. According to the calculation of WHO, about 80% of the world's inhabitants problem can be treated with plant based drug for the primary health care [2-4]. The active constituents of various plants such as *Campototheca acuminata, Ocrosia elliptica, Catharanthus roseus, Podophyllum peltatum Angelica gigas, Podophyllum emodii,* and *Taxus brevifolia,*, were utilized for the treatment of different stages of malignancies [5-6].

Various natural phytochemical components derived from fruits, vegetables and different herbs have been studied for their wide range of biological activities such as, antioxidant, antimicrobial, anti-inflammatory, anti diabetic and anti-cancer properties [7-9]. Most of the plant based constituents are used as antimicrobial agent in many fields like food preservation, phytopathology and pharmaceutics due to their less toxic properties. The increasing failure to various chemotherapeutic compounds and development of resistance against different drugs has led to the screening of various medicinal plants for the generation of new

antimicrobial agents either as a single entity or in combination forms [10].

The cancer is a result of uncontrolled cell growth and is characterized by the dysregulation of cell signaling pathways at various steps [11]. Despite, understanding the various factors involved in the development of cancer, its diagnosis and prevention, the response rate of the drug used and the long term survival of infected patients is still a disappointing fact [12]. Most of the cancer treatments involve surgery, radiotherapy and chemotherapy methods. The chemotherapy treatment applies drugs to cancerous cells which work either by destroying or delaying or avoiding spreading of the growth of cancerous cells [13-15]. The non-specific nature, adverse side effects, drug resistance and high cost are found to be the major drawback in treatment of cancer using chemotherapy methods. Hence there is an imperative need to develop novel anti cancer agents with less toxicity and diverse activity against the cancerous cells [11]. The development of active components of medicinal plants plays a important role in the treatment of cancer and over 3000 species of plants have been investigated so far for anticancer properties [16]. The National Cancer Institute, USA, has investigated the anti-cancerous activity of plant extracts collected from more than 35,000 plant samples from over 20 countries [17].

The aim of the present work is to determine the phytochemical constituents and antibacterial property of different solvent extracts from the plants, viz., *Cassia fistula L, Lantana camara L., and Wattakaka volubilis (Linn. f.).* Further, the methanolic extract of *L. camara* which showed better activity in antibacterial assay was selected and tested for its i*n vitro* antioxidant activity and cytotoxic activity against normal and cancer cell lines.

MATERIAL AND METHODS

Chemicals and Reagents:

The chemical and reagents used in this study were of AR grade purchased from SRL Ltd and Merck, India and all the microbiological media were procured from Himedia Laboratories, Mumbai, India.

Plant collection and processing

The fresh leaves of following plant materials, *C. fistula, L. camara, and W. volubilis* were collected near Tiruvallur district, TamilNadu. The plant materials were washed separately under the running tap water and rinsed with the distilled water to remove unwanted soil and other dust particles. Then the leaves were shade dried, coarsely powdered with a pre-cleaned grinder, sieved and used for further study.

Plant Extract preparation

The powder of each plant was soaked in different solvents, *viz*., aqueous, ethanol and methanol (concn. 1 g/10 ml), and kept at room temperature in a rotary shaker for 72 h. The preparation was then filtered using Whatman No. 1 filter paper and the filtrate obtained was further concentrated using rotary vacuum evaporator at 50 °C. They were finally concentrated using water bath and stored at 4 °C till further analyses.

Phytochemical Screening

The aqueous, methanolic and ethanolic extracts of each plant were subjected to various phytochemical analysis using standard procedures to analyze the presence different chemical constituents like carbohydrates (Benedict's test), proteins (Biuret test), alkaloid (Wagner' reagent test), flavonoid (ferric chloride test), saponin (foam test), steroids (Leibermann-Burchards test) tannin (ferric chloride test), protein (xanthoproteic test), aminoacids (ninhydrin test), alkaloids (Mayer's test), flavonoids (alkaline reagent and lead acetate test) [18]. The total flavonoid and phenolic content of different solvent extracts were determined using the method described by Zhishen et al. and Sidduraju et al. respectively [19-20] and expressed in equivalent of ß – carotene as mg/g dry weight of leaf.

