JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2021/25606

RESEARCH ARTICLE

Authenticating the Anti-cancer Properties of *Couroupita guianensis* in Western Ghats using HL60 Humanleukemia Cell Line

J. Bindhu*, R. Felicia Roshini, M. Monica Devi, Arunava Das, R. Balakrishna Raja and S. Tamilselvi

Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam – 638401, Tamil Nadu, India; bindhu@bitsathy.ac.in

Abstract

The target of this work is to evaluate the *in–vitro* anticancer activity and cellular toxicity of *Couroupita guianensis* (CG) towards the Human promyelocytic leukemia HL60 cells contrasted with the normal cell lines. The anti-cancer properties of *Couroupita guianensis* extracted with Chloroform and DMSO (Dimethyl sulfoxide) were analysed and studied throughout this paper. HL60 cell lines were permitted to develop in DMEM (Dulbecco's Modified Eagle's medium) and incubated with differed concentration of DMSO CG extract. MTT assay drew out the best approach to decide the cell feasibility and assessment was done with the optical absorbance at 570nm and 620nm as reference. The floral extract inhibited 50% growth (IC_{50}) of HL60 cell lines at 10 mg/ml of extract concentration. Inhibitory adequacy of CG showed the cell – plausibility in time and dosage relied route with predictable morphological changes. Flow cytometer assessed the apoptosis insisting the cell toxicity esteem for MTT at IC_{50} with 70 % cell suitability. Morphological investigation likewise plainly expresses that no apoptosis has been found in charge and comparably in CG treated when contrasted with destructive HL60 cell – line. Assessment of cell toxicity impact of CG demonstrated it can be an inert source of great transformation in human Acute promyelocytic leukemia indicating (revealing) that chemotherapeutic agent.

Keywords: Cellular Toxicity, Growth Inhibition, Leukemia DMSO

1. Introduction

Couroupita guianensis is a large deciduous tropical tree in Western Ghats belonging to the family of lecythidaceae which grows to a height of 35 m. The flowers have various medicinal properties which has antibiotic, antifungal, antiseptic and analgesic qualities. The flowers are usually yellow, pink and reddish in colour with sweet fragrance which grows on the trunk of the tree. These flowers are considered as most important in Buddhist culture[1]. The flowers are also called as *nagapushpam* since they resemble the *shivalingam* which is at the centre of the flower and the pollens mimic the snake structure used in *Shiva Pooja*. The stem, bark, and flowers showed antimicrobial properties. The leaves are used as antiseptic and also against toothache. The fruit pulp, bark and flowers have both antimicrobial and antifungal properties. The extract of the leaves cures the skin ailments and toothache[2]. The essential extracts from the flowers shows antibacterial and antifungal properties. It also cures the gastritis, scabies, piles, dysentery, antidote[3]. It is also used against acute promyelocytic leukemia studies. Human promyelocytic leukemia or lung carcinoma is a fatal disease worldwide. It is the most commonly occurring disease in both men and women. Human promyelocytic leukemia is the condition in which the cells divide enormously that leads to the growth of tumors that reduces the ability to breathe. The most common symptoms of lung carcinoma are uncontrollable cough that causes bleeding or rust coloured sputum, risky breathing, loss of appetite, feeling tired or week etc. Acute promyelocytic leukemia usually starts in the lining of bronchi and spreads through the respiratory tract. Human leukemia cell lines, HL-60 were widely used

