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## ASSESSMENT OF CYTOTOXIC ACTIVITY OF TWO MEDICINAL PLANTS USING BRINE SHRIMP (*ARTEMIA SALINA*) AS AN EXPERIMENTAL TOOL

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**ABSTRACT:** *Solanum sisymbriifolium* (Solanaceae) and *Jatropha gossypifolia* (Euphorbiaceae) have long been used in folkloric medicine for the treatment of various ailments. The purpose of the present study was to evaluate the cytotoxic activity of the plants and to make available the process of data analysis of cytotoxic studies to the researchers. A general toxicity of the extracts was assessed by a simple and low cost assay using brine shrimp lethality as an indicator of toxicity. Ethanol crude extract and fractions of the extract of leaves of *Solanum sisymbriifolium* exhibited relatively mild cytotoxic activity compared to that of flowers and fruits against brine shrimp larvae with median lethal concentration, LC<sub>50</sub> values ranging from 10.10 – 321.35 and 13.19 – 80.16 µg/ml, respectively. Methanol crude extract and fractions of the extract of leaves of *Jatropha gossypifolia* exhibited significant toxicity against the shrimps (LC<sub>50</sub> values 17.19 – 98.19 µg/ml), while that of potassium permanganate, a positive control, was 11.27 µg/ml. Among the fractions of the crude extracts of both plants, non-polar fractions such as *n*-hexane, chloroform, dichloromethane were the most cytotoxic. These results warrant follow-up through bioassay directed isolation of the active principles.

**INTRODUCTION:** Around half of the drugs currently in clinical use as anticancer drugs are of natural product origin, and it has been estimated that about 60% of new chemical entities (NCEs) introduced in the 1981-2002 period in this field were natural products or were derived from a natural lead compound<sup>1</sup>. Therefore, the screening of traditional medicinal plants has great importance to identify new medicinal plants and to isolate new cytotoxic compounds for life threatening diseases like cancer. Among the cytotoxicity screening processes, brine shrimp lethality (BSL) bioassay represents a rapid (24 hours), simple (e.g., no aseptic techniques are required), easily mastered and inexpensive method. Moreover it requires small amounts of test material (2–20 mg or less)<sup>2</sup>.

Brine shrimp has been used as a “bench top bioassay” for the discovery and purification of bioactive natural products<sup>3</sup>. BSL bioassay was developed by Michael et al.<sup>4</sup> and modified by others<sup>5,6</sup>. A positive correlation has been found between brine shrimp toxicity and 9KB (human nasopharyngeal carcinoma) cytotoxicity, and have been observed that ED<sub>50</sub> values for general cytotoxicities are about one-tenth LC<sub>50</sub> values in the brine shrimp test<sup>5,7</sup>.

Since its introduction, this *in vivo* lethality test has been successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays once the active compounds have been isolated.

*Solanum sisymbriifolium* is a well known remedy in the ayurvedic and folkloric system of medicine for the treatment of various types of disorders. The leaves of *S. sisymbriifolium* are used as diuretic in Brazil and febrifuge in Peru<sup>8</sup>, while in India, flowers are used in traditional medicine as analgesic. The fruits of the plant are edible and unripe fruits are used as a vegetable. The roots have been reported to use as an emenagogue, fertility regulator, analgesic, hysteria, contraceptive, antisyphilitic and hepatoprotective in Argentina<sup>8</sup>. The roots also possess diuretic and hypertensive properties and used in Paraguayan traditional medicine. In Argentina, aerial parts of the plant are used to treat diarrhea, infections of respiratory and urinary tracts<sup>8</sup>.

Much like other members of the family *Solanaceae*, *S. sisymbriifolium* contains several secondary metabolites such as alkaloids, flavonoids, steroids and tannins<sup>9</sup>. The main chemical components of this plant have been reported by other researchers include solasodine (leaves)<sup>8</sup>; sisymbriifolin, carpesterol and solasodine (fruits)<sup>10,11</sup>; cuscohygrine, solacaproine, isonuatigenin-3-O- $\beta$ -solutriose (roots)<sup>8,12</sup>. The wide variety of the traditional uses of the plant may be due to the presence of these phytochemicals. Pharmacological studies have demonstrated that *S. sisymbriifolium* known to possess antinociceptive<sup>9</sup>, antihypertensive<sup>12</sup>, cardiovascular<sup>13</sup>, molluscicidal<sup>14</sup>, cytotoxic<sup>15</sup>, sedative and anticonvulsant<sup>11</sup> activities.