Antibacterial activity

The antibacterial assay for the methanolic extract was performed using agar well diffusion test and broth dilution technique against six bacterial pathogens such as, *E. coli* ATCC 8739, *S. aureus* ATCC 29736, *K. pneumoniae ATCC 10031, B. subtilis ATCC 6633, S. pyogenes ATCC 19615 and P. aeruginosa ATCC 27853.* The modified agar well diffusion method by Olaleye *et al*. was followed to determine the antibacterial activity of methanolic plant extract [21]. Briefly, overnight bacterial suspension of each test pathogen were made lawn culture in Mueller

Hinton agar (MHA) medium, followed by 6 wells of 4 mm diameter were made using sterile glass borer. Twenty five microlitre of methanolic extracts of each plant (1 mg /1 ml) and a standard drug, streptomycin (1 mg/ 1 ml) were added into each well. The plates were kept at 37°C for 24 hours and observed for the presence of bacterial inhibition zone around each well. The zone of inhibition was measured and results were recorded in millimetres (mm). The methanolic extract of *L. camara* which showed good results in well diffusion assay was further subjected for the minimum inhibitory concentration using tube dilution method with a varying concentration from 1 to 1.95 µg/ml [22].

In vitro antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of methanolic extracts of each plant, C. *fistula, L. camara, and W. volubilis* was evaluated using DPPH free radical scavenging assay, based on the scavenging of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical [23]. Different concentrations of methanolic extract all the three plants (10, 20, 40, 60, 80, 100 µg/ml) and ascorbic acid (standard) were added to methanolic solution of DPPH, mixed well and kept at room temperature for 30 min in dark condition. After incubation, the absorbance values were measured at 517 nm using UV-Visible spectrophotometer and the percentage scavenging ability at different concentration was determined using the following formula:

% of radical scavenging activity = *(Abs control – Abs sample)/ Abs control × 100%*

The IC_{50} value was defined as the total antioxidant required to scavenge 50% of the initial DPPH radicals in the environment which can be determined using plotted graph of scavenging activity against the different concentrations of the tested extracts.

ABTS radical cation decolourisation assay

The method of Re *et al*., with slight modifications was adopted for ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) assay for the methanolic extract of three plants [24]. Briefly, the methanolic extracts of each plant (0.1 mL) with various concentrations (10, 20, 40, 60, 80, 100 µg/ml) were mixed with the ABTS working solution (1.9 mL) and the reaction mixture was kept at 30°C for 6 min, then the absorbance values were read using UV-Vis spectrophotometer at 734 nm. The scavenging activity of ABTS radical was calculated using following formula:

% of ABTS radical scavenging activity = (Abs control – Abs sample)/ Abs control × 100%

***In vitro* cytotoxic studies**

The methanolic extract of *L. camara* showing the maximum antioxidant activity in DPPH and ABTS assays was further investigated for its *in vitro* cytotoxic activity against normal cell line, Vero cells and cancer cell line, HeLa cells. The African monkey kidney cell line (Vero) and human cervix adenocarcinoma cell line (HeLa) was obtained from National Centre for Cell Science, Pune, India. Both the cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 mg/ml streptomycin, 0.1 mM sodium pyruvate and 0.14% sodium bicarbonate. The cells were maintained in CO_2 incubator at 37°C in a 5% CO_2 atmosphere with 95% humidity. Different concentrations (10 - 100 µg/ml) of aqueous leaf and methanolic extract of *L. camara* were prepared by reconstituting in dimethyl sulfoxide (DMSO). The cytotoxic effect was evaluated by tetrazolium- dye, MTT assay with slight modifications [25]. Briefly, both normal and cancerous cells (Vero and HeLa) were seeded in 96-well plates at a density of 5×10^3 cells/well in 200 µl culture medium. Following 24 h incubation, the monolayer cells were checked for 90 percent confluence; then the cells were treated with different concentrations of methanolic and aqueous leaf extracts of *L. camara* and incubated for 24 h. For positive control, cells were treated with doxorubicin, standard drug with different concentrations and incubated for 24 h. After incubation, the content in the microtiter wells were removed and 10 µl of MTT solution (5 mg/ml in PBS) was added to each well and incubated for 3 h at 37°C in a humidified incubator with 5% CO_2. Then, 100 µl of DMSO was added to each well and gently shaken for 1 min and absorbance was read at 600 nm, with reference 490 nm, by microtiter plate reader (MIOS Junior, Merck) [26]. The percentage cell viability of the cells was calculated using subsequent formula:

% cell viability = (Absorbance of treated cells / Absorbance of control cells) × 100%

Statistical analysis

Data are expressed as mean ± standard deviation from three separate experiments. The *in vitro* antioxidant assay and cytotoxicity assay was evaluated using GraphPad Prism version 6.0 software.

RESULTS AND DISCUSSION

The phytochemical constituents of three different plants, *C. fistula, L. camara and W. volubilis* were analyzed and tabulated in table 1. The obtained results confirmed the presence of medically active compounds in the plant extracts tested. All the plant extracts of different solvents tested demonstrated the presence of alkaloid, phytosterol, tannin and flavonoid. Absence of carbohydrates was observed in all the solvent extracts of three plants tested in the present study.

Table 1: Qualitative phytochemical analysis of the solvent extract of different plant leaves

Phytochemical tests	Plants Extracts								
	C. fistula			*L. camara*			*W. volubilis*		
	Meth (1:1)	Eth (1:1)	Aqu	Meth (1:1)	Eth (1:1)	Aqu	Meth (1:1)	Eth (1:1)	Aqu
Alkaloids	++	++	++	++	++	++	++	++	++
Carbohydrate	--	--	--	--	--	--	--	--	--
Phytosterols	++	++	++	++	++	++	++	++	++
Phenols	--	++	--	++	++	++	++	++	--
Tannins	++	++	++	++	++	++	++	++	++
Flavonoids	++	++	++	++	++	++	++	++	++
Protein and Amino acids	++	++	++	++	++	--	--	--	++

Meth – Methanolic extracts; Eth – Ethanolic extracts; Aqu – Aqueous extract; ++ = highly positive; -- = Negative

The screening of phytochemical contents such as, alkaloid, tannin, saponin, flavonoid and several components provides information about the plant and their medicinal importance [27]. Especially, polyphenolic compounds like flavonoid showed greater antioxidant and antimicrobial activity, tannin is reported for its spasmolytic activity in smooth muscle cells and antioxidant activity, alkaloid for analgesic, antioxidant, antiplasmodic and antibacterial activities, saponin and steroid for antimicrobial property against various microorganisms [28-32].

The total flavonoids and phenolic compounds were determined for the solvent extracts of all the three plants, *C. fistula, L. camara* and *W. volubilis*. The higher total flavonoid contents were observed in methanolic extracts of *C. fistula* (58 mg ß-carotene /g extract) followed by methanolic extract of *L. camara* and *W. volubilis* showing 52 and 49 mg ß-carotene /g extract respectively.

Similarly, the methanolic extracts of the all the three plants showed high phenolic contents. Among the three plants, high content of total phenolic compound was observed in *C. fistula* (194 mg ß-carotene /g extract) followed by *L. camara* and *W. volubilis* showing 178 and 176 mg ß-carotene /g extract respectively. In all the three plants tested, the aqueous extracts showed low amount of total phenolic and flavonoid compounds.

The presence of high phenolic and flavonoid components contributes to various biological activities such as anti carcinogenic, anti inflammatory, anti atherosclerotic, antifungal and also the free radical scavenging properties [33-34]. Stanojević and their co workers determined the total phenolic and flavonoid contents from *Hieracium pilosella* L. extracts and they observed that methanolic fraction of the plant possesses higher amount of phenolic and flavonoids compounds [35]. A number of previous studies also reported where methanolic fraction of the plants showed high content of

phenolic and flavonoid compounds which are in close association to our study [36-40].

