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Research Article

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In vitro Anticancer (Cervical Cell Line) and Antimicrobial Activity of Ethanolic Extract of *Acacia Chundra* Leaves

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ABSTRACT

***Objective**: To evaluate the in vitro activity of anticancer (cervical cell line) and antimicrobial activity of ethanolic extract of acacia chundra leaves.*
***Methods**: The ethanolic extract of acacia chundra leaves were evaluated for in vitro anticancer cell line studies by MTT assay and antimicrobial activity by Disc diffusion assay.*
***Results**: Acacia chundra willd is one of the indigenous plants, which has enormous traditional values against various diseases. A phytochemical study revealed the leaves shows the presence of Alkaloids, Flavanoids, Tannins, Phenols and Steroids. The physiochemical analysis was done. Antibacterial and antifungal activity revealed that ethanolic extract of Acacia chundra was effective against against staphylococcus aereus, pseudomonas aerugenosa and candida albicans zone of inhibition was found to be 10 mm (250 µg), 18 mm (500 µg), 10 mm (250 µg), 20 mm (500 µg) and 16 mm (250 µg), 24 mm (500 µg) respectively. In vitro anti-cancer activity (Cervical cell line) was shown that the IC_{50} value of EAC was found to be 51.27 which prove plant shown moderate activity against cervical cell line studies.*
***Conclusion**: It is concluded that even though the accessibility of the modern system of medicine for simple and complicated diseases is available, many people in the studied area still continue to depend on medicinal plants, for the treatment of different type of diseases. Further the plant should explore for phytochemical responsible for anticancer and antimicrobial activity.*

Keywords: *Acacia chundra*; Cervical cell line; *Staphylococcus aereus; Pseudomonas aerugenosa*; *Candida albicans*

INTRODUCTION

Herbal medicine is still the mainstay of about 75 -80% of the world population, mainly in the developing countries, for primary health care [1]. Natural plant products are perceived to be healthier than manufactured medicine". Plants are not optional; they are essential to life and central to the future of human well-being" According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times [2]. Cancer is a degenerative disease. Accumulation of toxins through carcinogenic food like fast food, colas, habits like smoking, drinking, paan chewing, stressful life style, toxic medicines and environmental pollutions lowers immunity causing cancer [3]. Internationally , the cancer burden doubled between 1975 to 2000 and is set to double again by 2020 and nearly triple by 2030 .There were around 12 million new cancer cases and 7million cancer death worldwide in 2008, with 20-26 million new cases and 13-17 million death's projected for 2030 [4]. In India cervical cancer is one of leading causes of cancer mortality among women 30 to 69 years of age, accounting for 17% of all cancer deaths [5].

MATERIALS AND METHODS

The leaves of *Acacia chundra*, were collected from Thirukkachur of Tamilnadu, India during January 2016. The plant material was identified and authenticated by professor Dr.P.Jayaraman, Ph.D, Director, Plant Anatomy Research Center, West Tambaram, Chennai [NO:PARC/2016/3218]. A voucher specimen was submitted at C.L.Baid Metha college of Pharmacy, Chennai-97. The shade dried leaves of *acacia chundra* were cleaned and the adherent sand and dust particle were removed and made into coarsely powder with the help of the electric grinder. About 83g of coarsely powder is placed in a porous bag or "thimble" made of strong filter paper which is placed in chamber E of the Soxhlet apparatus. The extracting solvent (ethanol) in flask is heated (50-60°C) and its vapor condensed in condenser. The condensed extracted drips into the thimble containing the crude drug. The crude drug was exhaustively extracted with solvent by hot continuous percolation and maceration method. When level of liquid in chamber rises to the top of Siphon tube the liquid content of chamber. Siphon into the flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. Again the process is repeated for several times (Figure 1). The solvent was evaporated at 40°C to obtain a viscous mass and the percentage yields of extract were determined and preliminary phytochemical test was carried out to identify the nature of phytoconstituents present in the extract [6,7].

