Vol 5 | Issue 3| 2014 | 158-164.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

INVITRO ANTICANCER AND ANTIOXIDANT ACTIVITY OF THE MEDICINAL PLANT *COUROUPITA GUIANENSIS*

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ABSTRACT

Couroupita guianensis is one of the medicinal plants which is native to South India and Malaysia, is commonly known as Nagalinga pushpam in Tamil. In traditional medicine, the leaves of this plant have been used in the treatment of skin diseases, stomachache, and intestinal gas formation, antithrombotic and vasodilatory actions. In the present investigation, In-vitro anti-cancer and In-vitro antioxidant activity were evaluated using the flower extract of the medicinal plant *Couroupita guianensis. In vitro* anti cancer activity was tested against the cancer cell lines *viz*, Caco2 (Human epithelial colorectal adenocarcinoma), MCF-7(Human Breast carcinoma), A-431(Human skin epidermis carcinoma), and HeLa (Cervix adenocarcinoma) using MTT assay. *In vitro* antioxidant activity was tested using free radicals viz., 2,2-diphenyl-1–picrylhydrazyl (DPPH)-radical, Nitric oxide, Hydroxyl radical. The MECG displayed potential anticancer activity (80.63±0.6%) at 1000 µg concentration against the Caco 2 human epithelial colorectal adenocarcinoma and the CTC_{50} value was recorded as 367.93 µg/ml. The anticancer activity was recorded 79.15±0.9 % against MCF-7(Human Breast carcinoma) and the CTC_{50} value was found to be 156.67 µg/ml. Against A-431(Human skin epidermis carcinoma) the compound showed 77.24±1.9% activity at the concentration of 1000 µg/ml and in the meantime the CTC_{50} value was found 166.67 µg/ml. Moderate anticancer activity 59.39±0.3% was recorded against HeLa Cervix cancer cell line at the concentration of 1000 µg/ml and the CTC_{50} value was 426.67 µg/ml. The DPPH radical scavenging activity was 95.00 ± 0.49 % at 200 µg /ml concentration and the IC_{50} value was 36.70 ± 0.07 µg/ml.The hydroxyl radical scavenging activity was found 77.66 ± 0.55 % at 200 µg/ml concentrations and the IC_{50} value was found 37.27 ± 0.28 µg/ml. Nitric oxide free radical scavenging activity was 83.77 ± 1.39 % at 200 µg/ml concentrations and in the due course the IC_{50} value was found 45.25 ± 0.20 µg/ml.

Keywords: *Couroupita guianensis*, Cancer cell lines, MTT assay, Free radicals, Antioxidants.

INTRODUCTION

Medicinal plants are the gifts presented by god to the human beings for the treatment of various diseases. Each and every part of the plants including leaves, flowers, stem, roots, and seeds still being used in the treatment of various diseases with specific type of pharmacological activity. These medicinal plants mostly find application in pharmaceutical, cosmetic, agricultural and food industry. The usage of medicinal herbs in curing disease has been documented in history of all civilizations. Sumarians and Akkaidians used medicinal plants about 2600 BC in the early history of the world [1]. In the present scenario, the medicinal plants are being utilized in incredible level in countries like China, Greece and India and more impressive number of drugs has been developed. The wide levels of usage of traditional medicinal plants are mainly due to their effectiveness, less side effects and relatively low cost [2]. *Couroupita guianensis* is one of the medicinal plants which is native to South India and Malaysia, is commonly known as Nagalinga pushpam in Tamil. It grows to a height of 20 meters. Leaves are alternate, oblong up to 20 cm long, entire to slightly serrate and hairy on the veins beneath. Inflorescence is racemose, arising from the trunk and other large branches. Flowers are reddish with a yellow tinge on the outside, fragrant, with stamens borne on an overarching androphore. Fruit is a large, reddish-brown globose, 15 to 24 cm, with a woody capsule, and each containing 200 to 300 seeds [3]. The leaf of this plant has been found with potential anti oxidant activity, anthelmintic activity, immuno modulator and anti-nociceptive activity [4-6].

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The bioactive compound isatin isolated from the medicinal plant *Couroupita guianensis* showed antioxidant activity and was cytotoxic against HL60 cancer cells [7]. In traditional medicine, the leaves of this plant have been used in the treatment of skin diseases, stomachache, and intestinal gas formation, antithrombotic and vasodilatory actions [8]. The flower extracts of this plant had been screened for larvicidal activity against vector [9]. Native Amazoniam people used the infusion or teas obtained from leaves, flower and bark of *C.guianensis* to treat hypertension, tumor, pain and inflammatory processes [10].The flower extracts of the plant *Couroupita guianensis* showed potential antimicrobial activity against the fish- borne pathogens *viz*., *Vibrio alginolyticus* and *Plesiomonas shigelloides* [11].