*Author for correspondence

Article Received on: 06.07.2020 Revised on: 15.12.2020 Accepted on: 13.02.2021

to find the cell morphology in laboratories[4]. Genetics of human promyelocytic leukemia cell lines reveals that chromosomal changes in human Acute promyelocytic leukemia with numerical abnormality including structural aberrations, deletion and translocation. Acute promyelocytic leukemia is one of the major types of acute myeloid leukemia. The External factors such as radiation exposure, petrochemicals, and the various other solvents like benzene, herbicides, carcinogens plays a major role in human acute promyelocytic leukemia. There are some increasing results which shows that diet plays a vital role in curing human acute promyelocytic leukemia[5]. Beta carotene had the highest protective role. Lowest intake of foods with beta carotene has the highest chances of human acute promyelocytic leukemia. Surgery, radiation therapy, chemotherapy are the major diagnostic treatments given for this cancerthan the natural cure for leukemia. Many biological studies have provided cures for human Acute promyelocytic leukemia. Some are ketogenic diet, hyperbaric oxygen chamber, juicing, vitamin D, enzymes, curcumin from turmeric, detoxification etc. Plants are used in medical industries as they possess natural curative properties. Compounds that have been extracted from plants for their anticancer properties include polyphenols, brassino steroids and taxols6. The secondary metabolites such as polyphenols, flavonoids and brassino steroids have shown potential anticancer properties. They appear to have anti-carcinogenic action, cell reinforcement action, concealment of disease cell development, incitement of apoptosis, target specificity, malignancy cytotoxicity. Anticancer properties of plants are successful inhibitors of cancer cell growth cell lines. Abuse of these agents should be kept up with demands and be sustainable7. The significant compounds responsible to treat against human Acute promyelocytic leukemia were explored through GCMS.

2. Materials and Methods

2.1 Identification and Handling of Sample

2.1.1 Plant Material

The samples of *Couroupita guianensis* were collected from the Bannari Amman institute of technology campus, Sathyamangalam and brought to the laboratory. The flowers were washed and cut into small pieces. Later the chopped floral parts were wilted in a hot air oven at 45°C for 48 hours. Then the wilted samples were turned to fine powder by using a blender and stocked in the air tight storage container.

2.1.2 Preparation of the Floral Extract

The obtained sample powder of about 10 g was packed in a filter paper. Then the Soxhlet's extraction was carried out at 65oC for 6 cycles and the solvent used was the chloroform with water (1:10) of 300 ml. After extraction was completed the extract was concentrated to 25 ml through steam distillation process. The steam distillation was preferred over other methods since the sample can be reused again for finding other non-volatile compounds.

2.1.3 Preliminary Qualitative Phytochemical Screening

The presence or absence of the phytochemical constituents were analyzed using the following procedures[9].

2.1.4 Test for Proteins: Biuret's Test

The floral extract of 1ml was added with 2% NaOH and 0.3% of CuSO4. The appearance of pink colour showed the presence of protein[8].

2.1.5 Test for Tannins: Ferric Chloride Test

The floral extract of 1ml and 5%ferric chloride were mixed together and the green colour occurrence revealed the existence of tannins[8].

2.1.6 Test for Saponins: Foam Test

The floral extract was agitated vigorously with 5 ml distilled water, the persistence of foam even after 15 minutes revealed the existence of saponins[8].

2.1.7 Test for Flavonoids: Alkaline Reagent Test

1ml of the floral extract was made to mix well with 1ml of 2N NaOH and the yellow colour formation proved the presence of flavonoids[8].

2.1.8 Test for Alkaloids: Mayer's Test

The floral extract of 1ml and 2ml of conc.HCl were mixed together and then few drops of Mayer's reagent was added. The white colour precipitate showed the presence of alkaloids[8].

2.1.9 Test for Glycosides: Borntrager's Reagent

1 ml of the floral extract was added to 3 ml chloroform and 10% ammonium solution. The pinkish red colour appearance indicates the presence of glycosides[8].

2.1.10 Test for Terpenoids

1ml of floral l extract and 2ml of chloroform were mixed

together and then few drops of conc.H_2SO_4 was added. The existence of terpenoids was confirmed by the formation of red brown colour at the interface[8].

2.1.11 Test for Phenols: Ferric Chloride Test

1 ml of floral extract and 2ml of distilled water were mixed together and then few drops of 10% ferric chloride was added. The existence of phenols was confirmed by formation of green colour[8].

2.1.12 Test for Steroids

To 1ml of the floral extract equal volume of acetic acid and choloroform was added followed by a few drops of con.H_2SO_4. The appearance of violet brown ring indicates the presence of steroids[8].

2.1.13 Test for Lipids

1 ml of the floral extract was added with 0.1N alcoholic potassium hydroxide along with the drops of phenolpthalein which is boiled in the water bath for 1 hour. The existence of soapy appearance indicates the existence of lipids[8].

