Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(2): 44-49
Received: 25-04-2015
Accepted: 28-05-2015

Priyanka Sarkar
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

Murshida Begum
Pharmacy department, Rajshahi University, Rajshahi - Dhaka Highway, Rajshahi 6205, Bangladesh.

Koly Aktar
Pharmacy department, Rajshahi University, Rajshahi - Dhaka Highway, Rajshahi 6205, Bangladesh.

Fatema-Tuz-Zohora
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

Abdul Jabbar
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

Choudhury Mahmood Hasan
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

Correspondence:
Priyanka Sarkar
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

Cytotoxic and Free radical scavenging activity of relatively polar fractions of *Couroupita guianensis* Aublet stem bark

Priyanka Sarkar, Murshida Begum, Koly Aktar, Fatema-Tuz-Zohora, Abdul Jabbar, Choudhury Mahmood Hasan

Abstract
The study was aimed at to evaluate the efficacy of relatively polar fraction of *Couroupita guianensis* Aubl. stem bark on free radical scavenging and brine shrimp lethality. The dried stem bark of *Couroupita guianensis* Aubl. was cold extracted with methanol and the crude extract obtained was subjected to vacuum liquid chromatography from which the portion eluted with 100% methanol and polar solvents was partitioned with petroleum ether (PEF), carbon tetrachloride (CTF), chloroform (CHF) and water (AQF). These were then subjected to investigation for DPPH free radical scavenging activity and cytotoxicity measurement by Brine shrimp lethality bioassay. From the results it was evident that for cytotoxicity measurement, chloroform fraction (CHF) having lethal concentration of 3.0439 µg/ml has significant activity than other fractions on brine shrimp lethality bioassay and the maximum scavenging activity of aqueous fraction (AQF) was highest, 22.401 µg/ml and petroleum ether fraction (PEF) having a value of 30.0367 µg/ml, showed better activity compared to other fractions. From the findings it was revealed that the polar fraction of the plant extract possesses cytotoxic activity and could be good source of cytotoxic compounds and the promising effect on free radical scavenging suggests that it may play an important role as potential natural source against free radical associated disease.

Keywords: *Couroupita guianensis* Aublet, polar fraction, DPPH free radical scavenging, Cytotoxicity

1. Introduction
Couroupita guianensis Aubl. (Cannon ball) is a large deciduous tree of 20-30m height with leaves, up to 6" long; fruits having a woody look like cannonballs and flowers having thick waxy petals with pale yellow on the outside, pinkish red inside, spreading strong fragrance when in full bloom (Shah G.N. *et al*., 2012) [10].
It is native to South India and Malaysia. The plant showed a broad spectrum of antibacterial and antifungal activities. 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 (Mariana *et al*., 2010) [6]. The biological activities of flavonoids include antioxidant, anti-inflammatory properties, protection against allergies, platelet aggregation, microbes, viruses, tumors, heptatotoxins, ulcers (S. Manimegalai *et al*., 2012) [11].
Phytochemical studies revealed the presence of triterpenoid glucoside, saponins (Massiot *et al*., 1992) [7], triterpenoid saponins (Das and Mahato, 1982) [4], flavonol glycosides and indol constitu- ents (Crublet *et al*., 2003) [3]. Some studies with this species had evidenced the presence of α-amirin, β-amirin, β-sitosterol, tannins (Row *et al*., 1966) [9] and cetoesteroids (Anjaneyulu and Rao, 1998). In the leaves, triterpenoid esters of fatty acids such as palmitate β-amiryn were characterized. In the flowers, eugenol, linalool and (*E, E*)-farnesol were identified (Eknat and Shivchandraji, 2002) [5].
Though different biological actitivities have already been investigated for different parts of the plant, this investigation was mainly focused on the evaluation of the efficacy of relatively polar fraction of *Couroupita guianensis* Aubl. stem bark on free radical scavenging and cytotoxic activity.

2. Methods and materials
2.1 Plant material
The stem bark of *Couroupita guianensis* Aubl. (Lecythidaceae) was collected during the month of August, 2011 from Dhaka, Bangladesh. A voucher specimen had been deposited in the herbarium of the Department of Botany, University of Dhaka under the voucher number DUH-7130. The samples were cut into small pieces and sun dried for 7 days followed by oven

drying for 24 h at 40 ºC for grinding purpose. The materials were grinded into coarse powder with the help of a grinder and stored in an air tight container for further use.

