Research Article

Studies on Antitumour and Antibacterial Activities of *Careya arborea* Roxb. *Invitro*

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ABSTRACT

Careya arborea Roxb is a plant traditionally used for cure and treatment of tumors, bronchitis, epileptic fits, diarrhoea, pyrexia, dysentery toothache, haemorrhoids, leucoderma, and eruptive fever particularly in smallpox. The present investigation was carried out to evaluate *in vitro* antitumour and antibacterial activities *Careya arborea* ethanolic leaves extract (*CAELE*). The cytotoxicity of *CAELE* based on inhibition of Vero cell line was screened by MTT assay. The per cent inhibition of vero cells at extract concentration of 100, 200, 400, 600, 800 and 1000 µg/ml was 16.74, 20.96, 44.27, 52.35, 62.62 and 63.47, respectively and that of standard drug curcumin at concentration of 10, 20, 40, 60, 80 and 100 µg/ml was 24.55, 29.2, 33.42, 46.65, 60.16, and 75.79, respectively. After 24 h, the highest growth inhibitory rate of Vero cells exposed to extract (1000 µg/ml) was 63.47 per cent and that of standard drug curcumin (100 µg/ml) was 75.79 per cent. The IC_{50} i.e. 50 percent growth inhibition of Vero cells by *CAELE* was found to be 640.4 µg/ml. The antitumour activity of *CAELE* was evaluated against HEp-2 cell lines. The per cent inhibition of HEp-2 cells by extract at 100, 200, 400, 600, 800 and 1000 µg/ml concentration was 5.6, 22.51, 25.07, 36.05, 57.64 and 74.66 respectively and that of curcumin at the concentration of 10, 20, 40, 60, 80 and 100 µg/ml was 30.67, 41.76, 50.35, 57.86, 67.43 and 80.64 respectively. The extract at 1000 µg/ml concentration showed prominent cytotoxic effect (74.66% inhibition) on HEp-2 cell lines. The IC_{50} of HEp-2 cells by *CAELE* was found to be 671.9 µg/ml. Four different concentrations of *CAELE* (10, 20, 40, and 80 mg/disc) were used to study antibacterial activity by disc diffusion and tube dilution method against gram positive and gram negative bacteria. Amongst the test bacteria *CAELE* induced highest zone of inhibition against *S. typhimurium* (20.33 ± 0.33 mm) followed by *E. coli* (19.66 ± 0.33 mm), *B. cereus* (18.33 ± 0.33 mm), *Z. mobilis* (18.00 ± 0.57 mm), *P. aeruginosa* (17.66 ± 0.88 mm) and *S. pyogenes* (17.66 ± 0.33 mm). The active phytoconstituents like alkaloids, amino acids, proteins, saponins, sterols, tannins and phenols, were found in extracts of *C. arborea*. The present investigation points out that *CAELE* can be a potential source to probable antitumour and antibacterial agent.

INTRODUCTION

Medicinal plants have been used from the early civilization onwards as a source of medicine for all type of diseases in man and animals. There has been an increasing awareness in recent years in ethnobotanical studies, because a large proportion of new medicinal compounds including chemotherapeutic agents have been discovered with the aid of ethnobotanical knowledge of their traditional uses. Cancer is one of the most dreadful disease, needs an integrated approach to treat using compound or formulations from medicinal plant through scientific developments. Cancer is a major public health burden in both developed and developing countries and claims over 6 million lives every year (Abdullaev *et al*., 2000). It is one of the 10 leading causes of death, in India. Similarly, there is continuous and urgent need to discover new antimicrobial compounds due to an alarming increase in the incidence of new and reemerging infectious diseases and development of resistance to the antibiotics in current clinical use (Bauer *et al*., 2003). Plants have great potential as antimicrobial compound and phytomolecules both with known antimicrobial properties, can be of great significance in therapeutics. The herb *Careya arborea* Roxb, belongs to the family Lecythidaceae is known by various vernacular names. In Hindi it is known as Kumbhi; in English, Wild Guava; Traditionally Stem bark of *Careya arborea* is used in the treatment of tumors, bronchitis, epileptic fits, astringents, as an anthelmintic, antidote to snake venom (Kirtikar and Basu, 1975). It is also used as remedy for diarrhoea, pyrexia and dysentery with bloody stools (Tessy *et al.* 2011).