In the present investigation we report *in-vitro* anticancer and antioxidant activity of flower extracts of the traditional medicinal plant *Couroupita guianensis.*

MATERIALS AND METHODS

Plant collection

The flowers of the medicinal plant *Couroupita guianensis*(Fig.1) (Elevation - 102 meters, latitude 8.7792^0 N, Longitude-77.4031^0 E) was collected from the temple premises of Kumararkoil located nearby to Tenkasi area, Tirunelveli District, Tamilnadu during the month of March 2013 .The flowers were brought about to the laboratory without the exposure of the plant material to scorching sun. Then the flowers were air dried under shade condition for about 10 days

Figure 1. Flowers of the medicinal plant *Couroupita guianensis*

Crude extract preparation

The powdered flowers of about 200g was teased onto the material backing unit of the soxhlet apparatus and the extraction process was carried out using methanol (boiling point 60- 80^o C) as solvent for 10 h. The extract obtained was condensed using a rotary vacuum evaporator under reduced pressure at 60^o C and the residue obtained (20g) was stored at 4 ^{o} C in a refrigerator.

ANTICANCER POTENTIAL OF MECG

Preparation of Test Solutions

The crude extract of the medicinal plant *Couroupita guianensis* was dissolved in distilled Dimethyl sulfoxide and the volumes were made up with Dulbecco's modified eagles medium supplemented with 2% inactivated Fetal bovine serum to prepare a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were followed for carrying out cytotoxic studies.

Cytotoxicity study using MTT assay

Caco2 (Human epithelial colorectal adenocarcinoma), MCF-7(Human Breast carcinoma), A-431(Human skin epidermis carcinoma) and HeLa (Cervix adenocarcinoma) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks .The mono layer cell cultures were trypsinized well and the cell count was adjusted to 1.0 x 10^5 cells/ml using Dulbecco's modified eagle medium containing 10% Foetal bovine serum . 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added in each well of the 96 well microtitre plates. After 24 h, the supernatant of the monolayer was removed and treated with 100 µl of different test concentrations of the extract of *Couroupita guianensis*. Then the plates were incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval.

After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed Formosan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of growth inhibition was calculated using the following formula and concentrations of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line [12].

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right)$$

ANTI OXIDANT POTENTIAL OF MECG

DPPH Free radical scavenging activity

The MECG was prepared in various concentrations (200-1000µg). Each concentrations were added with freshly prepared 5 ml of 0.1 Mm methanolic solution of DPPH and the reactions were carried out at 27 ^{0} C for 20 min. Then the reaction mixture was spectrophotometrically read at 517 nm [13]. The DPPH radical scavenging activity was calculated using the following formula

DPPH radical scavenging activity (%) =

(Control OD - Sample OD / Control OD) × 100.

The assay was carried out in triplicate. The IC_{50} value of the methanol extract of *Couroupita guianensis*

was calculated from the graph of inhibition percentage against sample concentration.

Hydroxyl radical scavenging activity **(HRSA)**

The hydroxyl radical scavenging activity of the methanol extract of *Couroupita guianensis* was tested as per the method of Klein et al [14]. The different concentrations of methanol extract (200-1000µg) were added with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1ml of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding ascorbic acid (0.22%) of about 0.5 ml and kept for incubation at 80-90°C for 15 min in a water bath.

After the completion of stipulated incubated time, the reaction was stopped with the addition o f 1ml of ice-cold TCA (17.5% w/v). Then three milliliter of Nash reagent was added and kept at room temperature for 15 min. The intensity of color formation in the reaction mixture was spectrometrically read at 412 nm against the reagent blank .The percentage of hydroxyl radical scavenging activity was calculated as follows.

HRSA (%) = (control OD-sample OD / control OD) × 100.

The assay was carried out in triplicate. The IC_{50} value of MECG was calculated from the graph of inhibition percentage against sample concentration.

Nitric oxide radical scavenging activity **(NO)**

Nitric oxide radical scavenging activity was measured using the method of Sreejayan and Rao [15].The prepared different concentrations of methanol extract of *Couroupita guianensis* (200-1000µg) was treated with three ml of 10mM sodium nitroprusside prepared in 0.2 M phosphate buffered saline (pH 7.4) and kept for incubation at room temperature for 150 min.