Among the three different extracts studied for the phytochemical analysis, the methanolic fractions of the three plants where found to be potential, thus they were subjected for antibacterial activity against selective pathogens by agar well diffusion and broth dilution method against six standard test strains namely; *E. coli*, *S. aureus*, *K. pneumoniae, B. subtilis, S. pyogenes and P. aeruginosa*. Among the methanolic extract of the three different plants tested, *L. camara* recorded the maximum zone of inhibition against all bacterial pathogens, followed by *W. volubilis and Cassia fistula* (table. 2).The zone of inhibition of standard drug streptomycin was also recorded (Fig. 1).

Table 2: Antibacterial evaluation of methanolic extracts of different plant leaves against bacterial pathogens

Methanolic extract Plant	Zone of inhibition (mm)					
	B. subtilis	***S.aureus***	***K. Pneumoniae***	***E. coli***	***P. aeruginosa***	***S.pyogenes***
Cassia fistula	8.07 ± 0.17	13.19 ± 0.34	5.98 ± 0.39	-	6.47 ± 0.21	7.24 ± 0.24
Wattakaka volubilis	6.01 ± 0.40	13.09 ± 0.55	-	-	5.31 ± 0.28	7.06 ± 0.33
Lantana camara	11.07 ± 0.43	16.23 ± 0.39	7.35 ± 0.21	-	7.02 ± 0.31	10.35 ± 0.32
Streptomycin (1mg/ml)	15.16 ± 0.44	18.18 ± 0.50	11.28 ± 0.23	-	12.14 ± 0.39	14.31 ± 0.32

Values are mean ± Standard deviation of three independent experiments; (-) No activity

The emergence of bacterial resistance against various drugs has necessitated an urgent need of developing new antibacterial agents with broad spectrum activity. The plant derived components were found to be low toxic and viable alternative due to their efficient activity against several pathogens, low cost and easy accessibility to poor communities [41]. The present results revealed that a significant antibacterial activity was found when methanolic extract was used against different pathogenic microorganisms.

Lagnika and co-workers evaluated the antimicrobial property of aqueous, dichloromethane and methanol extracts of *Acmella uliginosa* against *S. aureus, E. faecalis, S. epidermidis, Staphylococcus aureus Methicillin Resistant* (SARM), *P. aeruginosa* and *E. coli*. They also found that, the tested pathogens were more sensitive to extract of dichloromethane, followed by methanolic rather than the aqueous extract [42]. Similar kind of studies was reported by Ndhlala *et al*. who have investigated the antimicrobial property of various extracts of South African tree aloe (Aloe barberae) against microbial pathogens such as, *B. subtilis, S. aureus, E. coli, K. pneumoniae and Candida albicans* [43]. On the contrary, Narod and their co workers investigated the antimicrobial property of four medicinal plants namely, *Antidesma madagascariense* Lam., *Faujasiopsis flexuosa* (Lam.) C. Jeffrey, *Toddalia asiatica* (L.) Lam. and *Vepris lanceolata* (Lam.) G. Don against *E. coli, P. aeruginosa, S. typhimurium, S. aureus, C. albicans, Aspergillus niger* and they also found that methanolic fractions showed broad spectrum activity against the tested pathogenic strains [44]. Similar studies were reported by various researchers who reported antibacterial activity of different solvent extracts of plant leaves against different bacterial pathogens [45-49].