2.2 Preparation of plant extract

The air-dried and powdered plant materials (700 g) were taken in an amber colored reagent bottle and soaked in 5 liter of methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered off through a cotton plug followed by Whatman filter paper No. 1 and the filtrate thus obtained was concentrated at 40 ºC with a rotary evaporator. Then the solvent was evaporated to obtain a solid residue (15 g) or crude extract. This was then subjected to vacuum liquid chromatography for the initial rapid fractionation of the crude extract. The column was primarily eluted with comparatively non-polar solvents, then the mobile phases with progressively increasing polarity were passed through the column and finally with 100% methanol. The column fractions that were obtained from elution with non-polar solvents were investigated for compound separation whereas the fractions eluted with more polar solvent and 100% methanol (ME) was then partitioned with petroleum ether (PEF), carbon tetrachloride (CTF), chloroform (CHF) and water (AQF) and these fractions were subjected to biological investigation.

2.3 Brine shrimp lethality bioassay

This assay was based on the principle described by Meyer *et al.*, 1982. Brine shrimp eggs are hatched in simulated sea water to get nauplii. By the addition of calculated amount of Dimethyl sulfoxide (DMSO), desired concentrations of the test sample is prepared. The nauplii are counted by visual inspection and are taken in vials containing 5 ml of simulated sea water. Then samples of different concentrations are added to pre-marked vials using micropipettes. Then the vials are left for 24 hours. Survivors are counted after 24 hours.

2.3.1 Preparation of seawater

38 gm sea salt (pure NaCl) was weighed, dissolved in one liter of distilled water and filtered off to get a clear solution.

2.3.2 Hatching of Brine Shrimps

Artemia salina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Sea water was taken in a small tank and shrimp eggs were added to the one side of the tank and then this side was covered. One day was allowed to hatch the shrimps and to be matured as nauplii. Constant oxygen supply was carried throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated damn and they were taken for experiment. With the help of Pasteur pipette 10 living shrimps nauplii were added to each of the test tubes containing 5 ml of sea water.

2.3.3 Preparation of the Test sample

All the test samples (ME, PEF, CTF and CHF) were taken in vials and dissolved in 100 µl of pure Dimethyl sulfoxide (DMSO) to get stock solution. Then 50 µl of this solution was taken in the first test tube containing 5 ml of sea water and 10 shrimp nauplii. Thus the final concentration of the prepared of the first test tube was 800 µg/ ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In every case, 50 µl of the test samples were added to the test tube and fresh 50 µl DMSO was added to the vial.

2.3.4 Preparation of the positive control group

Positive control in a cytotoxity study is a widely accepted cytotoxic agent and the result of the test samples were compared with the result of obtained for the positive control. In the present study Vincristine Sulphate was used as the positive control. Measured amount of the Vincristine Sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ ml from which serial dilutions are made using DMSO to get 10 µg/ ml, 5 µg/ ml, 2.5 µg/ ml, 1.25 µg/ ml, 0.625 µg/ ml, 0.3125 µg/ ml, 0.15625 µg/ ml, 0.078125 µg/ ml, 0.039 µg/ ml. then the positive control solution were added to the pre-marked vials containing 10 living shrimps nauplii in 5 ml simulated sea water to get positive control groups.

2.3.5 Preparation of the negative control group

50 µl DMSO was added to each three pre-marked glass vials containing 10 living shrimps nauplii in 5 ml simulated sea water to used as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxity of the compounds.

2.3.6 Counting of nauplii

After 24 hours, the vials were using a magnifying glass and the numbers of survivors were counted. The percent (%) mortality was diluted for each dilution. The concentration- mortality data was analyzed statistically by using linear regression using a simple IBM-PC program. The effectiveness or the concentration- mortality relationship of plan product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

2.4 DPPH free radical scavenging activity

A simple method has been developed to determine the free radical scavengign activity of plant extract utilizing the stable 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical (Brand-Williams *et al*., 1995). 2.0 ml methanol solution of the extract, at different concentrations was mixed with 3.0 ml of a DPPH methanol solution (20 µg/ ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of *tert*-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer and the percentage inhibition activity was calculated from (I %) = (I- A_{sample}/A_{blank}) X 100, where A_{blank} is the absorbance of control reaction (containing all reagents except the test material).