MATERIALS AND METHODS

Collection of Plant Materials and Preparation of Extract

Careya arborea (Roxb.) leaves were collected from the forest of Patur in Akola District (M.S) and identified from expert taxonomist Dr. S. P. Rothe, Associate professor, Department of Botany, Shri Shivaji Science College, Akola (M.S.). Leaves were cut into small pieces and shade dried in the Department of Pharmacology and Toxicology PGIVAS, Akola. The shade dried leaves of *Careya arborea* were processed to get fine powder with the help of pulverizing machine. The freshly prepared powder of leaves (25 g) was immersed in hydro-ethanolic solution (40% distilled water + 60% ethanol) in a flask stoppered tightly with cotton plug and was kept at room temperature for 48 hours at 150 rpm in an orbital shaker. The filtrate thus obtained was filtered through Whatman No. 1 filter paper and transferred to rotary evaporator. The extract thus obtained was stored in airtight screw cap vials and kept in the desicator until further use in this study.

The *Careya arborea* ethanolic leaves extract is referred here after as *CAELE.*

Phytochemical Study

Phytochemical analysis (qualitative) of leaves of *Careya arborea* extracts in twelve different solvent viz. acetic acid, acetone, benzene, chloroform, distilled water, ethyl acetate, ethanol, hexane, hydro-ethanol, methanol, petroleum ether, and xylene was carried out, for the presence of the active phytochemical constituents (Roberts *et al.*, 1981).

In vitro studies on Anti-tumor Activity

Antitumor activity of *CAELE* was carried out using Vero and HEp-2 cell lines. The tumor cell lines i.e. HEp-2 (Human laryngeal epithelioma cells) and Vero cells (African green monkey kidney cells) were procured from the National Centre for Cell Sciences, (NCCS) Pune, India. Cells were cultured in flat-bottomed, 96-well tissue culture plates in Eagles's Minimum Essential Medium (HiMedia) supplemented with 10 per cent fetal bovine serum, glutamine and antibiotics (streptomycin, penicillin-G. kanamycin, amphotericin B), as per the method of Vyas and Kohli, (2002). *In vitro* cell cytotoxicity and cell viability was determined by MTT assay as per method of Mosmann, (1983). MTT (3-[4,5-dimethylthiazol-20yl]-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) reagent stored at 2-8°C in the dark. MTT dye was aseptically taken to appropriate volume for use during the experiment for prevention of contamination and placed into separate tube.

Cell viability assay

Vero cells were harvested, washed with phosphate buffer solution and 5×10^6 cells/ml were re-suspended. About 100 µl cell suspension (10^5 cells/ml) of Vero cell lines were seeded in each well and incubated at 37°C for 48 hours in 5 per cent CO_2 in CO_2 incubator as cells need time to recover and reattach (if adherent) for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions of the plant extract and further incubated at 37°C for 24 hours. The cells were viewed periodically for the appearance of punctuate, intracellular precipitate using an inverted microscope. 10 µl of MTT reagent was added to each well and the plate was incubated at 37°C for 4 hours. Curcumin and DMSO were used as positive and negative control respectively. Different concentration of test extract viz. 10, 50,100, 200, 400, 600,800 and 1000 µg/ml, reference drug and control were assayed in triplicates and the absorbance was recorded at 540 nm. The per cent inhibition (P.I.) was calculated by using following formula

P.I. = [(OD Control – OD test compound) / OD Control] x 100

The graph was plotted putting per cent inhibition of cells on Y-axis versus *CAELE* concentration on the X-axis and IC_{50} value of the extract was determined.

Antitumor assay

The HEp-2 Cells were treated with 10 µl of various concentrations of crude extract viz. 100, 200, 400, 600, 800 and 1000 µg/ml; Curcumin and DMSO were used as positive and negative control respectively, incubated at 37°C, in 5 per cent CO_2 for 24 hours. The per cent inhibition and IC_{50} of the extract was determined as described earlier.

