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Acute Toxic and Cytotoxic Studies of Ethanolic Extract of Fruit Rind of *Couroupita guianensis*

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Abstract: *Ethanolic extract of fruit rind of Couroupita guianensis was evaluated for the safety profile by acute toxicity study in Swiss albino mice. The oral medium lethal dose (LD50) of EECG was found to be 200mg/kg, 400mg/kg body weight for first 72 hours to 14 days. Different concentrations of ethanolic extract were tested on Hep G2, Vero cell lines, MCF 7 Cell lines and HT 29 cell lines by MTT assay. EECG showed a significant antiproliferative activity and a dose dependent effect was observed. Minimum inhibition of 12.5% (IC 50) was shown by EECG at concentration 10µg/ml and maximum inhibition (100%) was observed at 100µg/ml.*

Keywords: *Acute toxicity, Couroupita guianensis, IC 50, LD50, MTT*

1. INTRODUCTION

Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. In recent times, medicinal plants occupy an important position for being the paramount sources of drug discovery, irrespective of its categorized groups – herbs, shrub or tree. Plants have been indispensable in treating diverse forms of diseases. **(Christine A., et al., 2002) [4]**

Acute toxicity study was performed in Swiss Albino mice and administered only as a single dose. The root extract of C.guianensis treated and animals showed no stereotypical symptoms reported by Juvekar, et al. 2009.[6]C.guianensis flower extracts (2000mg/kg) were administered once orally to the mice. (Nelson E.K et al., 1937) [8] The antidepressant activities of the flower extract were evaluated using forced swim test and tail suspension test, animal models of depression. (Vine.K.L.et al., 2009).[11] Mice were responded well for the flower extracts reported by (Shaijesh S et al., 2009).[9]C.guianensis leaf extract was tested in Swiss Albino mice for the assessment of antidepressant activity .(Kulkarni et al., 2009).

Hence the Swiss Albino mice were considered to be suited for acute toxicity study, to be selected for the present study as experimental animal to treat with the ethanolic extract of fruit rind of C.guianensis.The rich and diverse plant sources of India are likely to provide effective anticancer agents. Medicinal plants can reduce or minimize the toxic side effect of chemotherapy and radiation treatment by reinforcing their cancer killing action. (Arcamone.F, et al (1969).[1]

2. MATERIAL & METHOD

Acute toxicity tests provide preliminary information on the toxic nature of ethanolic extract of fruit rind for which no other toxicology information is available. Before deciding on the dose of a test compound that will be used in studying its acute toxicity. (Lorke, 1983).[7]

Acute oral toxicity test was performed as per OECD – 423 guidelines. All the 18 mice (Swiss Albino) were randomly distributed into one control group and two treated groups 200mg/Kg and 400mg/Kg body weight six mice per group three male and three female. They were housed in groups in stainless steel cages and kept under standard environmental conditions. They were given pelleted food and drinking water ad libitum. The mice were acclimatized to the laboratory conditions for at least 5 days prior to commencement of the experiments. The institutional animal ethics committee approved the experimental protocol. (Reg. No.115/PHARMA/SCRI, 8th July, 2011).

The animals were observed continuously for first 72 hours to 14 days for any signs of behavioral changes, toxicity salivation, lachrymation, respiration, hypersensitivity stimulant, muscular grip,

Ataxia, abdomen, contraction, straub tail, licking, body weight and mortality. The purpose was to identify the lowest dose level. (Diener et al., 1994). [5] Before and after the drug (ethanolic extract of fruit rind) given, the body weight was calculated.

Human breast cancer MCF – 7 (GDC055), Human colon adenocarcinoma (GDC033) and African Green monkey kidney (VERO) cell lines were obtained from national centre for cell sciences Pune (NCCS). The cells were maintained in minimal essential media (MEM) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100µg/mL) in a humidified atmosphere of 50ug/mL CO2 at 37°C. After 48 hours of inculcation, 1µL of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4 hours. The medium with MTT was then flicked off and the formed Formazan crystals were solubilized in 100µL of DMSO and then measured the absorbance at 57m nm using micro plate reader. (Velliangiri Prabhu., 2012) [10]

The % cell inhibition was determined using the formula. % cell inhibition = 100 – Abs (sayde) Abs (control) x100. Non linear regression graph was plotted between % cell inhibition and log to concentration and IC50 was determined using graph pad Prism Software.