2.4.1 Control preparation

Tert-butyl-1-hydroxytoluene (BHT) was used as positive control. Calculated amount of BHT were dissolved in methanol to get a mother solution having a concentration 1000 µg/ml. Serial dilution was made using the mother solution to get different concentration from 500.0 to 0.977 µg/ml.

2.4.2 Test sample preparation

Calculated amount of different extractives were measured and were dissolved in methanol to get a mother solution having a concentration 1000 µg/ml. Serial dilution was made using the mother solution to get different concentration from 500.0 to 0.977 µg/ml.

2.4.3 DPPH solution preparation
20 mg of DPPH was weighed and dissolved in methanol to get a DPPH solution having a concentration 20 µg/ml. The solution was prepared in the amber reagent bottle and kept in the light-proof box.

3. Results and discussion
3.1 Determination of Brine shrimp lethality
The lethal concentration (LC_{50}) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. Vincristine Sulphate (VS) was used as positive control and the LC_{50} was found as 0.451 µg/ ml for VS. Compared with the negative control VS (positive control) gave significant mortality and the LC_{50} values of the different extractives were compared with negative control. The LC_{50} values of ME, PEF and CHF were found to be 4.2331µg/ ml, 10.8451µg/ ml and 3.0439 µg/ ml respectively (Tab-2, Fig: 2a, 2b, 2c, 2d and Fig: 3).
Based on the results, the brine shrimp lethality of different fractions was found to be concentration-dependent. The observed lethality to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components of the plant.

Table 2: LC_{50} values of the test samples of *Couroupita guianensis* Aubl.

Test Samples	Regression line	R^2	Conc. (µg/ ml)	Log_{10} Conc.	% Mortality	LC_{50} values
VS (Vincristine Sulphate)	y = 30.79x+60.640	0.972	00	-	20	
			0.0390	-1.40894	20	
			0.078125	-1.10721	30	
			0.15625	-0.80618	30	
			0.3125	-0.50515	40	0.451
			0.625	-0.20142	50	
			1.25	0.09691	70	
			2.5	0.39794	80	
			5	0.69897	80	
			10	1	90	
			20	1.30103	100	
Methanolic extract (ME)	y =25.773x+33.849	0.9475	00	-	10	
			0.78125	-0.10721	20	
			1.5625	0.19382	40	
			3.125	0.49485	50	
			6.25	0.79588	60	
			12.5	1.09691	70	4.2331
			25	1.39794	70	
			50	1.69897	80	
			100	2	80	
			200	2.30103	90	
			400	2.60206	100	
Pet-ether fraction (PEF)	y = 23.558x+25.612	0.9322	00	-	20	
			0.78125	-0.10721	20	
			1.5625	0.19382	40	
			3.125	0.49485	40	
			6.25	0.79588	40	
			12.5	1.09691	50	
			25	1.39794	50	10.8451
			50	1.69897	60	
			100	2	80	
			200	2.30103	80	
			400	2.60206	90	
Chloroform fraction (CHF)	y = 26.175x+37.346	0.9484	00	-	10	
			0.78125	-0.10721	30	
			1.5625	0.19382	50	
			3.125	0.49485	50	
			6.25	0.79588	60	
			12.5	1.09691	60	3.0439
			25	1.39794	70	
			50	1.69897	80	
			100	2	100	
			200	2.30103	100	

			400	2.60206	100	

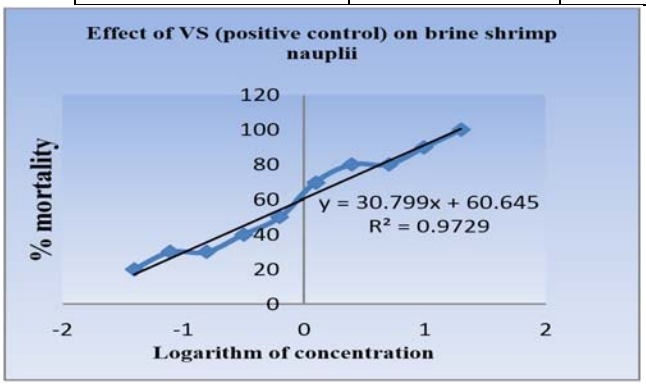

Fig 2(a):

