Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(1); 113-117

ISSN: 0975-4873

Research Article

In vitro Cytotoxic and Antibacterial Activity of Various Flower Extracts of *Couroupita Guianensis*

*Pusapati Madan Ranjit[1], V.Harika[1], Soumya. M, Y.A.Chowdary[1], K.Phanikumar[2], T.Bhagyasri[1], B.Sunitha[1], Girijasankar Guntuku[3]

[1]NRI College of Pharmacy, Pothavarappadu (V), Agiripalli Mandal, Krishna District, A.P, India.
[2]ANU College of Pharmaceutical Sciences, Nagarjuna Nagar, Nagarjuna University, Guntur (Dist.), A.P, India.
[3]University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, AP, India.

Available online: 1st March 2014

ABSTRACT

Aim: The present study was focused on the screening of the phytochemical, *in vitro* cytotoxic and antimicrobial activities of various flower extracts [ethanolic (ECG), Ethyl acetate (EACG) and aqueous (ACG)] of *Couroupita guianensis* Aubl. (Family: Lecythiadaceae).

Method*:* Preliminary phytochemical analysis of extract revealed the presence of alkaloids, flavonoids, carbohydrates, glycosides, steroids and terpenoids. *In vitro* cytotoxic activity was performed against MCF-7 cell lines by using MTT assay. All these extracts (12.5, 25, 50, 100 and 200µg/ml) in dose manner showed a significant percentage inhibition of cancer cells. Antimicrobial activity was performed using cup-plate method at concentration of 50µg/ml, 100µg/ml, 200µg/ml and 300µg/ml in dimethyl sulphoxide (DMSO) against selected strains of the National Collection of Industrial Microorganisms Sparfloxacin was used as the reference standards.

Results: The IC_{50} values of ECG (59.95), EACG (64.81), ACG (685.6) against standard tamoxifen (37.79) was determined. All extracts of *Couroupita guianensis* flowers were treated on MCF-7 cell lines which observed, both ethanolic extract (ECG), ethyl acetate extract (EACG) showed *in vitro* cytotoxic activity.Similarly all these extracts also showed good antimicrobial properties in dose dependent manner.

Conclusion: The results suggested that *Couroupita guianensis* flowers are a potent natural source of new chemical agents, further studies are needed for isolation of pure chemical constituents and establish their molecular mechanism of cytotoxic and antimicrobial properties.

Key words: *Couroupita guianensis* Aubl., flavonoids, MCF-7 cell lines, MTT assay, cup-plate method

INTRODUCTION

Couroupita guianensis. Aubl. (Family: Lecythiadaceae) commonly known as Canaball tree, Shivalingam (Hindi) and Nagalingam (Tamil) is a large tree of 20-30m height with wide spreading branches bearing a peculiar flower found throughout India. It is native to South India and Malaysia. The flowers are used to cure cold, intestinal gas formation and stomach ache. The leaves have found to show antioxidant activity, anthelmintic activity, immunomodulator and anti-nociceptive activity[1]. Traditionally various parts of this plant were used as an antibiotic, antifungal, antiseptic, analgesic, and for treating skin diseases[2]. Previous studies reported that, methanolic and aqueous extracts of leaves showed the presence of alkaloids, phenolics, flavonoids, saponins and tannins in the medicinal plant. Likewise, the fruit pulp contained alkaloids, carbohydrates, tannins, glycosides, proteins, fixed oils, steroids, triterpenes[3]**.** In case of bark, the benzene extract showed the presence of phytosterols, alcohol where as the water extract showed the presence of alkaloid, saponins, tannins and glycosides[4]. Methanolic extract of *Couroupita guianensis* root showed the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc by employing standard screening tests[1]. Both aqueous and methanol extracts of flowers are showing the presence of alkaloids, carbohydrates, glycosides phenonlic compounds like tannins, flavonoids and saponins. Benzene extract of flowers showed the presence of fixed oil and phytosterols, whereas ethanol extract showed carbohydrates, saponins and flavonoids where as water extract showed the presence of flavonoids, tannins and glycosides[5]. Hence, the present study was focused on investigating the preliminary phytochemical constituents of in various extracts, evaluating the *in vitro* cytotoxic activity of *Couroupita guianensis* flowers against MCF-7cell lines and antimicrobial evaluation of the flowers extracts.