Human Breast cancer MCF-7 (GDC055), Human Colon Adenocarcinoma (GDC033) and African Green Monkey Kidney (VERO) cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C. (Aubl Chandolu, et al., 2011),[2] MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

2.1. In vitro assay for Cytotoxicity activity (MTT assay).

The Cytotoxicity of samples on MCF-7 cells, HT 29 and VERO was determined by the MTT assay (Velliangiri Prabhu., 2012)).]10] Cells (1 × 105/well) were plated in 100 µl of medium/well in 96-well plates (Costar Corning, Rochester, NY). 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 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. (Bader G, et al., 1996), [3]

Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of human breast cancer cells was expressed as the % cell viability, using the following formula: % cell viability = A570 of treated cells / A570 of control cells × 100%.

3. RESULTS & DISCUSSION

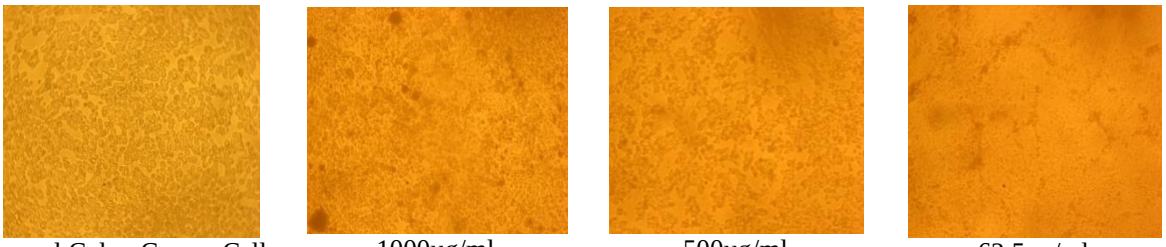
The animals were observed continuously for first 72 hours to 14 days for any signs of behavioral changes, toxicity salivation, lachrymation, respiration, hypersensitivity stimulant, muscular grip, Ataxia, abdomen, contraction, straub tail, licking, body weight and mortality (Fig.1)

Figures1. *Acute Toxicity – Mouse Dosage Fixation*

The purpose was to identify the lowest dose level. (Diener, et al., 1994). [5] All animals were normal and the dosages were fixed in two ways via. 200mg/kg and 400mg/kg body weight of the mice. (Table –1)

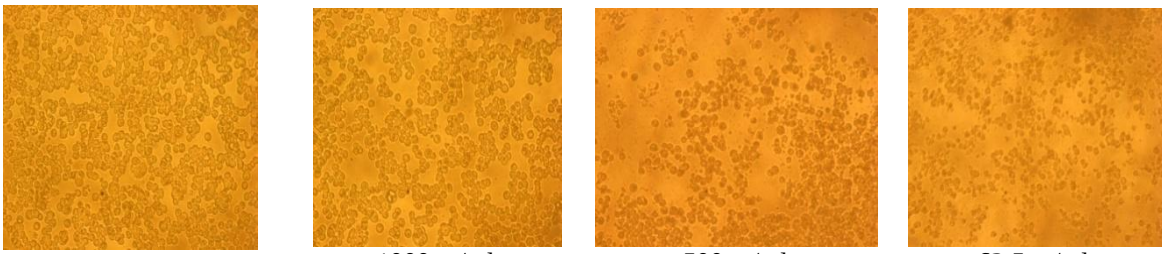
Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. (Fig.2)

Cytotoxicity Effect on Colon cancer Cell lines (HT 29)



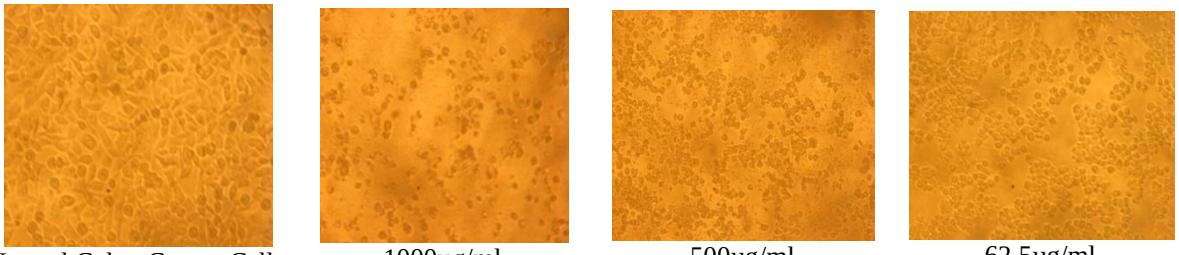
Normal Colon Cancer Cells 1000µg/ml 500µg/ml 62.5µg/ml