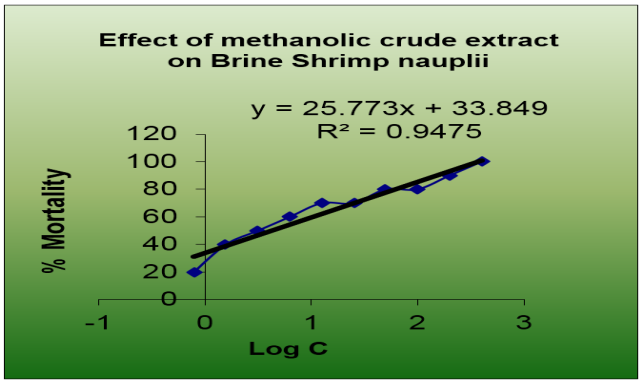

Fig 2(b):

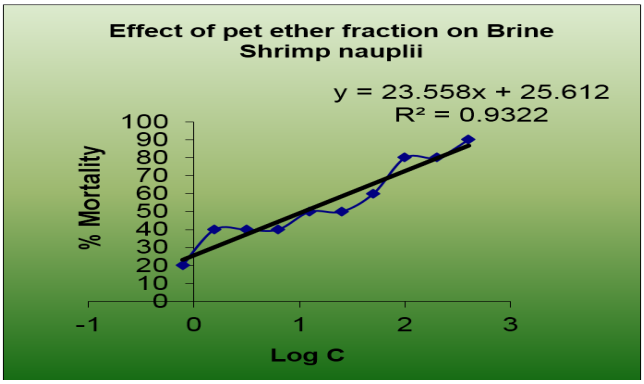

Fig 2 (c):

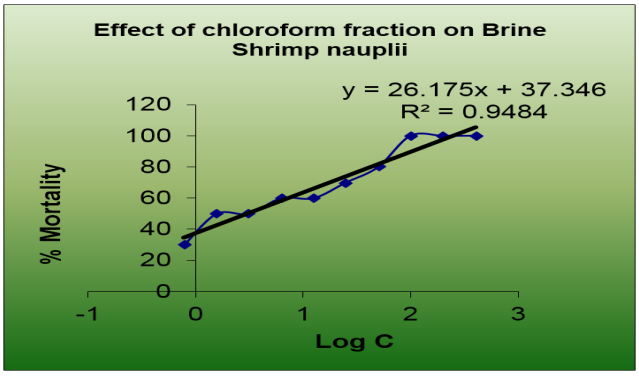

Fig 2(d):

Fig 2(a): Plot of % mortality and predicted regression line of VS, Fig: 2(b) Plot of % mortality predicted and regression line of ME Fig: 2(c) Plot of % mortality and predicted regression line of PEF and Fig: 2(d) Plot of % mortality and predicted regression line of CHF.

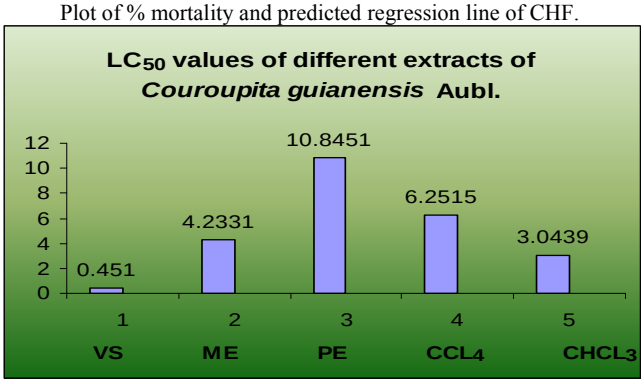

Fig 3: LC_{50} values of the different extractives of *Couroupita guianensis* Aubl.