2.1.14 GC-MS Analysis

The Bio-chemical nature of the floral sample was assessed through Gas chromatography mass spectrometry. The analysis was carried out using GC–MS Perkin Elmer model: Clarus 680 which is equipped with mass spectrometer Clarus 600 (EI) and analyzed using Turbo Massver 5.4.2 software. The Fused silica capillary column which is covered with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane 30 m × 0.25mm ID × 250µm df). The constant flow rate is about 1ml/min and carrier gas such as helium was used to separate the components[10]. The temperature of the injector was kept at 260^{0}C while performing the experiment. The extract sample of 1µl was injected into the equipment and the temperatures of the oven were 60^{0}C for about 2 minutes followed by 300^{0}C at the rate of 10^{0}C min-1, and 300^{0}C for 6mins. The conditions of the mass detector were: the temperature of transfer line was 240^{0}C and ionization mode electron impact at 70eV, the duration time of search interval is 0.2sec and the search interval is 0.1 sec. The fragments were from 40 to 600Da. The spectrum of components was relating to the information of the spectrum of previously build up components assembled in the GC-MS NIST (2008) library[11].

2.1.15 Antibacterial Assay

The antibacterial assay of *Couroupita guianensis* was performed against five-gram positive bacteria such as *Listeria monocytogenes, Bacillus subtilis, Streptococcus agalactiae, Staphylococcus aureus, Streptococcus faecalis* and 3-gram negative bacteria like *Escherichia* coli, *Klebsiella oxytoca, Klebsiella aerogenes* by following Agar well diffusion method These bacteria was used to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections[12]. The following bacteria were inoculated in the sterile nutrient plates individually in triplicates by swabbing technique[13]. The wells in diameter of 3.5mm were made using the sterile agar well puncher. The flower extract sample was diluted at varied concentration (20%, 40%, 60%, 80%, 100%) using distilled water. Then the agar plates were placed in an incubator at ±37oC. After 24 hours of incubation, the zone of incubation was measured in mm[14].

2.1.16 Antioxidant Activity

The antioxidant activity of the extract was determined by 2, 2-Diphenyl- 1-picrylhydrazyl (DPPH) assay. Both Samples and Standards (Ascorbic acid) were taken in different concentrations and the volume was adjusted to 100µl using methanol. The DPPH solution of 0.1 mM was prepared by using methanol as solvent. About 3ml of 0.1 mM DPPH solution was mixed with samples of different concentrations and the negative control was prepared by adding 100 µl of methanol to the 3ml of solution. The tubes with mixtures were made to stand in dark at surrounding temperature for 30 min[15]. The shading change from violet to yellow shows the nearness of cancer prevention agents and the evaluation was by estimating its absorbance at 517nm against the clear. The IC50 esteem (inhibitory focus) was determined for both example and standard. The level of hindrance was determined utilizing the accompanying recipe:

$$\% \text{ of hindrance} = [A0\text{-}A1/A0] * 100$$

Where A0 is absorbance of control (for example DPPH arrangement without test) and A1 is absorbance of test or on the other hand standard (for example DPPH arrangement with test/standard)[16].

2.1.17 Cytotoxic Assay (MTT Method)

Anticancer assay was proceeded according to ISO 10993:5. HL60 (Human promyelocytic leukemia) cell line was treated with these compounds at one essential cytotoxic assay dose of 100µM for 48 h (MTT anticancer test). MTT assay provides about both cell death and viability. In the current procedure, all cell lines were pre-

incubated on a microtiter plate.The after-effects of each test were seen as the advancement level of treated cells which stood out from untreated control cells. A 0.1 mL aliquot of the cell suspension (5 × 106 cells/100 µl) and 0.1 mL of the test solution (10-50 µl) were added to the wells, with the plates kept for incubation (5% CO^2) at 37 °C for 18 h. After 18 h, 1mg/ml of MTT was included, and the plates were kept in the CO^2 hatchery for 4h, followed by including propanol (100µl). The plates were covered with the aluminum foil to shield them from light and therefore simultaneously agitated in the shaker for 10–20 min, from that point the well plates were set up on an ELISA reader to get absorption information at 517 nm[17].