Cytotoxicity Effect on Breast cancer Cell lines (MCF-7)



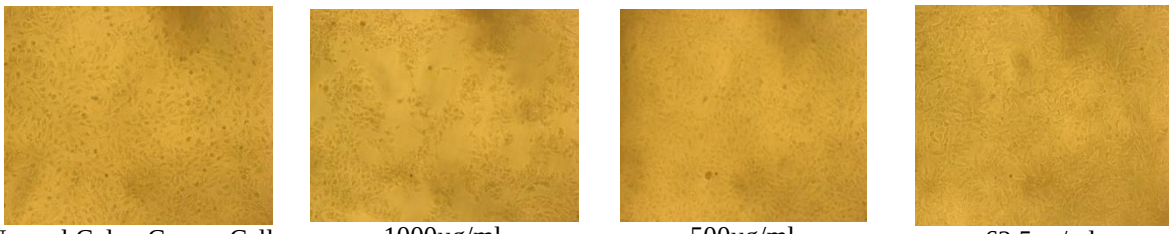
Normal Colon Cancer Cells 1000µg/ml 500µg/ml 62.5µg/ml

Cytotoxicity Effect on Liver cancer Cell lines (Hep G2)



Normal Colon Cancer Cells 1000µg/ml 500µg/ml 62.5µg/ml

Cytotoxicity Effect on VERO Cell lines



Normal Colon Cancer Cells 1000µg/ml 500µg/ml 62.5µg/ml

Figures2.

The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. (Table 2-6) The effect of the samples on the proliferation of human breast cancer cells was expressed as the 83% cell viability, using the following formula: % cell viability = A570 of treated cells / A570 of control cells × 100%.

Table2. *MTT assay of Couroupita guianensis on Hep G2 cell lines*

S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	1000	Neat	15.79
2.	500	1:1	22.37
3.	250	1:2	30.26
4.	125	1:4	32.89
5.	62.5	1:8	40.79
6.	31.25	1:16	51.32
7.	15.625	1:32	55.26
8.	7.8125	1:64	59.21
9.	3.906	1:128	67.10
10.	1.953	1:256	81.58
11.	Cell control	-	100

Table3. *MTT assay on Ethanolic extract of fruit rind of Couroupita guianensis on Vero cell lines*

S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	1000	Neat	34.51
2.	500	1:1	45.67
3.	250	1:2	49.80
4.	125	1:4	54.44
5.	62.5	1:8	69.01
6.	31.25	1:16	78.72
7.	15.625	1:32	85.61
8.	7.8125	1:64	87.55
9.	3.906	1:128	89.01
10.	1.953	1:256	96.71
11.	Cell control	-	100

Table4. *MTT assay on Ethanolic extract of fruit rind of Couroupita guianensis on MCF 7 cell lines*

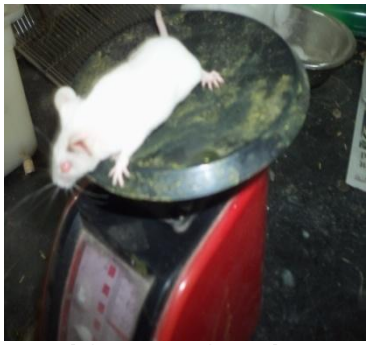
S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	1000	Neat	12.33
2.	500	1:1	17.88
3.	250	1:2	23.33
4.	125	1:4	27.89
5.	62.5	1:8	35.11
6.	31.25	1:16	39.08
7.	15.625	1:32	45.63
8.	7.8125	1:64	53.99
9.	3.906	1:128	67.89
10.	1.953	1:256	71.56
11.	Cell control	-	100

Table5. *MTT assay on Ethanolic extract of fruit rind of Couroupita guianensis on HT 29 cell lines*