3.2 Assay of Free Radical Scavenging Activity

In DPPH radical scavenging assay SC_{50} values of the methanol extract (ME), petroleum ether fraction (PEF), carbon tetrachloride fraction (CTF), chloroform fraction (CHF) and aqueous fraction (AQF) were evaluated. From the results it was observed that the scavenging activity of aqueous fraction (AQF) was higher compared with other fractions and very much close to the value of standard. The SC_{50} values of the *Tert*-butyl-1-hydroxytoluene (BHT), methanol extract (ME), petroleum ether fraction (PEF), carbon tetrachloride fraction (CTF), chloroform fraction (CHF) and aqueous fraction (AQF) were found to be 17.03 µg/ml, 62.295 µg/ml, 30.037 µg/ml, 54.03 µg/ml, 60.618 µg/ml and 22.401 µg/ml respectively. (Tab-1, Fig: 1.a and 1.b).

Figure 2 shows the dose–response curves of DPPH radical activities of extracts from *Couroupita guianensis* Aubl. It was found that all test samples exhibited potent scavenging activities in a concentration dependent manner. At a concentration of 500 µg/mL, the scavenging activities of AQF reached a plateau of 94.15% while, at same concentration, the scavenging effects of ME, PEF, CTF and CHF were 79.53%, 76.61%, 69%, 70.76% respectively.

Table 1: DPPH radical scavenging activity of the *Tert*-butyl-1-hydroxytoluene (BHT), methanol extract (ME), petroleum ether fraction (PEF), carbon tetrachloride fraction (CTF), chloroform fraction (CHF) and aqueous fraction (AQF) at different concentrations.

Sample	Concentration (µg/ml)	Absorbance	% of scavenging Mean (n=3)	SC_{50}(µg/ml)
Tert-butyl-1-hydroxytoluene (BHT)	500	0.018	94.73	
	250	0.068	80.11	
	125	0.097	71.63	
	62.5	0.135	60.52	
	31.25	0.159	53.51	17.03
	15.625	0.175	48.83	
	7.813	0.206	39.77	
	3.906	0.225	34.21	
	1.953	0.238	30.41	
	0.977	0.287	16.08	
Methanol extract (ME)	500	0.007	79.53	
	250	0.02	94.15	

	125	0.078	77.19	
	62.5	0.234	31.58	
	31.25	0.281	17.84	62.2953
	15.625	0.299	12.57	
	7.813	0.304	11.11	
	3.906	0.306	10.53	
	1.953	0.309	9.65	
	0.977	0.321	6.14	
Petroleum ether fraction (PEF)	500	0.08	76.61	
	250	0.100	70.76	
	125	0.112	67.25	
	62.5	0.129	62.28	
	31.25	0.145	57.60	
	15.625	0.191	44.15	30.0367
	7.813	0.229	33.04	
	3.906	0.253	26.02	
	1.953	0.281	17.84	
	0.977	0.307	0.307	
Tetrachloride fraction (CTF)	500	0.106	69	
	250	0.107	68.71	
	125	0.121	64.62	
	62.5	0.145	57.60	
	31.25	0.191	44.15	54.0395
	15.625	0.234	31.58	
	7.813	0.259	24.27	
	3.906	0.286	16.37	
	1.953	0.291	14.91	
	0.977	0.306	10.53	
Chloroform fraction (CHF)	500	0.100	70.76	
	250	0.107	68.71	
	125	0.119	65.20	
	62.5	0.151	55.85	
	31.25	0.191	44.15	
	15.625	0.253	26.02	60.6175
	7.813	0.286	16.37	
	3.906	0.291	14.91	
	1.953	0.305	12.13	
	0.977	0.333	2.63	
Aqueous fraction (AQF)	500	0.02	94.15	
	250	0.049	85.67	
	125	0.078	77.19	
	62.5	0.107	68.71	
	31.25	0.144	57.89	22.401
	15.625	0.191	44.15	
	7.813	0.234	31.58	
	3.906	0.269	21.35	
	1.953	0.308	9.94	
	0.977	0.317	7.31	

Here, Results of the assay were presented as mean (n=3).