Cytotoxicity = [(Control- Treated) / Control] * 100
Cell viability = (Treated /Control) * 100

3. Results and Discussion

Researches have shown that fruit, bark, stem and leaves of cannonball tree are effective for the treatment of High Blood Pressure, Malaria, Stomach Ache, Tumours, Inflammation, Pain, Toothache, Wounds etc., They are used as Analgesic, Antifungal, Antimalarial, Antiseptic, Local Antibiotic, Anti Inflammatory and Anti-cancerous agents. The effective nutrients that are present in cannonball tree are Couroupitone, Henolic Substances, Isatin, Volatile Oil, Glycosides, Indirubcin and Keto Steroids. Chewing of cannonball leaves helps in curing of toothache. The flesh of cannonball fruit acts as a local curative for wounds. The leaf extract treats skin diseases. Unripe fruit is harmful and might cause an allergic reaction. The isatin present in flower of *Couroupita guianensisis* known to have cancer cell treating properties and anti- cell poisoning properties against carcinoma cell lines[10]. This compound has a significant chemotherapeutic agent. They show anticancer activity and cytotoxicity against HL60 cells. The leaf extract was filtered against six different human pathogenic bacteria and four fungal strains. This shows the antimicrobial activity if the plant. The anti-ulcer activity of the plant is reported by Elumalai et al says that the ethanolic extract of *Couroupita guianensis* inhibhits the gastric lesions formation. Anti-inflammatory activity is reported by Pinheiro et al as the ethanolic extract was able to suppress the leucocyte migration into the pheripheral cavity. Stigma sterol and flavonoids present in the extract has antioxidant activity which decreases the time of wound healing process.

The phytochemical studies revealed the existence of proteins, tannins and phenols, glycosides, Alkaloids and flavonoids whereas it shows the absence of saponins, Triterpenoids, steroids, terpenoids in aqueous extract. Triterpenoids and terpenoids were absent in the ethanol extract whereas all others are present. The results were shown in Table 1. The glycosides have Anti-inflammatory, anti-cancer and immune boosting properties. The alkaloids have antimicrobial properties whereas the flavonoids have anti-oxidant properties[17].

Table 1. The phytochemical constituents of *Courou pita guianensis*

Phytochemical constituents	Presence
Tannins	+
Saponins	+
Lipids	+
Flavonoids	+
Alkaloids	+
Glycosides	+
Proteins	+
Terpenoids	-
Phenols	+
Steroids	+

(+ present, - absent)

The results of the GCMS showed the components present in the extract are shown in the Table 2. The molecule 2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-Hexamethyl-(All-E) showed maximum peak which is followed by Hexatriacontane, Octacosane, Tritetracontane, Hexatriacontane 1-chloro showed highest peaks[11]. These compounds were found to have several antimicrobial, anti-tumour, immuno stimulant and cancer preventive properties. Other than the molecule Hexatriacontane and Heptacosane1-chloro, all other molecules contains oxygen group. The presence of oxygen may be the reason for anti-oxidant properties.

The antimicrobial properties were performed to identify the presence of antibacterial activity in the floral extracts. The disc diffusion method was performed to carry out to find the antibacterial properties[16]. Disc containing 5 mg of floral extracts were made by immersing the disc in floral extracts that were priorly dissolved in pure solution of Dimethylsulfoxide (DMSO). Then the discs were moved onto the plates and diameter of the inhibition zone around the disc reveals the antibacterial activity. The clear region around the disc shows no bacterial growth and this is the zone of inhibition. The variety of bacterial strains used to perform the antibacterial activity are *Listeria monocytogenes, Bacillus subtilis, Streptococcus agalactiae, Staphylococcus aureus, Streptococcus faecalis, Escherichia*

coli, Klebsiella oxytoca, Klebsiella aerogenes . Among these strains *Listeria monocytogenes* and *Streptococcus faecalis* have shown highest susceptibility to the floral extract. The bacteria such as *Escherichia coli, Klebsiella oxytoca, Klebsiella aerogeneshas* exhibited moderate sensitivity. The bacterial species *Streptococcus agalactiae* showed