S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	100	Neat	18.85±1.32
2.	50	1:1	32.45±0.95
3.	25	1:2	45.65±0.85
4.	12.5	1:4	58.32±0.45
5.	6.25	1.8	67.95±1.35
6.	3.125	1:16	78.95±1.49
7.	1.56	1:32	91.15±0.385
8.	Cell control	-	100

Acute oral toxicity studies (OECD 423) revealed that the ethanolic extract of fruit rind is relatively nontoxic upto 400 mg/kg b.w. pronouncing the safety profile of the fruit rind extracts and fractions. (Table.1)

Table1. *Acute Toxicity – Mouse Dosage Fixation*

Cage	Position	Body weight in gms	Dosage in ml	Period of observation			
				24hrs	3rd day	7th day	14th day
1	H	30	0.12				
	B	30	0.12				
	T	35	0,12				
2	H	25	0.10				
	B	27	0.11				
	T	28	0.11				
3	H	30	0.21				
	B	35	0.25				
	T	30	0.21				
4	H	27	0.20				
	B	28	0.21				
	T	25	0.21				

ACTIVE NORMAL

In our cell line culture the ethanolic extract of fruit rind showed activity in the MTT assay using tumor cell lines, in HEP G2, 1000 µg / ml. 250 µg / ml. 62.5 µg / ml. and 100% viability, in Vero Cell line, 1000 µg / ml. 250 µg / ml. 125 µg / ml. 62.5 µg / ml. and 100% viability in Breast cancer cell line1000 µg / ml. 250 µg / ml. 31.25µg / ml. 15.625µg / ml. and 100% viability in HT 29-Colon cancer cell line 1000 µg / ml. 500 µg / ml. 62.5 µg / ml. and 100% viability, (Fig. 2-6) indicating the presence of 83% cytotoxic compounds in the ethanolic extract, IC 50 values for our tested cell lines.

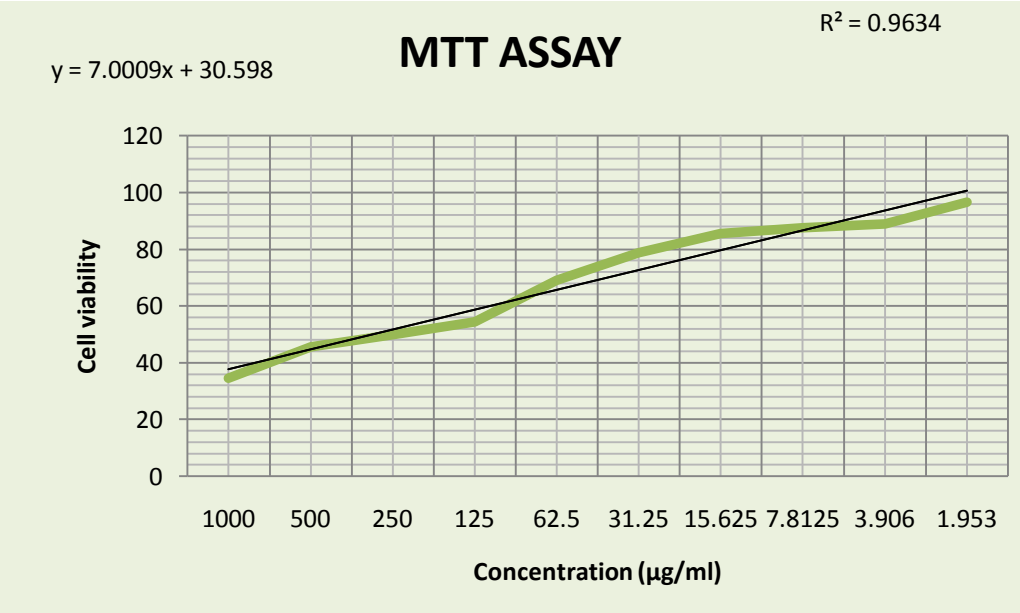


Figure3. *Graph of MTT assay on the ethanolic extract of fruit rind Couroupita guianensis on Hep G2 Cell lines*