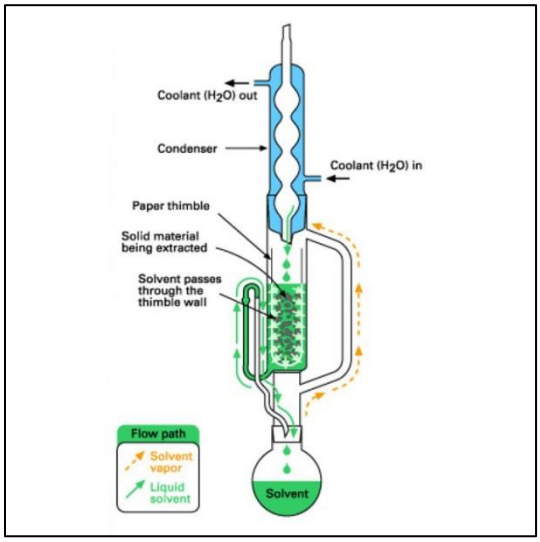

Figure 1: Phytochemical evaluation for identifying nature of phytoconstituents present in the extract

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis and physicochemical constant of ethanolic extract of *Acacia chundra* was carried out by employing standard procedure [8,9].

BIOLOGICAL STUDIES

In vitro Anticancer Activity

Cell lines and cultural conditions:

Hela cell lines (human cervical cancer cells) were cultured in RPMI-1640 medium with 20% FBS, 2 mM L-glutamine, 1% penicillin/streptomycin under a fully humidified atmosphere 5% CO_2 at 37°C.

MTT assay:

The effect of plant extract on the viability of human cancer cell lines Hela were determined by MTT (3-(4,5-dimethyl thiozole-2-yl)-2-5-diphenyl tetrazolium bromide) assay. 100 µl of cell suspensions in growth medium were plated in 96-well microtitre plate at concentrations of 1×10^4 cells/well and incubated for 48h at 37°C in a humidified incubator. After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 72 h at 37°C. After removal of sample solution, followed by washing with phosphate buffered saline (pH 7.4), 20 µL of MTT (5 mg/mL) was added to each well of the plate. The plate was incubated for 4h at 37°C. The solution in each well including MTT was aspirated and 100 µL of buffered DMSO was added to dissolve formazone. The plates were shaken for 5 min.

Optical density was measured on a microplate ELISA reader at 540 nm with DMSO as control. The cytotoxicity was obtained by comparing the absorbance between the samples and control (Table 1). The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = (\text{Abs (control)} - \text{Abs (Test)}/\text{Abs (control)}) \times 100$$

IC_{50} was calculated from dose-response curves [10-16].

***In vitro* Antimicrobial Activity**

Antibacterial activity:

Acacia chundra extract were screened for antimicrobial activities by disc diffusion technique. Compounds are screened *in vitro* for their anti-microbial activity against *S. aureus, P. aeruginosa and* are compared with standard drug amikacin (30 µg). The zones of inhibition formed for the compounds against organisms were calculated.

Table 1: Detail of the organism used for the study

Grams strain	Name of the organism	Std Code
Gram-negative	*Pseudomonas aeruginosa*	(ATCC-2853)
Gram-positive spherical bacteria	*Staphylococcus aureus*	(ATCC-9144)

Disc-diffusion assay:

The antibacterial activity of the extract was carried out by disc diffusion method. The concentrations of the test compounds were taken in DMSO and used in the concentration of 250 µg and 500 µg/disc. The target microorganisms were cultured in Mueller–Hinton broth (MHB) (Figure 2). After 24 h the suspensions were adjusted to standard sub culture dilution. The petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain [17-20].

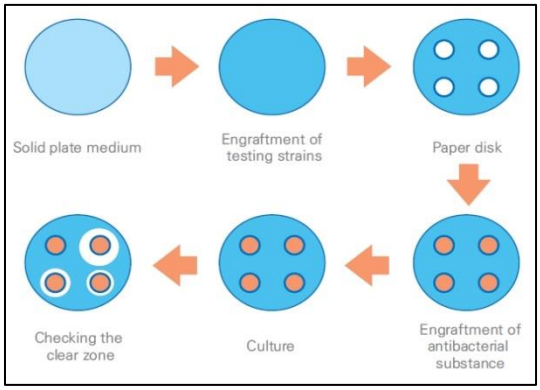

Figure 2: Disc- diffusion method

Disc made of Whattman No.1, diameter 6 mm was pre sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug amikacin (30 µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37°C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial activity. Antibacterial activity of test compound against the *S. aureus, P. aeruginosa.* Antibacterial activity of test compound against the *S. aureus, P. aeruginosa.*

Antifungal Activity

Acacia chundra extract were screened for antifungal activity by disc diffusion technique. Compounds are screened *in vitro* for their antifungal activity against *Candida albicans* and compared with standard drug ketoconazole. The zone of inhibition formed for the compounds against organisms were calculated (Table 2) [18].