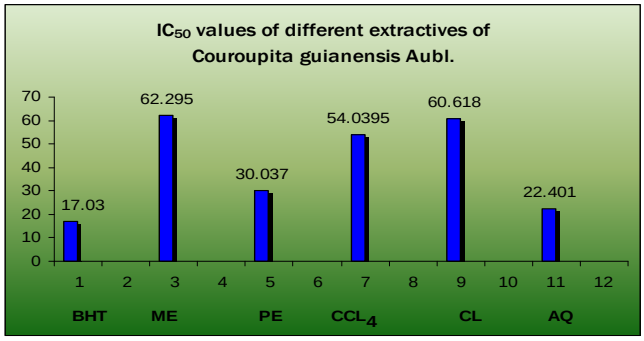

Fig 1(a):

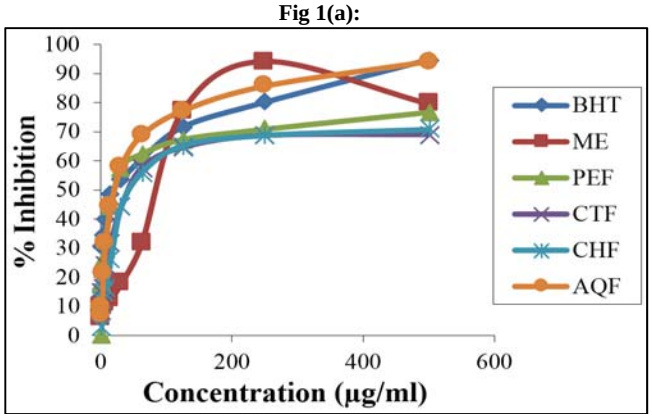

Fig 1(b):

Fig 1(a): SC_{50} values of different extractives of *Couroupita guianensis* Aubl. at different concentrations presented in bar diagram. 1(b) SC_{50} values of different extractives of *Couroupita guianensis* Aubl. at different concentrations presented graphically.

4. Conclusion

In the present study different extractives of *Couroupita guianensis* Aubl. stem bark were investigated for cytotoxic and free radical scavenging and activity. The chloroform soluble fraction of the methanolic extract showed significant cytotoxic activity which suggests it could contain potent or active components. The aqueous soluble fraction as well as the pet ether of the methanolic extract showed strong free radical scavenging activity. These extracts may be rich in radical scavengers. Further investigation on the chemical composition of those extracts, as well as studies with other models, such as lipid peroxidation and in vivo assays are essential to characterize them as biological antioxidants. From these findings we can conclude that this plant has potential therapeutic efficacy and may serve as potential source of chemically interesting and biologically important drug candidates and thus can be further screened against various diseases or using different in vivo model in order to find out its unexplored efficacy as well as the mechanism of action of the explored bioactivities.

5. References

1. Anjaneyulu ASR, Rao SS. A new ketosteroid from the bark of *Couroupita guianensis* Aubl. Indian Journal Chemistry Section B: Organic Chemistry1998; 37:382-386.
2. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol. 1995; 28:25–30.
3. Crublet M, Long C, Sevenet T, Hadi HA, Lauvaud C. Acylatedflavonol glycosides from leaves of *Planchonia*

grandis. Phytochemistry 2003; 64:589-594.
4. Das MC, Mahato SB. Triterpenoids apogenols from the leaves of *Careya arborea*: structure of careyagenolide. Phytochemistry 1982; 21:2069-2073.
5. Eknat AA, Shivchandraji LK. b-Amirin palmitate isolation on from *Couroupita guianensis* Aubl. Leaves. Indian Drugs 2002; 39:213-216.
6. Mariana Pinheiro MG, Sidnei Bessa O, Catharina Fingolo E, Ricardo Kuster M, Maria Eline Matheus, Fábio Menezes S. Patrícia Dias Fernandes Antinociceptive activity of fractions from *Couroupita guianensis Aubl* leaves. Journal of Ethno pharmacology 2010; 127(2):407-413.
7. Massiot G, Chen X, Lavaud C, Men-Olivier LL, Delaude C, Viari A *et al.* Saponins from stembark of *Petersianthus acrocarpus*. Phyto- chemistry 1992; 31:3571-3576.
8. Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plants constituents. Planta Med 1982; 45:31-34.
9. Row LR, Santry CSPP, Suryananayana PP. Chemical examination of Couroupita guianensis. CurrentScience 1966; 35:146-147.
10. Shah GN, Shete SA, Patil VS, Patil KD, Killedar SG. Standardization and Anti-Bacterial Activity of *Couroupita guianensis* Fruit Pulp Extract. International Journal of Pharmacognosy and Phytochemical Research 2012-13; 4(4):185-189.
11. Manimegalai S, Rakkimuthu G. Phytochemical Screening of Stem of *Couroupita guianensis*. IJPSR, 2012, 3(11).