MATERIALS AND METHODS

Collection of plant material: *Couroupita guianensis Aubl*. flowers were collected in the month of July to August from the area Rajiv Gandhi Park, Near Prakasham barrage, Vijayawada. The collected flowers were authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati. Herbarium

**Author for correspondence: E-mail: madanranjit@gmail.com*

Table 1: Showed the presence of phytochemical constituents of various extracts of flowers of *Couroupita guianensis.*

S.no.	Test for	Ethanolic extract (ECG)	Ethyl acetate extract (EACG)	Aqueous extract(ACG)
1	Alkaloids	+	+	+
2	Tannins	+	_	+
3	Flavonoids	+	+	+
4	Phenols	+	_	_
5	Carbohydrates	_	+	+
6	Glycosides	_	+	_
7	Proteins	_	_	_
8	Saponins	_	_	_
9	Sterols	+	+	_
10	Terpenoids	+	+	_

"+": Presence, "–": Absence.

Table 2: showed the Average percentage inhibition of triplicates ± SEM.

S.no.	Conc µg/ml	standard (TM)	` Average % of Inhibition ± SEMs of various extracts (ECG)	(EACG)	(ACG)
1	12.5	10.5±0.05	17.46 ±0.05	10.33± 0.023	0.63±0.05
2	25	33.6±0.23	28.36±0.11	25.3±0.023	9.73±0.04
3	50	66±0.17	41.3±0.05	46.86±0.023	19.73±0.023
4	100	81.9 ±0.41	64.16±0.02	69.16±0.046	25.7±0.023
5	200	84.9±0.17	72.73±0.41	74±0.046	27.5±0.05

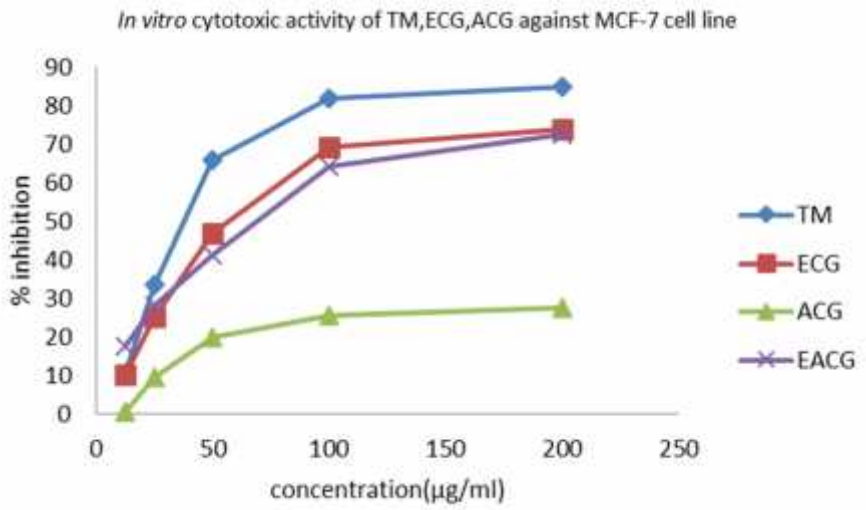

Fig 1: Anti cancer activity of TM,ECG,ACG against MCF-7 cell line at concentration 12.5, 25, 50, 100, 200 µg/ml concentrations

specimen was deposited in the department of Pharmacognosy with specimen No: 002.NRI/COL/P.COG/ (Flowers).

Preparation of extracts: The shade-dried flowers were powdered and subjected to extraction in soxhlet extractor with 70% ethanolic (ECG), Ethyl acetate (EACG) and aqueous (ACG) solvents for 48 hours and the extracts were collected. The collected extract was evaporated to dryness and stored at 4°C until use.

Preliminary phytochemical screening: Preliminary phytochemical screening of ethanolic (ECG), ethyl acetate (EACG) and aqueous (ACG) extracts using standard methods for identification of reducing sugars[6], protein[7], fats[7], resins[8],tannins[8],steroids[8],flavonoids[9],alkaloids[8],saponins[6], and phenols[10].

In Vitro Cytotoxic Activity

Cell culture: Breast cancer cells (MCF-7) were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose, 2mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

MTT assay: The MTT assay developed by Mosmann[11] was modified and used to determine the inhibitory effect of test compounds on cell growth *in vitro*. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test samples (12.5, 25, 50, 100 and

Table 3: IC_{50} Values of standrded tamoxifen (TM) and various flower extracts (ECG, ACG, EACG) against MCF-7 Cell lines at log concentration of 12.5, 25, 50, 100, 200 µg/ml. Results are expressed as mean ±SEM. *p<0.05(DUNNET'S test).