Table 2. GCMS analysis result for major phytocomponents in *Couroupita guianensis* extract

SI.No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structure
1	18.67	N-Hexadecanoic acid	C16H32O2	256	6.305	
2	19.94	9,12-Octadecadienoic acid	C18H32O2	280	21.35	
3	20.64	Hexatriacontane	C36H74	506	3.015	
4	21.37	Tritetracontane	C43H88	604	3.381	
5	22.19	Heptacosane	C27H56	380	2.302	
6	22.26	Heptacosane,1-chloro-	C27H55Cl	414	1.572	
7	22.95	Hexatriacontane	C36H74	506	4.15	
8	23.64	Hexatriacontane	C36H74	506	4.41	
9	24.31	Tetratetracontane	C44H90	618	4.263	
10	24.46	2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-Hexamethyl-(All-E)-	C30H50	410	28.94	
11	25.02	Tritetracontane	C43H88	604	4.301	
12	25.66	Octacosane	C28H58	394	4.225	
13	26.3	Octacosane	C28H58	394	3.941	
14	26.91	Hexatriacontane	C36H74	506	3.569	
15	27.57	Pentacosane	C44H90	618	2.23	
16	28.34	Heptacosane,1-chloro-	C27H55Cl	414	2.051	

lower susceptibility. The zone of inhibitions is represented in the Figure 2.

The DPPH results indicate the high number of antioxidants were present in the sample. The IC50 value for both standard (Ascorbic acid) and sample (floral extract) were measured from the graph reveals that standard has 400.2µg/ml and the sample have 604µg/ml[16]. The obtained values are represented in Figure 3.

The MTT results showed that the floral extract has shown mild effects over the HL60 cancer cells. The cell toxicity activity of the concentrates of *Couroupita guianensis* on HL60 cells from human promyelocytic leukemia was investigated using in vitro 3-(4) 5-Dimethyl-thiazol-Zyl) - 2,5 biphenyl tetrazolium bromide (MTT) dye. The outcomes demonstrated the diminished cell suitability and cell development hindrance in a dose dependent manner[17]. Cytotoxicity activity of DMSO *Couroupita guianensis* extracts were carried out against HL60 cell line at different concentrations to determine the IC_{50} (50% growth inhibition) by MTT assay. Results