Table 2: Detail of the organism used for the study

Name of the organism	Code
Candida albicans	(MTCC-227)

Antifungal Assay

Potato dextrose agar (PDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Potato dextrose broth. The synthesized compounds were applied on sterile disc. Standard

antibiotic (ketoconazole 30 µg) was used as positive control and fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition observed were measured (Figures 3-8 and Tables 3-7) [21,22].

RESULTS

Table 3: Preliminary phytochemical studies of *Acacia chundra* leaves

S. No	Phyto Constituents	Results
1.	Alkaloids	+ve
2.	Glycosides	-ve
3.	Anthraquinones	-ve
4.	Flavanoids	+ve
5.	Terpenoids	-ve
6.	Steroids	+ve
7.	Tannins	+ve
8.	Phenol	+ve
9.	Quinones	-ve
10.	Saponins	-ve
11.	Sugars	-ve
12.	Proteins	-ve
13.	Resins	-ve

Table 4: Physiochemical analysis of acacia chundra leaves

S No.	Parameters	Results
1	**Ash Values**	
	Total ash	1.50%
	Water soluble ash	0.19%
	Acid insoluble ash	0.22%
	Sulphated ash	2.40%
2	**Extractive Values**	
	Water soluble	38%
	Alcohol soluble	28.00%
3	Loss on drying	2.50%

Biological Studies

Table 5*:* Antibacterial activity

Compounds	Zone of Inhibition (mm)			
	S. aereus		*P. aerugenosa*	
	250 µg	500 µg	250 µg	500 µg
EEAC	10	18	10	20
G	-	12	-	10
Amikacin (30 µg)	34		36	
EEAC : Ethanolic Extract Of Acacia Chundra G: Ethanolic extract of *Gracilaria Corticata*				

Figure 3: Zone of inhibition against *Staphylococcus aureus*

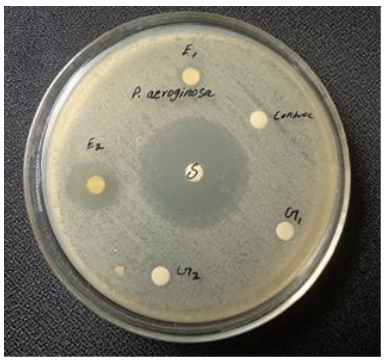

Figure 4: Zone of inhibition against *Pseudomonas aeroginosa*

Table 6: Antifungal activity

Compounds	Zone of inhibition (mm)	
	C.albicans	
	250 µg	500 µg
EEAC	16	24
G	-	12
Ketoconazole (30 µg)	32	
Ethanolic Extract of *Acacia Corticata*		
G: Ethanolic extract of *Gracilaria Corticata*		

Figure 5: Zone Of Inhibition against *Candida albicans*

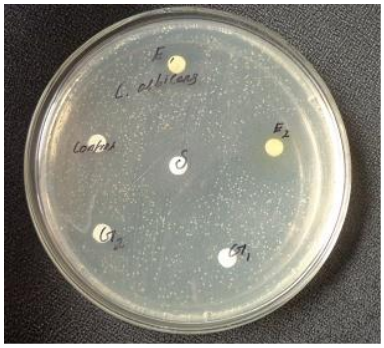

Table 7: Anticancer activity

Concentration	% Inhibition			Average
(µg/ml)	Trial 1	Trial 2	Trial 3	
Control	100	100	100	100
5	82.38	85.16	81.59	83.0433
10	71.82	68.94	69.16	69.97333
25	63.72	59.86	61.24	61.60667
50	55.29	49.97	54.03	53.09667
100	21.54	18.95	23.36	21.28333
				IC50 = 51.27