Name of cell line	Standared (TM)	IC_{50} Values ECG	EACG	ACG
MCF-7 Cell line	37.79±0.21	59.95±0.24*	64.81±0.06*	685.6±0.11*

Table 4: Showed mean inhibition zone diameter (mm) ± SEM against *Bacillus subtilis* NCIM 2063(BS), *Bacillus pumilis* NCIM 2327(BP), *Micrococcus lutes* NCIM 2871(ML), *Pseudomonas aeruginosa* NCIM 2037(PA), *Staphylococcus aureus* NCIM 20799(SA), *Escherichia coli* NCIM 2067(EC), *Proteus vulgaris* NCIM 2027(PV) by using ethanolic extracts (ECG). Sparfloxacin was used as positive control and DMSO was used as negative control (C).

S.no	Type of Strain	Inhibition zone diameter(mm)				Standard	*C*
		50µg/ml	100 µg/ml	200 µg/ml	300 µg/ml		
1	BC	12.6±0.72	17.3±0.28	21±0.48	23±0.48	28	7
2	BP	12±0.48	16.3±0.28	22.6±0.28	24.3±0.28	26	7
3	EC	14±0.48	15.3±0.28	22.3±0.28	24.6±0.28	27	7
4	PV	14.3±0.277	15.6±0.28	22±0.48	24.3±0.28	27	7
5	SA	12.6±0.277	15.3±0.28	20.6±0.28	24.3±0.28	28	7
6	BS	11.3±0.277	13.6±0.28	18.3±0.54	23.3±0.28	29	7
7	ML	13±0.48	14.6±0.28	18.6±0.28	22.3±0.28	27	7

Table 5: Showed mean inhibition zone diameter (mm) ± SEM against *Bacillus subtilis* NCIM 2063(BS), *Bacillus pumilis* NCIM 2327(BP), *Micrococcus lutes* NCIM 2871(ML), *Pseudomonas aeruginosa* NCIM 2037(PA), *Staphylococcus aureus* NCIM 20799(SA), *Escherichia coli* NCIM 2067(EC), *Proteus vulgaris* NCIM 2027(PV) by using Ethyl actate extract (EACG). Sparfloxacin was used as positive control and DMSO was used as negative control (C).

S.no	Type of Strain	Inhibition zone diameter (mm)				Standard	*C*
		50µg/ml	100 µg/ml	200 µg/ml	300 µg/ml		
1	BC	12± 0.47	14.3±0.27	18.3± 0.27	22.6±0.27	28	7
2	BP	12±0.47	16.3±0.27	18.6± 0.27	23.3±0.27	26	7
3	EC	12.3±0.27	14.6±0.27	17.3±0.27	22.6± 0.41	27	7
4	PV	12.3±0.27	15.6±0.27	17.6± 0.54	23± 0.47	27	7
5	SA	12.6±0.27	15±0.47	22.6±0.72	24.3±0.27	28	7
6	BS	12±0.27	16.6±0.27	20.3± 0.27	25± 0.47	29	7
7	ML	12± 0.27	14.6±0.27	18± 0.47	23.6±0.27	27	7

Table 6: Showed mean inhibition zone diameter (mm) ± SEM against *Bacillus subtilis* NCIM 2063(BS), *Bacillus pumilis* NCIM 2327(BP), *Micrococcus lutes* NCIM 2871(ML), *Pseudomonas aeruginosa* NCIM 2037(PA), *Staphylococcus aureus* NCIM 20799(SA), *Escherichia coli* NCIM 2067(EC), *Proteus vulgaris* NCIM 2027(PV) by using aqueous extract (ACG). Sparfloxacin was used as positive control and DMSO was used as negative control (C).

S.no	Type of Strain	Inhibition zone diameter (mm)				Standard	*C*
		50µg/ml	100 µg/ml	200 µg/ml	300 µg/ml		
1	BC	12.6±0.72	17.3±0.27	21±0.47	23±0.47	28	7
2	BP	12± 0.47	16.3±0.27	22.6±0.27	24.3±0.27	26	7
3	EC	14±0.47	15.3±0.27	22.3±0.27	24.6±0.27	27	7
4	PV	14.3±0.27	15.6±0.27	15.6±0.27	24.3±0.27	27	7
5	SA	12.6±0.27	15.3±0.27	20.6±0.27	24.3±0.72	28	7
6	BS	11.3±0.27	13.6±0.27	18.3±0.54	23.3±0.27	29	7
7	ML	13±0.47	14.6±0.27	18.6±0.27	22.3± 0.27	27	7

200µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent were used as controls. Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer. The percent cell viability was determined with respect to control, was calculated using formula.

% Viability = corrected OD of sample /Control OD * 100 and percentage of inhibition was determined by using formula, % Inhibition = 100-%viability.