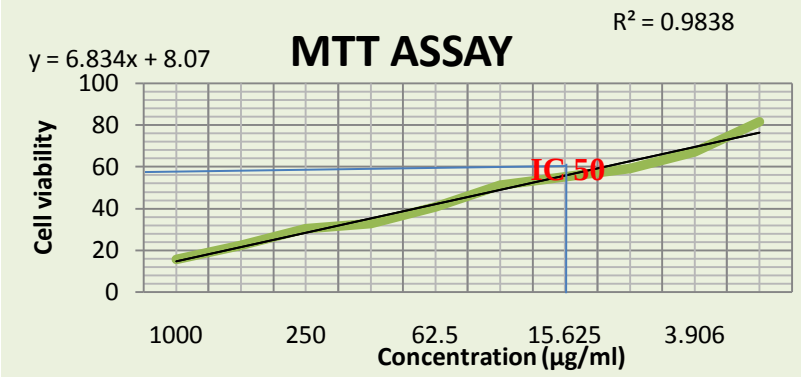


Figure4. *Graph of MTT assay on the Ethanolic extract of fruit rind Couroupita guianensis on Vero Cell lines*

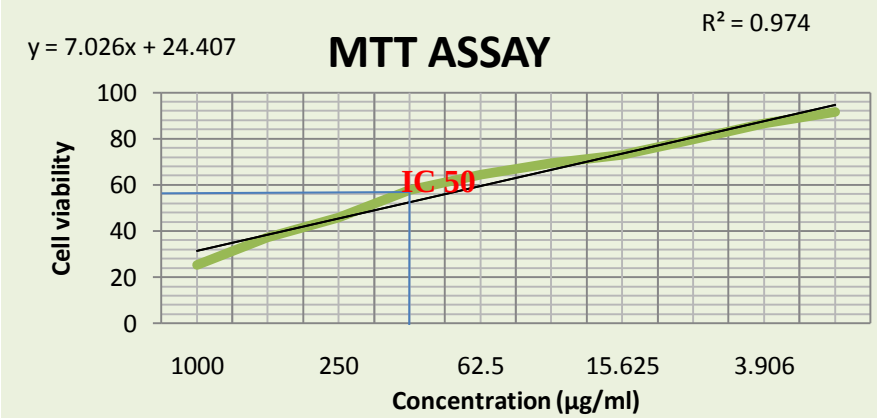


Figure5. *Graph of MTT assay on the Ethanolic extract of fruit rind Couroupita guianensis on MCF 7 Cell lines*

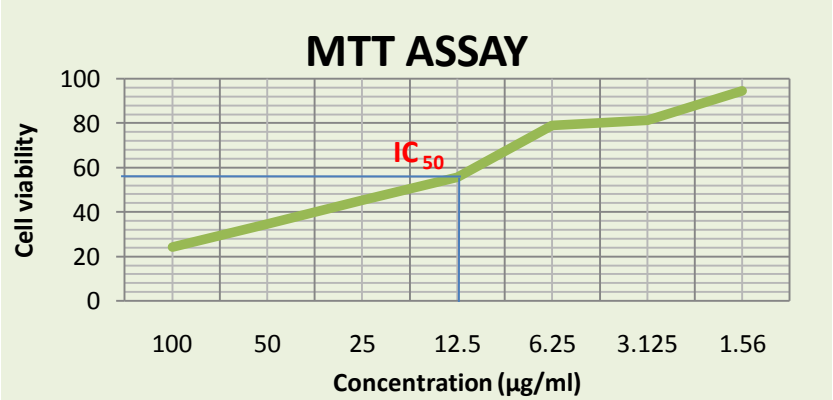


Figure6. *Graph of MTT assay on the Ethanolic extract of fruit rind Couroupita guianensis on HT 29 Cell lines*

4. CONCLUSION

In the present study based on the earlier reports the adverse effect of a hopane in the ethanolic extract of fruit rind of C.guianensis, within 14 days of the administration of the crude extract. There was no mortality; hence the dosage was fixed as 200mg / kg and 400mg/kg.

We determined the cytotoxicity of fruit rind with an 83 % cytotoxic concentration for a variety of human and animal cell lines in vitro. Concomitantly, we observed an improvement in proliferation rates and changes in the morphology of contaminated mammalian cells after treatment with this phytodrug.

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REFERENCES

[1] **Arcamone.F, G.Cassinelli, G.Fantini, A.Grein, P.Orezzi, C.Pol and C.Spalla (1969).** Chemical constituents of Lecythipisons and cytotoxic activity. Revista Brasileira de Farmacognosis (Rev.Bras.Faemacop). Vol.22 (5).