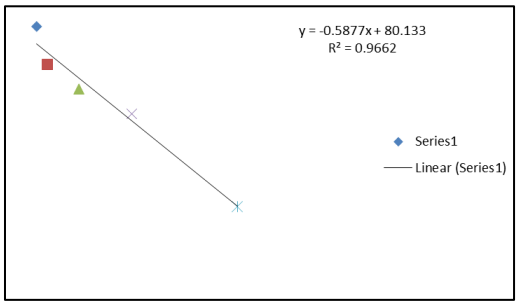

Figure 6: Concentration vs. % inhibition

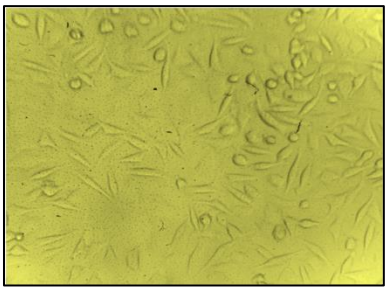

Figure 7: Control HeLa cell line

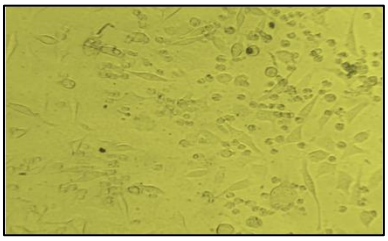

Figure 8: Zone of inhibition on HeLa cell line

DISCUSSION

Acacia chundra willd is one of the indigenous plants, which has enormous traditional values against various diseases. It is a tree which is normally 8m height with bark rusty brown .Leaves are bipinate, alternate, stipulate, opposite, sessile with entire margin. Flowers yellowish-white, calyx 5 lobed; corolla lobes are linear with stamens and stigma were present. Fruits are stipitate pod, flat, thin, horned, seeds with ovoid shape. Plant is distributed throughout India mainly in Western Ghats and in the places of Tamilnadu, Maharastra, Kerala, Orissa, and Pondicherry on dry and rocky soils. Phytochemicals identified in the species of *Acacia* are Flavanoids, Alkaloids, Sugars, Tannins and Glycosides. It has medicinal uses including the treatment of painful throat and cough. It is employed in Astringent products and use to promote digestion. It also used to sooth infection and severe irritation of the skin. Wood is used for certain application in ship building. It is also used as food for bees. In India, it is now necessary to get permit before cutting the tree down. The leaves of *Acacia chundra* were collected, shade dried, milled and extracted by Soxhlet extraction method. The yield of extract was found to be 48.1% w/w. The preliminary Phytochemical studies reveals the leaves shows the presence of Alkaloids, Flavanoids, Tannins, Phenols and Steroids.

Physicochemical and Phytochemical Analysis

The physiochemical analysis was done. The ash of any organic material is compressed of their non-volatile inorganic components (metallic salts and silica). It usually represents the naturally occurring inorganic salts and organic matter added for the purpose of adulteration. Hence ash determination furnishes a basis for judging the identity and cleanliness of the drug. Total ash, water soluble ash, acid insoluble ash, sulphated ash was found to be 1.5%, 0.19%, 0.22%, 0.24% respectively. The water soluble and alcohol soluble extractives were found to be 38% and 28% respectively. It indicates the polar water soluble organic compounds such as flavanoids, alkaloids, sugars and inorganic substances present in plant. The alcohol soluble extractive value indicates the present of considerable amount of polar organic salts present in plant. These constant would help to identify and to standardize the plant.The TLC studies of *Acacia chundra* ethanolic extract shows the presence of max. 6 spots (a) Chloroform : Petroleum ether (9:1) with Rf values of 0.92, 0.88, 0.83, 0.79, 0.66, 0.43 (b) Ethyl Acetate : Petroleum ether (3:7) with Rf values of 0.92, 0.88, 0.84, 0.41, 0.52, 0.32.

Biological Studies

Anti-bacterial activity was done by Disc Diffusion Method. Ethanolic extract was compared with standard and Gracilaria corticata (red algae). The zone of inhibition against *staphylococcus aureus* and *pseudomonas aerugeniosa* was found to be 10 mm (500 µg), 18 mm (500 µg) and 10 mm (250 µg), 20 mm (500 µg) respectively. Anti-fungal activity was done by Disc Diffusion Method. Ethanolic extract was compared with standard and *gracilaria corticata*

(red algae). The zone of inhibition against *candida albicans* was found to be 16 mm (250 µg), 24 mm (500 µg). *In vitro* anti-cancer activity was done by cell line studies with cervical cell line (HeLa cell line) as control. The inhibitory concentration of ethanolic extract was found to be 51.27. Thus the drug has proved to be potent anticancer activity and anti-microbial activity.

CONCLUSION

The present study has provided a new incentive to the traditional system of health care and also will be helpful to find out the other use of the plant which would be helpful to modern healthcare system. It is concluded that even though the accessibility of the modern system of medicine for simple and complicated diseases is available, many people in the studied area still continue to depend on medicinal plants, for the treatment of different type of diseases. Further the plant should explore for phytochemical responsible for anticancer and antimicrobial activity.

CONFLICT OF INTEREST

The authors confirm this paper content has no conflict of interest.

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