Screening of Antimicrobial Activity Against Human Pathogens

Test organisms: Antibacterial activity was performed by using various extracts of flowers against selected NCIM (National Collection of Industrial Microorganisms) type bacterial stains *Bacillus subtilis* NCIM 2063(BS), *Bacillus pumilis* NCIM 2327(BP),*Micrococcus luteus* NCIM 2871(ML), *Pseudomonas aeruginosa* NCIM 2037(PA), *Staphylococcus aureus* NCIM 20799(SA),*Escherichia coli* NCIM 2067(EC),*Proteus vulgaris* NCIM 2027(PV).

Preparation of inoculums: The *in vitro* screening of antibacterial activity was carried out using Cylinder-plate assay method. For antibacterial activity, the inoculums or microbial suspension was prepared according to the procedure given in the I.P (Indian pharmacopoeia-2010).The test organism (one loop full) was seeded into the nutrient agar medium (HIMEDIA) at temperature between 40° to 50° and immediately the inoculated medium into the Petri plate (8 Inch) to give a depth of 3 to 4 mm, allowed to solidify and punched with a sterile cork borer (6.0 mm diameter) to make open cavities. Each plate should have cavities with appropriate distances.

Preparation of test and standard solutions**:** All the test sample extracts were prepared at concentration of 50µg/ml, 100µg/ml, 200µg/ml and 300µg/ml in dimethyl sulphoxide (DMSO). The stock solution of reference standard (Sparfloxacin) was prepared at the same concentration like test samples. Antimicrobial activity was screened by adding 0.05 ml stock solution to each cup by micropipette. The sparfloxacin was used as positive control (50µg/ml) and 0.05 ml of DMSO was used as negative control. Antimicrobial activity was screened by adding 0.05 ml of both test and standard solution to each cavity of the plate using micropipette. Each microbial culture was inoculated into three petri plates. All the plates were kept for 1 to 4 hours at room temperature and then incubated for about 24 hours at the incubator (33-34°C). After incubation, the bacterial inhibition zone diameters were measured and the average of inhibition zone diameters of three plates of each organism was noted.

RESULTS AND DISCUSSION

Prelimanary phytochemical constituents: Ethanolic extract of flowers showed the presences of alkaloids, tannins, flavonoids, phenols, steroids and terpernoids. Likewise ethyl acetate extract were showed the presences of the alkaloids, flavanoids carbohydrates, glycosides, steroids and terpenoids. Similarly aqueous extract showed the presence of alkaoids, tannins, flaoanoids.These results indicated *Couroupita guianensis* is a rich source of pharmacologically important chemical constituents. Some other reports also explained the phytochemical contents of the methanolic extract of flowers showed the presence of glycosides, alkaloids, tannins and flavonoids[12]. Table 1 showed the presence of phytochemical constituents of various flower extracts.

In vitro cytotoxic activity**:** All extracts of *Couroupita guianensis* flowers treated on MCF-7 cell lines observed that, both ethanolic extract (ECG), Ethyl acetate extract (EACG) showed *in vitro* cytotoxic activity. Table 2 showed percentage inhibition of cancer cell with tamoxifen (TM), ethanolic extract (ECG), aqueous extract (ACG), and ethyl acetate extract (EACG). Figure 1 showed the comparison of percentage inhibition of cancer cell against tamoxifen. Table 2 showed the percentage inhibition (Average of the triplicate and SEM) value of ECG is 17.46 ± 0.05 at 12.5µg/ml and 72.73 ± 0.41at 200µg/ml. Likewise; the percentage inhibition (Average of the triplicate and SEM) value of EACG is 10.33± 0.023 at 12.5µg/ml and 74±0.046 at 200µg/ml. Similarly the Percentage of inhibition of cancer cells with ECG and EACG was less when compared with standard tamoxifen. Tamoxifen showed the percentage of inhibition 10.5 ± 0.05 at 2.5µg/ml and 84.9 ± 0.17 at 200µg/ml, but aqueous extract showed very less percentage inhibition of cancer cell (27.5 ± 0.05 at 200µg/ml). Table 3 showed the comparison of IC_{50} values of ECG (59.95), EACG (64.81), ACG (685.6) against standard tomaxifen (37.79) were determined by using Graph pad prism. Version 6.

The above results indicated that, both ethanolic and ethyl acetate extracts of *Couroupita guianensis* flowers showed *in vitro* cytotoxic activity against MCF-7 cell lines, when compared with standard tamoxifen, whose cytotoxic activity is less. This may be due to its crude form. Cytotoxic properties observed may be due the presence of a number phenolic chemical constituents in the extracts. Prior reports suggested that flavonoids and phenolic constituents have exhibited antineoplastic activity[13]and antioxidant activity[14].