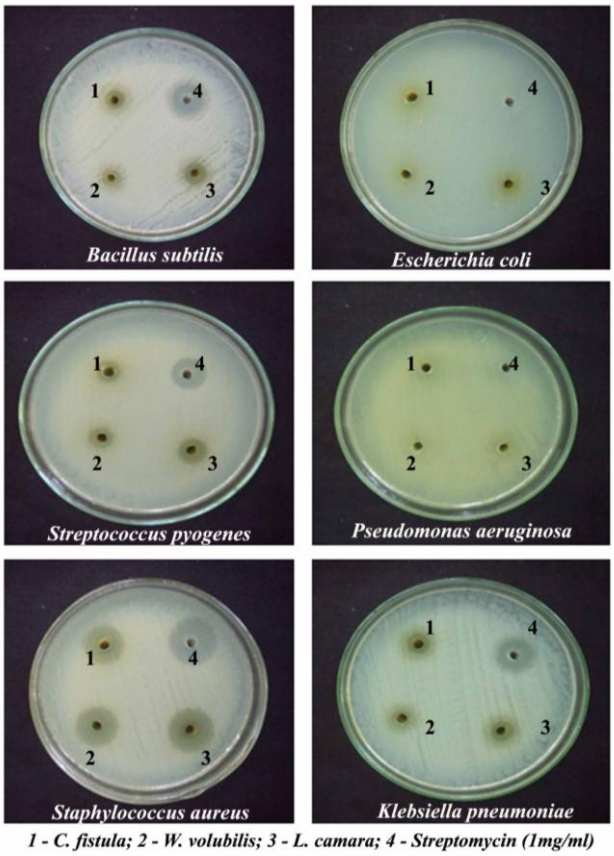

Fig. 1: Antibacterial activity of methanolic extract of different plant leaves against different bacterial pathogens

The methanolic extracts of *Lantana camara* was found to be most effective among the extracts tested for antibacterial activity using well diffusion method; hence it was further subjected for the determination MIC against the test pathogens (table 3). The results confirmed that, maximum activity was found when methanolic extract of *L. camara* was used against *S. aureus* showing a MIC value of 31.25 µg/ml, where all other tested strains showed a MIC value of 62.5 µg/ml.

Table 3: Determination of MIC of the methanolic extract of *L. camara leaves* on bacterial pathogens

Strains	MIC (µg/ml)
B. subtilis	62.5
P. aeruginosa	62.5
S. aureus	31.25
K. Pneumoniae	62.5
S.pyogenes	62.5

The *in vitro* antioxidant activity of the methanolic extract of the three plants was tested using DPPH radical scavenging activity and ABTS radical cation decolorization assay method (Fig. 2). The percentage of radical scavenging activity of the extract against the stable DPPH was calculated and the IC_{50} value of standard ascorbic acid, methanolic extract *Cassia fistula, Lantana camara* and *Wattakaka volubilis* were found to be 19.45 (µg/ml), 87.4 (µg/ml), 76.5 (µg/ml) and 91.8 (µg/ml) respectively. The percentage of radical scavenging activity of the

extract against the stable ABTS was also determined, (Fig. 3) and the IC_{50} value of standard ascorbic acid, methanolic extract of *Cassia fistula, Lantana camara* and *Wattakaka volubilis* were found to be 21.08 (µg/ml), 95.32 (µg/ml), 70.68 (µg/ml) and 85.24 (µg/ml) respectively. Based on the *in vitro* antioxidant assays, the methanolic extracts of *L. camara* were found more efficient than the other two plants; therefore it was preceded for the *in vitro* cytotoxicity studies against different cell lines.