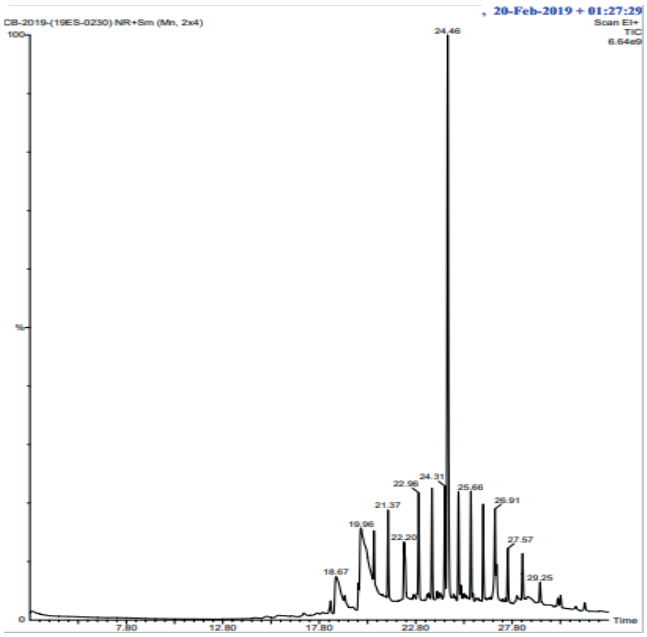
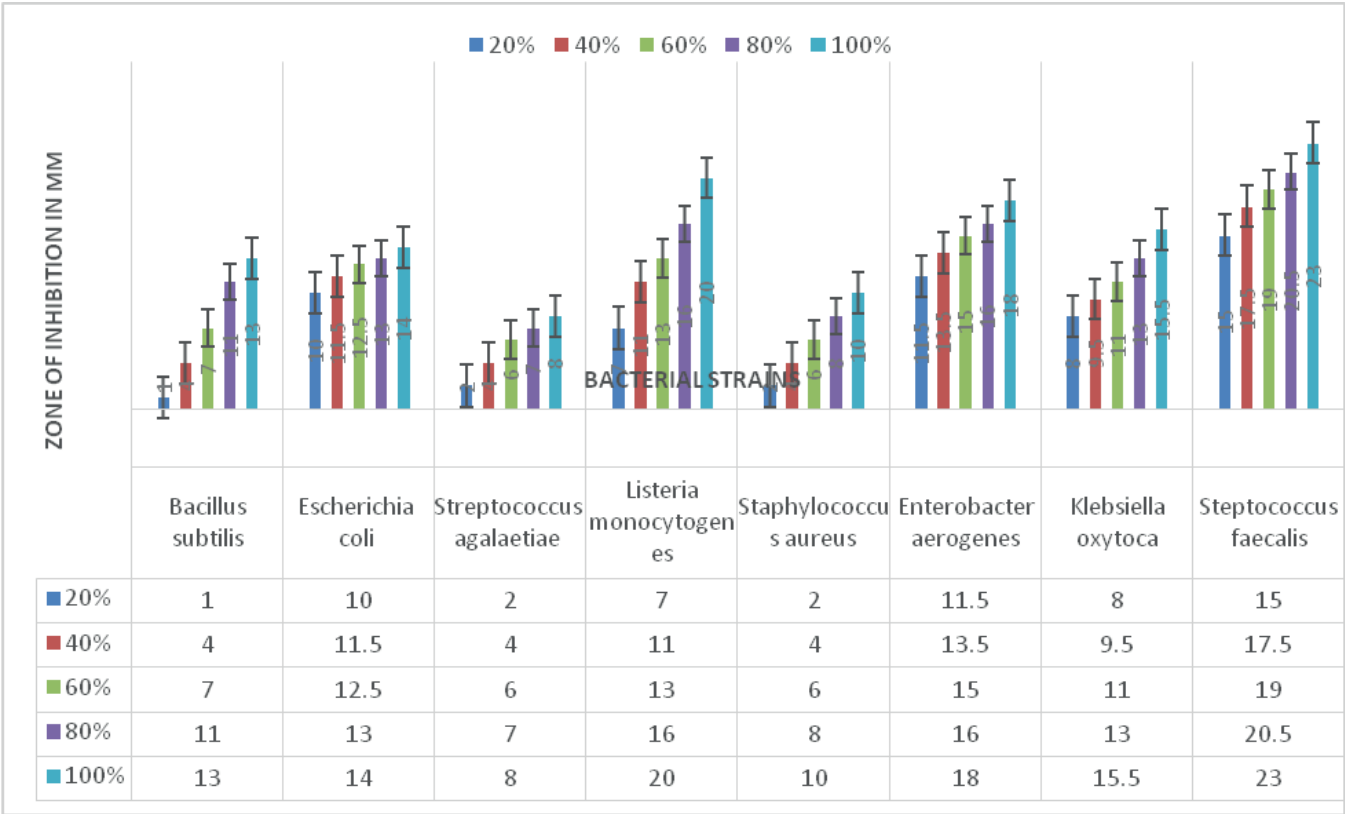


Figure 1. Chromatogram of compounds present in Couroupita guianensis. The sharp peak indicates the fractions involved in anticancer activity. Maximum anticancer activity was detected in retention time of 24.46.

of different concentrations of *Couroupita guianensis* L. including 100,50,25,12.5,6.25,3.125,1.562,0.781 µg/ml are tabulated in Table 3, and graphically represented in Figure 4. MTT assay of Couroupita guianensis L. showed significant impact on HL60 in concentration range between 10 mg/ml to 1 mg/ml compared with control. The most noteworthy cytotoxicity of this extract against HeLa cell was found in 1 and 0.05 mg/ml concentration with 70 and 55 percent of cell growth inhibition.