Antimicrobial activity against human pathogens: *Couroupita guianensis* flowers extracts (ethanolic, aqueous and ethyl acetate) showed dose dependent inhbition zone diameter.Table 4, 5, and 6 showed that mean inhibition zone diameter (mm) ± SEM against *Bacillus subtilis* NCIM 2063(BS), *Bacillus pumilis* NCIM 2327(BP), *Micrococcus luteus* NCIM 2871(ML), *Pseudomonas aeruginosa* NCIM 2037(PA), *Staphylococcus aureus* NCIM 20799(SA), *Escherichia coli* NCIM 2067(EC), *Proteus vulgaris* NCIM 2027(PV) by using ethanolic extracts (ECG). Sparfloxacin was used as a positive control and DMSO was used as a negative control (C).The results confirmed that all these extracts have good antimicrobial properties against both gram positive and negative bacteria.

CONCLUSION

Isatin was isolated from the chloroform extract of flowers of *C. guianensis* showed antioxidant and anticancer activities against human promyelocytic leukemia (HL60) cells[15]. This is one of the advience for cytotoxic properties of *Couroupita guianensis.* Further studies are required to isolate the active components of ethanolic (ECG), aqueous (ACG), and ethyl acetate (EACG) extracts responsible for both anticancer and antimicrobial activity.

ACKNOWLEDGEMENTS

The authors are thankful to the management and principal, NRI College of pharmacy, Pothavarappadu, Agiripalli for providing the research facilities and financial support (Ref no. NRIP/2012/Project-27) to carry out this research work.

REFERENCES:

1. Mariana MG. Pinheiro, Sidnei O. Bessa, Catharina E. Fingolo,Ricardo M. Kuster, Maria Eline Matheus, Fábio S. Menezes and Patrícia Dias Fernandes Antinociceptive activity of fractions from *Couroupita guianensis Aubl leaves*. *Journal of Ethnopharmacology* 2010; 127 (2): 407-413.
2. The useful plants of India, Publication of information directorate, CSIR, New Delhi
3. Kavitha R, Kamalakannan P, Deepa T, Elamathi R, Sridhar S, Suresh Kumar J. In vitro Antimicrobial Activity and Phytochemical Analysis of Indian Medicinal Plant *Couroupita guianensis Aub*l. J. *Chem. Pharm. Res.*, 2011, 3(6):115-121.
4. Pradhan D, Panda PK, Tripathy G. Evaluation of Immunomodulatory activity of the methanolic extract of *Couroupita guianensis Aubl* flowers in rats. *Natural product radiance* 2009; 8(1): 37-42.
5. M. Geetha, AK. Saluja, MB. Shankar, RS. Mehta. Analgesic and anti-inflammatory activity of *Couroupita guianensis Aubl*. Journal of Natural Remedies 2004; 4/1; 52 –55.
6. Brain KR. and Tuner TD. The practical evaluation of phytopharmaceuticals. *Wright Scientectica Publishers, Bristo*l 1975; 57-58.
7. Debela A. Manual for Phytochemical Screening of Medicinal Plants. *Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia* 2002; 35-47.
8. Cuilel I. Methodology for the analysis of vegetables and drugs. Chemical Industry Division, *NNIDO Romania* 1994; 24-67.
9. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum books limited, Ibadan, Nigeria 1993; 220-224.
10. William C Evan. Trease and Evans Pharmacognosy. Elsevier limited 16th Indian edition: 2009.
11. osmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*1983; 65; 55.
12. Gupta VH, P, Wankhede SS, Gunjal MA, Juvekar AR Antidepressant like effect of *Couroupita guianensis aubl*. Flowers in animal model of depression, International Journal of *Toxicological and Pharmacological Research* 2012; 4(2):12-16
13. Kim HK, Namgoong SY, Kim HP. Biological actions of flavonoids-I. *Arch Pharmacol Res* 1993; 16:18-27.
14. Pier-Giorgio Pietta, Flavonoids as Antioxidants. *J. Nat. Prod*. 2000; 63:1035 1042.
15. Mariappan Premanathan , Srinivasan Radhakrishnan , Kumarasamy Kulangiappar., et al, Antioxidant & anticancer activities of isatin (1H-indole-2,3-dione), isolated from the flowers of Couroupita guianensis Aubl., *Indian J Med Res* 2012; 136,822-826.