[2] **Aubl Chandolu, Saikumar, Gaddala Naresh, Vanamala Sudheer, Naresh Veldi, A.Elumalai 2011.** A short Review on Therapeutic uses of *Couroupita guianensis*. International Research Journal of Pharmaceutical and Applied Sciences. Vol.1 (1); 105 – 108.

[3] **Bader G, Plohmann B, Hiller K, Franz G. 1996** Cytotoxicity of triterpenoid saponins. Part 1: activities against tumor cells in vitro and hemolytic index. Pharmazie; 51:414–7.

[4] **Christine A Haller, Joewen Dyes, Richard K.O, and Kent R O/S on 2002.** Making a diagnosis of herbal related toxic hepatitis . West J med. 176 (1) 39 – 44.

[5] **Diener W., Sidha L., Mischke U., Kayserd and schlede E. 1994.** The Biometric Evaluation of the Acute - toxic class method (oral) Arch. Toxicol. 68(10):599-610.

[6] **Juvekar, M. A.Juvekar, M.Kulkarni, A.Wakhade, R.Ambaye S.Wankhade 2009.** "Phytochemical and pharmacological studies on the leaves of *Couroupita guianensis* Aubl." Planta med. 75 – PJ168.

[7] **Lorke, D. 1983**. A new approach to acute toxicity testing Arch Toxicol. 54, 275-287.

[8] **Nelson E.K, Wheeler D.H (1937**) "Some constituents of the cannon ball fruit *Couroupita guianensis* Aubl." J.Am. Chem.Soc. Vol.59 (12): 2499 – 250.

[9] **Shaijesh S Wankhede, Manoj Gambhire, Archana Juvekar**; **2009**. Anxiolytic Effect of Methanolic Root Extract of *Couroupita guianensis* Aubl. in Mouse Models of Anxiety; Pharmacology online 3; pp193-206.

[10] **Velliangiri Prabhu and Subban Ravi 2012.** "Quantification of quercetin and stigmasterol of *Couroupita guianensis* aubl by HPTL method and invitro cytototoxic activity by MTT assay of the methanol Extract against Hela, NIH 3T2 and HepG2 cancer cell lines." International Journal of Pharmacy and Pharmaceutical sciences" Vol. 4(4). 126-130.

[11] **Vine.K.L. Matesic.L., Locke.J.M, Ranson.M. Skropeta.D 2009**. "Cytotoxic and anti cancer activities of Isatin and its derivatives: A comprehensive review from 2000 – 2008. "Anticancer agents in medicinal chemistry, anticancer agents". Vol.9 (4): 397 – 414.

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1. Regina V and K.M.Uma Rajan (2012) "***Phytochemical analysis, antioxidant and antimicrobial studies of fruit rind of Couroupita guianensis (AUBL)"*** INTJ CURR SCI pg. 262-267. ISSN: 2250 -1770.

2. Regina V and K.M.Uma Rajan (2012) ***"Pharmacognostical, Preliminary phytochemical, GC-MS and docking analyses of fruit rind of Couroupita guianensis (AUBL)"***INTJ CURR SCI. 3:101-107. ISSN: 2250-1770.

3. Regina V (2014) "***Docking Analysis of Fruit Rind of Couroupita guianensis Aublet"*** AARJMD 19 (1):574-584.ISSN:2319-2801.

4. Regina V (2014) "***Pharmacognostical Studies of Various Parts of Couroupita guianensis Aubl.*"** INT.J.CURR.RES.BIOSCI.PLANT BIOL. 1(3):17-26.ISSN:2349-8080.

5. Regina V and K.M.Uma Rajan (2014) ***"In-vitro assay for Cytotoxicity activity in ethonolic extract of Fruit rind of Couroupita guianensis Aubl"***INT.J.CURR.MICROBIOL.APP.SCI. 3(10):169-176.ISSN:2319-7706.

15 years of service as a PG Assistant in Botany in a various esteemed institutions and 5 years of service as a Lecturer in various reputed colleges for UG and PG students, From 2012 till now producing teacher trainees. The Author's objective is to establish herself in the field of education, to utilize her skills and experience to help students to achieve high improvements in academics. Her interest is to be a reviewer in the field of research and development of experimental Botany.