It was observed that the percentage of growth inhibition was increasing with increase in concentration of test compound, and IC_{50} value of this assay was 1 mg/ml. DMSO extracts of *Couroupita guianensis* demonstrated strong antioxidant and anti-proliferative activities. Amassing proof obviously shows that apoptosis is a basic sub-atomic focus by dietary bioactive operators, in the aversion of malignant growth. Since the phytochemical investigation has demonstrated the nearness of intense phytochemicals like alkaloids, phenols, flavonoids, terpenoids, glycosides, saponin, steroids, tannin and sugars, and so forth. Consequently, it is clear from the report that phenolic, acids, flavonoids, steroids, terpenoids are known to be bioactive standards behind the anticancer property.



	Bacillus subtilis	Escherichia coli	Streptococcus agalaetiae	Listeria monocytogenes	Staphylococcus aureus	Enterobacter aerogenes	Klebsiella oxytoca	Steptococcus faecalis
20%	1	10	2	7	2	11.5	8	15
40%	4	11.5	4	11	4	13.5	9.5	17.5
60%	7	12.5	6	13	6	15	11	19
80%	11	13	7	16	8	16	13	20.5
100%	13	14	8	20	10	18	15.5	23

Figure 2. Different Bacteria and its zone of inhibition.

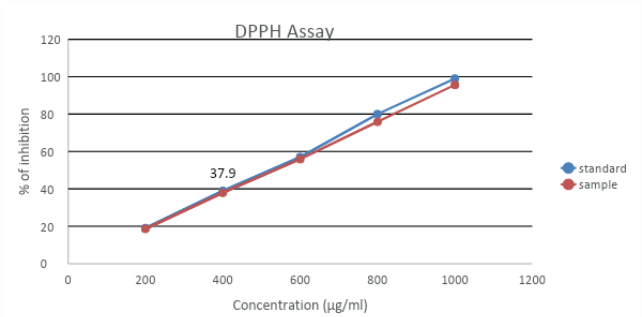


Figure 3. DPPH scavenging activity.

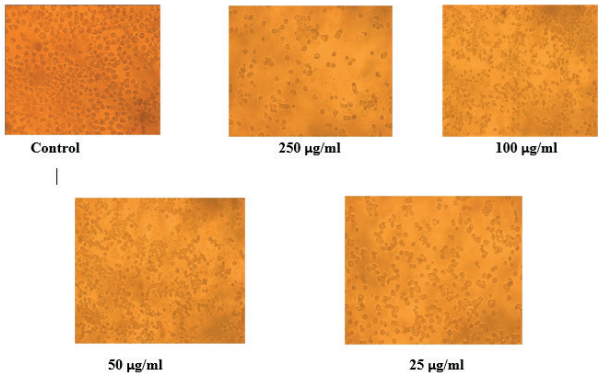


Figure 4. HL60 cells reactions at different sample concentrations.

Table 3. Cytotoxic activity of *Couroupita guianensis*

S.No	Concentration (µg/ml)	% of Cell Death	% of Live Cells
1	100	69.61	30.52
2	50	51.97	45.64
3	25	44.97	56.09
4	12.5	42.09	61.97
5	6.25	11.12	87.89
6	3.125	6.23	94.18
7	1.562	6.04	93.42
8	0.781	5.08	94.50