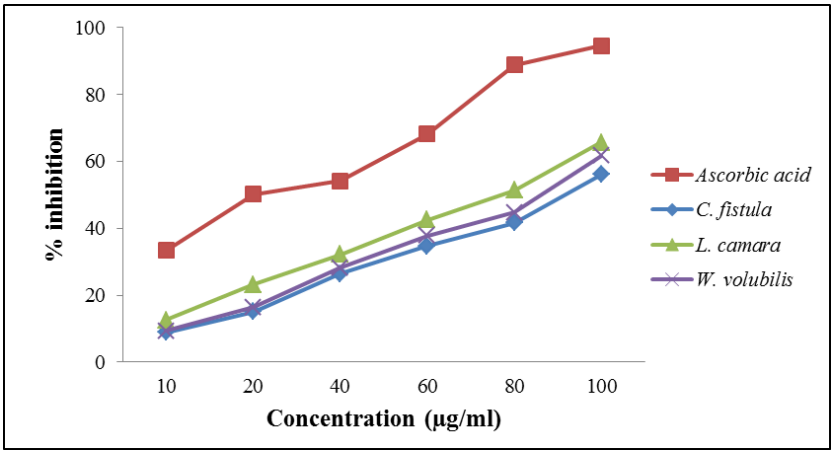

Fig. 2: DPPH scavenging activity of methanolic extract of different plants

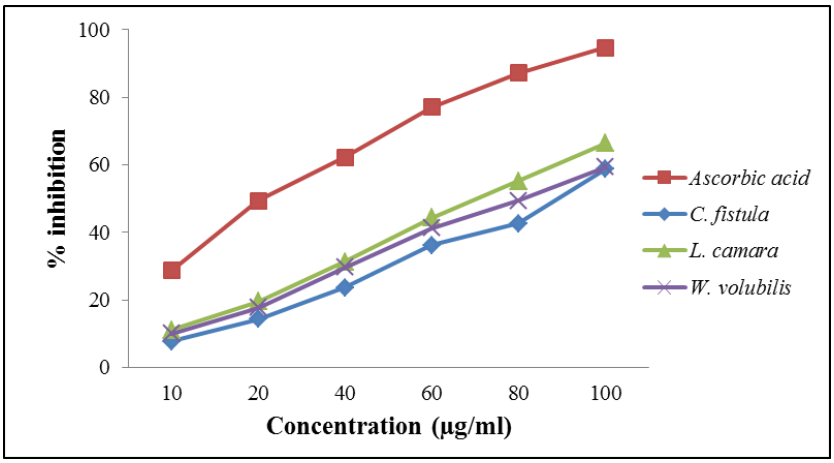

Fig. 3: ABTS scavenging activity of methanolic extract of different plants

Several plant extracts where found to have manifold biological effects that include antioxidant activities which is majorly due to their different phytochemical constituents. Various studies have reported that the antioxidant property of phenolics is primarily due to the redox properties which aid in adsorbing and neutralizing free radicals present in the environment, quenching the singlet and triplet oxygen, or by decomposing peroxides [50-51]. Similar results were reported by various researchers who have investigated the antioxidant potential of various plants extracts such as *Datura metel* L., *Cynodon dactylon* L., *Barringtonia racemosa*, *Hibiscus sabdariffa* [52-54].

Cancer, one of the most feared diseases in the modern society is one of the leading causes of mortality worldwide [55]. An ideal anticancer agent should inhibit the progression of various cancer through its cytotoxic properties and study of these compounds contributes a key development in the field of anticancer therapeutics [56]. The plant based medicine, generally considered as traditional still plays a foremost role in the development of new therapeutic compounds with broad spectrum and less toxic [57]. Till date, the natural based products are found to be most prolific source of biologically active compounds playing a major role in the drug development.

Both the methanolic and aqueous leaf extracts of *L. camara* were studied for their *in vitro* cytotoxic activity against normal Vero cells and HeLa cells. Cell viability of about 70% and above was observed when Vero cells were treated with aqueous extract of *L. camara* with a concentration of 80 µg/ml, similarly in case of methanolic extract, 70 % viability was observed when treated with 60 µg/ml. (Fig. 4 & 5). The present study also revealed that, an IC_{50} of 170.7 µg/ml and 58.53 µg/ml was observed when HeLa cells were treated with aqueous and methanolic extracts of *L. camara*, respectively. In case of doxorubicin treated HeLa cells, the IC_{50} value was found to be 16.14 µg/ml. The above results clearly revealed the significant cytotoxic activity of the methanolic extract of *L. camara* against HeLa cell lines creating an opportunity to explore the potential of these components in the healthcare and pharmaceutical industries.