After incubation, 0.5 ml of Griess reagent was added and the absorbance of the reaction mixture was read at 546 nm. The Percentage of radical scavenging activity by MECG was calculated as follows

Nitric oxide radical scavenging activity (%) =

(Control OD - Sample OD / Control OD) x 100.

The assay was carried out in triplicate. The IC_{50} value of the MECG was calculated from the graph of inhibition percentage against sample concentration.

Determination of total phenolics

The total phenolic content of MECG was measured according to the method of Siddhuraju and Becker [16].The methanol extracts of *Coroupita guianensis* of about ten microlitre (2mg/2ml) were taken in test tubes and the volume was made into 1 ml with the addition of distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Subsequently the reaction mixture vortexed and the test tubes were kept in a dark room for about 40 min.

After the incubation time was over, the absorbance of the reaction mixture was read at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

Determination of total tannins

The tannins were estimated as per the method of Siddhuraju and Manian [17].One hundred milligrams of polyvinyl polypyrrolidone (PVPP) was taken in a test tube and was added with 1 ml distilled water .Then 1 ml of the methanol extract of *Couroupita guianensis* (2mg/2ml) was added and vortexed .The reaction was carried out at 4°C for 4 h and subsequently centrifuged at 3000 rpm for 10 min at room temperature. The supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured as mentioned above and expressed as the content of non-tannin phenolics (tannic acid equivalents) on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) =

Total phenolics (%) – Non-tannin phenolics (%)

Determination of total flavonoid content

The flavonoid content was determined as per the method of Zhishen et al [18]. A 0.5ml aliquot of appropriately (2mg/2ml) diluted methanol extract of *Couroupita guianensis* was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% NaNO2 solution. After 6 min, 10% AlCl3 solution of about 0.15 ml of was added and allowed to stand for 6 min, and was followed with the addition of 2ml of 4% NaOH solution. The final volume of the reaction mixture was made into 5ml as by adding water and mixed thoroughly. The reaction mixture was allowed to stand for another 15min.The absorbance value of the reaction mixture was carried out at 510 nm against the water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent

RESULTS

Anticancer Potential of MECG

In the present investigation invitro anti cancer activity of the medicinal plant *Couroupita guianensis* was evaluated against the cancer cell lines *viz*, Caco2 (Human epithelial colorectal adenocarcinoma), MCF-7(Human Breast carcinoma), A-431(Human skin epidermis carcinoma), and HeLa (Cervix adenocarcinoma). The anticancer activity displayed by extract of this plant was found 80.63±0.6% at 1000 µg concentration against the Caco2 human epithelial colorectal adenocarcinoma and the $CTC_{50\ value}$ was recorded as 367.93 µg/ml (Table 1). The anticancer activity was recorded 79.15±0.9 % against MCF-7(Human Breast carcinoma) and the $CTC_{50\ value}$ was 156.67 µg/ml (Table 2). Against A-431(Human skin epidermis carcinoma) the extract showed 77.24±1.9 % activity at the concentration of 1000 µg/ml and in the mean time the CTC_{50} was found 166.67 µg/ml (Table 3). Moderate anticancer activity 59.39±0.3% was recorded against HeLa Cervix cancer cell line at the concentration of 1000 µg/ml and the CTC_{50} value was 426.67 µg/ml (Table 4). The anticancer activity was recorded in a dose dependent manner in all types of cancer cell lines treated with extracts of this medicinal plant.

Table 1. Cytotoxic potential flower extracts of the medicinal plant *Couroupita guianensis* against Caco2 cancer cell line

Sl. No	Extract of the medicinal plant*Couroupita guianensis* (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	1000	80.63±0.6	367.93±1.1
	500	79.70±1.0	
	250	16.29±2.9	
	125	13.61±0.6	
	62.5	10.24±0.2	

Values are means of three independent analyses ± standard deviation (n = 3).

Table 2. Cytotoxic potential flower extracts of the medicinal plant *Couroupita guianensis* against MCF-7 cancer cell line

Sl. No	Extract of the medicinal plant *Couroupita guianensis* (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	1000	79.15±0.9	156.67±0.4
	500	68.91±0.3	
	250	56.46±0.3	
	125	49.07±0.2	
	62.5	38.01±0.5	

Values are means of three independent analyses ± standard deviation (n = 3).