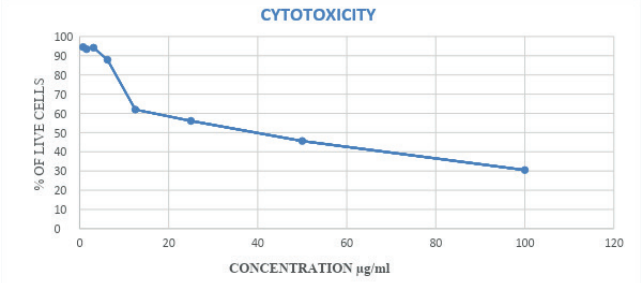


Figure 5 . Live cell percentage of HL60 cells against *Couroupita guianensis.*

4. References

1. Moorthi V, Jasmine SF. *Couroupita guianensis*: the reservoir of medicinal compounds of human welfare. Asian J Pharm Clin Res. 2017; 10:50–2. https://doi.org/10.22159/ajpcr.2017.v10i3.16174
2. Kekuda TP. Antimicrobial, radical scavenging, and insecticidal activity of leaf and flower extracts of *Couroupita guianensis* Aubl. Int. J. Green Pharm. 2017; 11:55–8.
3. Gousia SK, Kumar KA, Kumar TV, Latha JNL. Biological activities and medicinal properties of *Couroupita guianensis*. Int J Pharm Pharm Sci. 2013; 3:140–3.
4. Bergman J, Lindström JO, Tilstam ULF. The structure and properties of some indolic constituents in *Couroupita guianensis* Aubl. Tetrahedron. 1985; 41:2879–81. https://doi.org/10.1016/S0040-4020(01)96609-8
5. Ramachandran RP, Madhivanan S, Sundararajan R, Lin CWY, Sankaranarayanan K. An in vitro study of electroporation of leukemia and cervical cancer cells. Electroporation-Based Therapies for Cancer: From Basics to Clinical Applications. 2014; 12:161–83. https://doi.org/10.1533/9781908818294.161
6. Collins SJ. The HL-60 promyelocytic leukemia cell line: Proliferation, differentiation, and cellular oncogene expression. Blood. 1987; 70(5):1233–44. https://doi.org/10.1182/blood.V70.5.1233.1233. PMid:3311197
7. Panchal HN, Desai SD, Soni MK, Mishra P, Meshram DB. In-vitro antiurolithiatic and antioxidant activity of *Couroupita guianensis* aubl leaves. Pelagia Research Library Der Pharmacia Sinica. 2012; 35:3455–77.
8. Uppala PK, Kumar KA, Dadi JVR, Gorla US. Evaluation of antimicrobial activity of the aqueous and chloroform extracts of leaves of *Couroupita guianensis* by well diffusion method. Eur J Pharm Med Res. 2016; 3(11).
9. Sumathi S, Anuradha R. *Couroupita guianensis* Aubl: An updated review of its phytochemistry and pharmacology. Asian J Pharm Pharmacol. 2017; 3:1–8.
10. Premanathan M, Radhakrishnan S, Kulangiappar K, Singaravelu G, Thirumalaiarasu V, Sivakumar T, et al. Antioxidant and anticancer activities of isatin (1H-indole-2, 3-dione), isolated from the flowers of *Couroupita guianensis* Aubl. Indian J Med Res. 2012; 136:822–4.

11. Bharathi TR. International Journal of Phytopharmacy Research Article. The Indian journal of medical research, 2015; 12:22-26.
12. Keerthana V, Devakumar J, Sudha SS. Qualitative analysis of phytochemicals, antibacterial activity, and mycelial growth inhibition of three different plants. Drug Invent. Today. 2018; 10.
13. Singh R, Kumari N, Gangwar M, Nath G. Qualitative characterization of phytochemicals and in vitro antimicrobial evaluation of leaf extract of *Couroupita guianensis* Aubl. - A threatened medicinal tree. Int. J. Pharm. Pharm. Sci. 2015; 7:212–15.
14. Al-Dhabi NA, Balachandran C, Raj MK, Duraipandiyan V, Muthukumar C, Ignacimuthu S, Rajput VS. Antimicrobial, antimycobacterial and antibiofilm properties of *Couroupita guianensis* Aubl. fruit extract. BMC Complement Altern Med. 2012; 12:242. https://doi.org/10.1186/1472-6882-12-242. PMid:23206492. PMCid:PMC3537531
15. Vaijanathappa J. Phytochemical screening, antioxidant and antiproliferative activities of successive extracts of *Couroupita guianensis* Aubl. plant. Indian J Nat Prod Resour. 2018; 9:22–7.
16. Kavitha R, Kamalakannan P, Deepa T, Elamathi R, Sridhar S, Kumar JS. In vitro antimicrobial activity and phytochemical analysis of Indian medicinal plant *Couroupita guianensis* Aubl. J Chem Pharm Res. 2011; 3:115–21.
17. Bahuguna A, Khan I, Bajpai VK, Kang SC. MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J. Pharmacol. 2017; 12:234–84. https://doi.org/10.3329/bjp.v12i2.30892