Antibacterial Study

The *CAELE* impregnated discs of 10, 20, 40, and 80 mg/disc concentration were evaluated for antibacterial activity against six different bacteria as per the disc diffusion method described by Bauer *et al.* (1966). The amount of extract in each disc was assessed by weighing the disc before and after impregnating the extracts. Lyophilized pure bacterial strains of some common pathogenic bacteria viz. *Salmonella typhimurium* (MTCC-1251), *Streptococcus pyogenes* (MTCC-442), *Bacillus cereus* (MTCC-430), *Escherichia coli*

(MTCC-739), *Pseudomonas aeruginosa* (MTCC-2453) and *Zymomonas mobilis* (MTCC-91) were procured from the Microbial Type Culture Collection and Gene bank, Institute of Microbial Technology Chandigarh (MTCC) India. They were sub-cultured and maintained on nutrient broth in the department of Veterinary Pharmacology and Toxicology. PGIVAS, Akola and were employed for screening antibacterial activity.

Disc diffusion test and MIC (minimum Inhibitory concentration) Evaluation

The lowest concentration of the extract that inhibited the growth of bacteria *in vitro* referred as MIC. Therefore MIC of *CAELE* determined spectrophotometerically observing optical density as per the method described by Pelczar *et al*., (1986) involving tube dilution technique. A set of test tubes containing bacterial culture added with increasing amount of extract (1, 1.5, 2, 2.5, 3 and 3.5 mg/ml). In each set one test tube was kept as bacterial control (no extract) and other test tubes as extract control. The bacterial control and respective bacterial culture added with graded amount of the extract with the extract control were incubated at 37°C for 24 hrs. The concentration of the extract of *Careya arborea* inhibiting growth of bacteria was assessed spectrophotometrically at 490 nm. The absence of growth or change in turbidity was assessed by measuring Optical Density (OD). Thus, the MIC of the extract that prevents the growth of bacteria *in vitro* was determined.

Statistical Analysis

Statistically interpreted the data of present investigations employing Randomized Block Design, as per Snedecor and Cochran (1967).

OBSERVATIONS AND RESULTS

Phytochemical Study

The results of Phytochemical analysis are depicted in Table 1. The alkaloids, amino acids, proteins, saponins, sterols, tannins and phenols, were found in the twelve different solvent extracts of *C. arborea.*

Table 1: Phytochemical analysis (qualitative) of *CAELE*

Solvent Used	Active Principles										
	Alkaloids	Flavonoids	Anthra-quinones	Amino Acids	Proteins	Saponins	Tanins	Sterols	Reducing sugars	Glycosides	Phenolics
Acetic Acid	+	+	-	+	+	-	-	-	-	-	+
Acetone	-	+	-	+	+	+	-	+	-	-	+
Benzene	+	-	-	+	+	-	-	+	-	-	+
Chloroform	+	-	-	+	+	+	+	+	+	+	+
Ethyl Acetate	-	+	-	+	+	+	-	-	+	-	+
Ethanol	+	+	-	+	+	+	+	+	-	-	+
Hexane	+	+	-	-	-	+	+	+	+	+	+
Hydrothanol	+	+	-	+	+	+	-	-	-	+	+
Methanol	+	-	-	+	+	-	-	+	+	+	+
Petroleum Ether	+	+	-	-	-	+	+	+	+	+	+
Xylene	+	+	-	+	+	+	+	+	-	+	+
Water	+	+	-	+	+	+	+	+	-	-	+

Antitumor Study

The *in vitro* antitumor activity of *CAELE* at different concentration was ascertained using Vero and HEp-2 cell lines by MTT assay. The reduction of MTT into blue formazan was quantified by measuring absorbance at 540 nm by ELISA reader and antitumor activity and IC_{50} of Vero and HEp-2 was determined based upon per cent inhibition i.e. cytotoxicity.