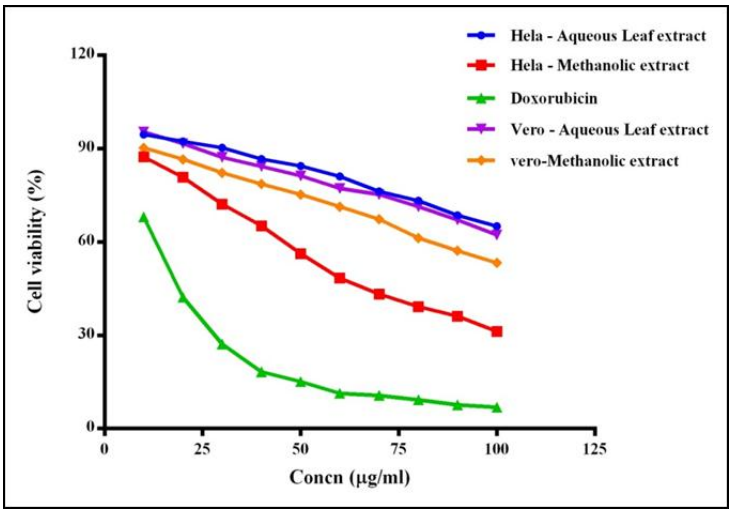

Fig. 4: *In vitro* cytotoxicity study of methanolic extract of *L. camara* leaves using MTT assay

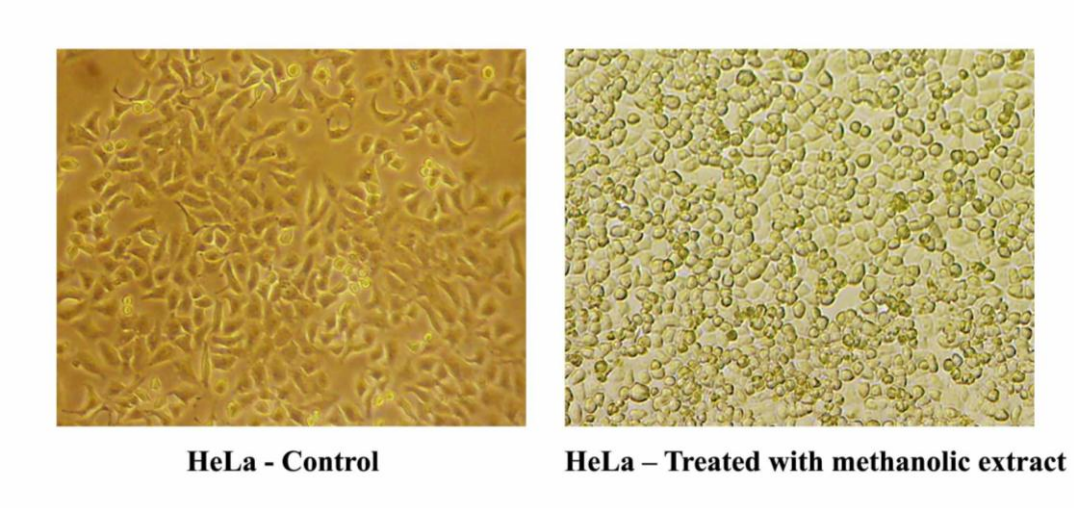

Fig. 5: *In vitro* cytotoxicity study of methanolic extract of *L. camara* leaves against HeLa cells

Pour and co workers have also investigated the cytotoxic effect of different concentration of methanolic extract of *L. camara* against Vero cell lines. They found that the leaf extract of *L. camara* at concentrations up to 500 µg/mL inhibited the growth of Vero cells 2.5 times less than triton 100x. In another study, Srivastava et al. used *in vitro* cell culture method to study the effect of secondary metabolites, pentacyclic triterpenoids, oleanolic acid, betulinic acid, and ursolic acid extracted from *L. camara* leaf extracts against HeLa and BHK-21 cells [58-59].

Similar kind of results showing the bioactivity of the plant derived extract were demonstrated by various researchers against different cancer cell such as HeLa and MCF-7 cell lines [53, 60].

CONCLUSION

The methanolic extract of three different plants, *C. fistula, L. camara and W. volubilis* exhibited varying amount of total phenolic and flavonoid content. The methanolic extract of *L. camara* possess greater antioxidant activity and also showed good antibacterial activity against various tested pathogens. Further the extract was also tested for in vitro cytotoxic property against Vero and HeLa cells which also confirmed their potential activity. Thus the present finding provides a preliminary data of *L. camara* extract to have potential antibacterial and cytotoxic activity. Further studies is necessary to identify the active components involved in the biological activity which leads to the further development of the plant components into promising antibacterial and anticancer drugs.

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CONFLICT OF INTEREST

The authors confirmed that there is no conflict of interest for this research paper.

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