Table 3. Cytotoxic potential flower extracts of the medicinal plant *Couroupita guianensis* against A-431 cancer cell line

Sl. No	Extract of the medicinal plant *Couroupita guianensis* (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	1000	77.24±1.9	166.67±1.0
	500	60.13±0.7	
	250	57.32±1.1	
	125	48.05±0.2	
	62.5	44.17±1.2	

Values are means of three independent analyses ± standard deviation (n = 3).

Table 4. Cytotoxic potential flower extracts of the medicinal plant *Couroupita guianensis* against HeLa cancer cell line

Sl. No	Extract of the medicinal plant *Couroupita guianensis* (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	1000	59.39±0.3	426.67±0.4
	500	52.77±0.2	
	250	43.86±0.9	
	125	23.50±0.1	
	62.5	17.97±0.5	

Values are means of three independent analyses ± standard deviation (n = 3)

Table 5. DPPH radical scavenging activity of flower extracts of the medicinal plant *Couroupita guianensis*

Sample Concentration (µg)	Percentage activity (%)	IC50 (µg/ml)
40	35.81 ± 0.20	36.70 ± 0.07
80	55.70 ± 0.49	
120	74.15 ± 0.45	
160	89.00 ± 0.22	
200	95.00 ± 0.49	

Values are means of three independent analyses ± standard deviation (n = 3).

Table 6. Hydroxyl radical scavenging activity of flower extracts of the medicinal plant *Couroupita guianensis*

Sample Concentration (µg)	Percentage activity (%)	IC50 (µg/ml)
40	15.05 ± 0.72	37.27 ± 0.28
80	27.00 ± 0.90	
120	45.76 ± 0.55	
160	62.49 ± 0.75	
200	77.66 ± 0.55	

Values are means of three independent analyses ± standard deviation (n = 3).

Table 7. Nitric oxide radical scavenging activity of flower extracts of the medicinal plant *Couroupita guianensis*

Sample Concentration (µg)	Percentage activity (%)	IC50 (µg/ml)
40	34.90 ± 0.80	45.25 ± 0.20
80	46.60 ± 1.05	
120	54.28 ± 1.60	
160	65.97 ± 0.52	
200	83.77 ± 1.39	

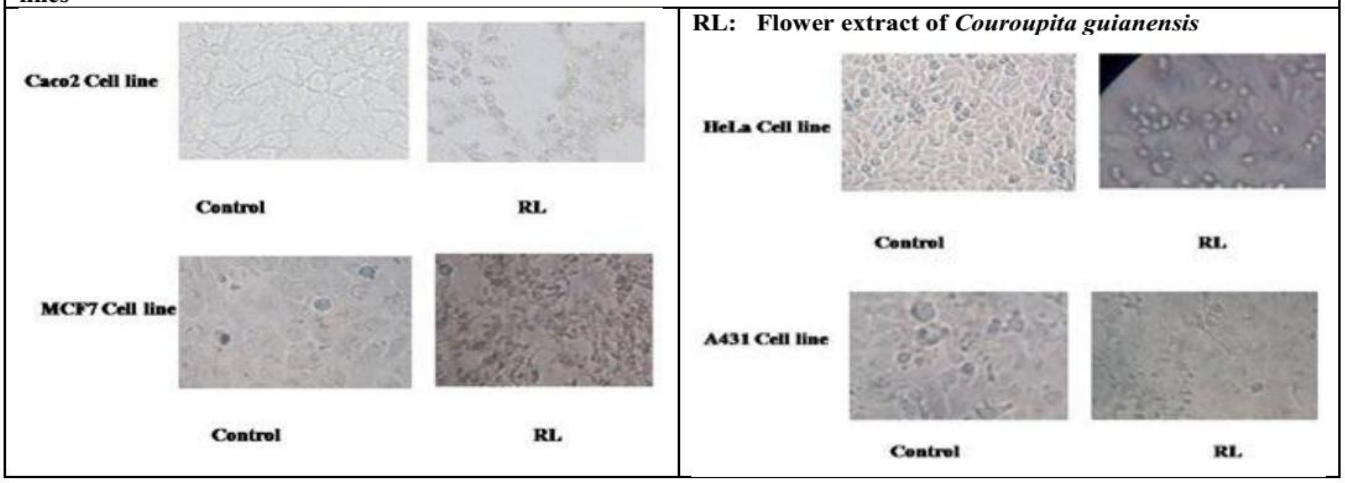
Values are means of three independent analyses ± standard deviation (n = 3).