Cell viability assay

Table 2 shows mean absorbance and per cent inhibition by *CAELE* and curcumin against Vero cell lines. After 24 h, the highest growth inhibitory rate of Vero cells exposed to extract (1000 µg/ml) was 63.47 per cent and that of standard drug curcumin (100 µg/ml) was 75.79 per cent.

Table 2: Effect of *CAELE* and curcumin on OD values and percent inhibition of Vero cells in MTT assay

Sr. No.	Test comp	Conc. (µg/ml)	Absorbance (Mean ± SE)^m	Per cent Inhibition (%)
1	*CAELE*	100	0.394 ± 0.02	16.74
2		200	0.374 ± 0.001	20.96
3		400	0.263 ± 0.01	44.27
4		600	0.225 ± 0.01	52.35
5		800	0.173 ± 0.01	62.62
6		1000	0.177 ± 0.009	63.47
7	Curcumin	10	0.357 ± 0.01	24.55
8		20	0.335 ± 0.03	29.2
9		40	0.315 ± 0.03	33.42
10		60	0.252 ± 0.01	46.62
11		80	0.188 ± 0.002	60.16
12		100	0.114 ± 0.006	75.79
13	DMSO	0.5%	0.473 ± 0.04	-

m - Mean of three observations

The graph is plotted with the extract concentration versus the per cent inhibition i.e. per cent of cell death. The IC_{50} of the plant extract and standard drug curcumin calculated was 640.4 µg/ml and 60.63 µg/ml, respectively.

Antitumor assay

Table 3 shows mean OD and per cent Inhibition of *CAELE* and curcumin against HEp-2 cell lines. After 24 h, the highest growth inhibiton of HEp-2 cells exposed to extract (1000 µg/ml) and standard drug curcumin (100 µg/ml) was 74.66 per cent and 80.64 per cent, respectively.

Table 3: Effect of *CAELE* and curcumin on OD values and per cent inhibition of HEp-2 cells in MTT assay

Sr. No.	Test comp	Conc. (µg/ml)	Absorbance (Mean ± SE)^m	Per cent Inhibition (%)
1	*CAELE*	100	0.578 ± 0.07	5.6
2		200	0.475 ± 0.01	22.51
3		400	0.459 ± 0.02	25.07
4		600	0.392 ± 0.03	36.05
5		800	0.259 ± 0.02	57.64
6		1000	0.155 ± 0.02	74.66
7	Curcumin	10	0.425 ± 0.01	30.67
8		20	0.357 ± 0.02	41.76
9		40	0.304 ± 0.01	50.35
10		60	0.258 ± 0.009	57.86
11		80	0.199 ± 0.01	67.43
12		100	0.118 ± 0.008	80.64
13	DMSO	0.5%	0.613 ± 0.03	-

m - Mean of three observations

The inhibitory effect of *CAELE* in HEp-2 cells is shown in Table 3. The CAELE was found to have remarkable cytotoxic activity against HEp-2 cells but the activity was comparatively lesses than the standard drug curcumin employed in this assay. The IC_{50} determined graphically was 640.4 µg/ml and 671.9 µg/ml for Vero and HEp-2 cell lines.

Antibacterial activity

Amongst the test bacteria *CAELE* induced highest zone of inhibition against *S. typhimurium* (20.33 ± 0.33 mm) followed by *E. coli* (19.66 ± 0.33 mm), *B. cereus* (18.33 ± 0.33 mm), *Z. mobilis* (18.00 ± 0.57 mm), *P. aeruginosa* (17.66 ± 0.88 mm) and *S. pyogenes* (17.66 ± 0.33 mm) indicating these bacteria to be moderately sensitive to the extract. Standard reference drug ciprofloxacin (5 µg/disc) showed highest sensitivity against all pathogenic bacteria Table 4.

Table 4: Zone of inhibition by *CAELE* and reference standard drug (ciprofloxacin) against different bacteria

CAELE (mg/disc) /drug	Zone of inhibition (mm) ± SE[m]						
	S. typhimu-rium	*S. pyogenes*	*B. cereus*	*E. coli*	*P. aerugi-nosa*	*Z. mobilis*	Pooled mean
10	13.66 ± 0.33	13.00 ± 0.57	13.33 ± 0.33	12.33 ± 0.33	13.66 ± 0.33	12.66 ± 0.33	13.10[e] ± 0.37
20	14.66 ± 0.66	14.33 ± 0.66	15.33 ± 0.33	15.00 ± 0.57	15.33 ± 0.33	14.66 ± 0.33	14.88[d] ± 0.48
40	17.66 ± 0.33	16.66 ± 0.33	16.33± 0.33	16.66 ± 0.66	17.00 ± 0.57	15.57 ± 0.57	16.64[c] ± 0.46
80	20.33 ± 0.33	17.66 ± 0.33	18.33 ± 0.33	19.66 ± 0.33	17.66 ± 0.88	18.00 ± 0.57	18.60[b] ± 0.46
Pooled mean	16.57 ± 0.41	15.41 ± 0.47	15.83 ± 0.33	15.91 ± 0.47	15.91± 0.52	15.22 ± 0.45	
Cipro-floxacin	35.66 ±0.33**	38.0 ±0.57**	36.33 ±0.88**	37.33 ±0.88**	36.66 ± 0.66**	33.33 ± 0.33**	36.21[a]± 0.60

[m] = mean of three observations
Means with different superscripts are significantly different
**significantly higher (P<0.01) than extract

Minimum inhibitory concentration

MIC of hydro-ethanolic leaves extract of, *S. typhimurium, S. pyogenes, B. cereus, E. coli, P. aeruginosa, Z. mobilis* was found to be at 2.0, 3.0, 2.0, 2.0, 1.5 and 1.5 mg/ml, respectively.

DISCUSSION

The preliminary phytochemical screening of *Careya arborea* showed the presence alkaloids, glycosides, saponins, tanins, sterols and phenols which might be responsible for antibacterial activity of the extract which is supported by the related reports of Cowan (1999) documented that plant rich in tannins, terpenoids, alkaloids and flavonoids have been found *in vitro* to have antimicrobial properties similarly, Kamal *et al.* (2010) reported flavonoids and phenolic compounds act as antibacterial agent against many pathogens. Gallic acid exhibited antimicrobial activity against *S. aureus*, *B. cereus*, *E. coli* and *C. albicans* (Panizzi *et al*., 2002). In present study the antimicrobial potential of the *C. arborea* might be due to presence of phenolic acid like gallic acid and other phytoconstituents present in the crude extracts of *C. arborea.*

From the above result, *CAELE* showed pronounced antitumour activity against HEp-2 cells at concentration 1000 µg/ml (74.66 %), however, at this concentration *CAELE* showed 63.47% inhibition against Vero cell line indicating comparatively lesser inhibition in normal cells and the antitumour and antiproliferative activity against tumour cells at this concentration. From the literature reviewed there is no previous work reported on antitumour activity of leaves of *C.arborea*, Subhadradevi *et al.* (2010) had also reported apoptotic and cytotoxic activities of methanolic extract of bark of *Careya arborea* against DLA, EAC and L929 cell lines. In preliminary phytochemical analysis saponins, sterols and phenols (flaovnoids) found to present in *CAELE* which might be responsible for anticancer activity of the extract.

From the above results it is apperent that the *CAELE* had moderate broad spectrum antibacterial activity against both Gram positive and Gram negative bacteria employed in this study. From the literature reviewed there is no previous work reported on antimicrobial activity of leaves of *Careya arborea.*

CONCLUSION

Careya arborea showed prominent antitumor activity against tumor cells and moderate to mild antibacterial activity against test pathogens indicates the possible use of leaves of *Careya arborea* in the control of enteric infections and some degenerative diseases such as cancer which also supports the traditional claim of this plant made in ethnomedicines and by folk practitioners. It is therefore recommended that more work be conducted to optimize and test bioactive compounds which could ultimately lead to inclusion of these compounds for possible pharmaceutical applications.

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Place of work
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