Table 8. Total phenolics, tannin and flavonoid content of flower extracts of the medicinal plant *Couroupita guianensis*

Phytoconstituents	Content
Total phenolic (mg TAE/g extract)	55.20 ± 0.37
Tannin (mg TAE/g extract)	20.34 ± 0.92
Flavanoid (mg RE/g extract)	0.35 ± 0.02

Values are means of three independent analyses ± standard deviation (n = 6). TAE –Tannic acid equivalent
RE –Rutin equivalent

Fig. 2:- Anticancer potential of flower extract of the medicinal plant *Couroupita guianensis* against various cancer cell lines



Antioxidant Potential of MECG

The results of antioxidant activity showed the potential of the methanol extract of *Couroupita guianensis* in scavenging free radicals. The DPPH radical scavenging activity was 95.00 ± 0.49 % at 200 µg /ml and the IC_{50} value was 36.70 ± 0.07µg/ml (Table 5), The hydroxyl radical scavenging activity was found to be 77.66 ± 0.55 % at 200 µg/ml concentration and the IC_{50} value was found 37.27 ± 0.28 µg/ml(Table 6). For nitric oxide, the radical scavenging activity was 83.77 ± 1.39 % at 200 µg/ml concentrations and in the due course the IC_{50} value was 45.25 ± 0.20 µg/ml (Table 7). The antioxidant activity of MECG was recorded in a dose dependant mode. The presence of phytoconstituents tannins (20.34 ± 0.92 mg Tannic acid equivalent/ g extract) and flavonoids (0.35 ± 0.02 mg Rutin equivalent/g extract) present in the methanol extract of Couroupita *guianensis* may be the major reason for its potential antioxidant activity (Table 8).

DISCUSSION

In the present investigation the traditional medicinal plant *Couroupita guianensis* was tested for its potential anticancer, antioxidant activity. The activity displayed by methanol extract of the plant *Couroupita guianensis* was potential in all the determined assays. The anticancer activity tested against the cancer cell lines *viz.*, Caco2 (Human epithelial colorectal adenocarcinoma), MCF-7 (Human Breast carcinoma),A-431(Human skin epidermoid carcinoma), and HeLa (Cervix adeno carcinoma) using MTT assay. Excellent activity has been displayed by MECG against almost all the tested cancer cell lines which may be possibly due to the presence of terpenoids and Phenolic compounds. The Polyphenols present in herbal extracts showed potential anti-proliferative activity against various human cancer cell lines [19].Terpenoids derived from plants showed potential cytotoxic and chemo preventive effects [20,21] .The above given reports supporting the reason for the potential anticancer activity displayed by the extract of the medicinal plant *Couroupita guianensis* . The presence of phytochemicals such as phenolics and terpenoids in the flower extract of Courouptia *guianensis* was earlier reported by Ramalakshmi *et al* [22]. More over MECG showed potential anti oxidant activity in scavenging free radicals viz., 2, 2-diphenyl-1 –picrylhydrazyl (DPPH), Nitric oxide and Hydroxyl radical. This antioxidant activity

may be due to the presence of phytochemicals such as terpenoids and flavonoids.Terpenoids present in the petroleum ether extract of *C.officinalis* flowers showed potential antioxidant activity[23].Hydroxyl radicals react with all biomolecules in the cells of the living system[24].As the flower extract of the medicinal plant *Couroupita guianensis* showed potential hydroxyl radical scavenging activity ,it's expected to control the formation of cancer cells and mutation in the living system. In the gastro intestinal tract (GIT), nitric oxide takes part in the regulation of intestinal peristaltism, gastric emptying, antral motor activity [25]. However, overproduction of NO associated with the tissue injury in the gut during inflammatory reactions including peptic ulcer, chronic gastritis, gastrointestinal cancer, bacterial gastro enteritis, celiac or chronic inflammatory bowel diseases [26]. As the methanol extract of *Couroupita guianensis* displayed potential nitric oxide radical scavenging activity, the MECG would exhibit possible gastro protective activity via the modulation of NO.

CONCLUSION

In the present investigation in vitro anticancer and anti oxidant potential of flower extracts of the medicinal plant *Couroupita guianensis* was evaluated. The extract of this plant displayed potential invitro anticancer and antioxidant activity. Further research is needed to identify the exact bioactive compound responsible for the above said activity.

CONFLICT OF INTEREST

We declare that we have no conflict of interest

ACKNOWLEDGEMENT

The authors are thankful to Sri Paramakalyani College management, Alwarkurichi, Tirunelveli district, Tamilnadu for the provided research facilities in the Department of Zoology laboratory.

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