Different parts of *Jatropha gossypifolia* are used in traditional medicine in different countries to alleviate several ailments. The bark is considered an emmenagogue<sup>16</sup> and had been used to procure abortions. The leaves and seeds of *J. gossypifolia* are considered a purgative and are widely used to treat obstinate constipation. The leaves are also traditionally employed as febrifuge in intermittent fevers and swollen mammae<sup>16</sup> and also applied to sores, rash, boils, carbuncles, eczema, itches and venereal diseases in Latin America and the Caribbean<sup>17</sup>. Seeds are emetic, purgative and used for cancer and body pain<sup>16</sup>.

The oil obtained from them is used in indigenous medicine as an external stimulation, application in constipation, rheumatism and in paralytic affections, also in skin diseases<sup>16</sup>. Roots are used to treat leprosy<sup>16</sup>.

Stem sap used to stop bleeding and itching of cuts and scratches<sup>17</sup>. The stem latex also has been shown to possess coagulant activity<sup>18</sup>.

The wide variety of the traditional uses of the plant may be due to the presence of various phytochemicals in the plant like alkaloids (jatrophine), lignan (jatroiden)<sup>18</sup>, flavonoids, saponin, histamine, resin, tannin, curcin, jatrophone, jatropholones A and B, jatrophatrione, apigenin Cyclogossine A<sup>19</sup>. To establish its traditional uses, *J. gossypifolia* has been investigated for its anti-allergic, molluscicidal, antimicrobial, insect repellent<sup>19</sup>, larvicidal activity and antifeeding<sup>20</sup>, coagulating and anti-coagulating<sup>17</sup> activities.

The objective of this research work was to investigate the cytotoxic activity of the crude extracts and different solvent soluble fractions of crude extracts of *Jatropha gossypifolia* and *Solanum sisymbriifolium*. Moreover, through this report the process of calculation of LC<sub>50</sub> i.e., the dose that will kill 50% of animals of a particular species would be more available to the researchers.

## MATERIALS AND METHODS:

**Plant Collection and Identification:** The leaves, flowers and fruits of *S. sisymbriifolium* and leaves of *J. gossypifolia* were collected in the month of August, 2011 from Dhaka and Jhenidah, Bangladesh respectively. Collected plants were identified by a taxonomist (Dr. Bushra Khan, Principal Scientific Officer) from Bangladesh National Herbarium, Mirpur, where duplicate specimens for *S. sisymbriifolium* (Accession no. DACB-35894) and *J. gossypifolia* (Accession no. DACB-35937) have been deposited for future reference.

**Preparation of Crude Extract:** The dried, coarsely powdered plant materials (1 kg) were extracted by cold extraction process (5 L) over 72 hour period using ethanol as solvent for *S. sisymbriifolium* leaves, flowers & fruits and methanol as solvent for *J. gossypifolia* leaves at room temperature. The extracts were concentrated with a rotary evaporator (IKA, Germany) at low temperature (40-50°C) and reduced pressure. The dry crude extracts were kept in a refrigerator until use.

**Partitioning of the Crude Extracts:** Solvent-solvent partitioning was done by the modified Kupchan and Tsou method<sup>21</sup> by using a separating funnel. A portion (20 gm) of the crude extracts was triturated with 90% extraction solvents (methanol/ethanol) and the prepared solutions were then fractionated successively using solvents of increasing polarity. From each of the crude extract four fractions were obtained and fractions were evaporated to dryness by using rotary evaporator and kept in air tight containers for further analysis.

**Brine Shrimp Lethality Bioassay:** *Artemia salina* leaches (brine shrimp eggs) were collected and placed in one side of a small tank divided by a net containing simulated sea water (3.8% NaCl solution) at a temperature around 37°C equipped with constant oxygen supply by using an air pump for hatching and the pH of the hatching solution was maintained at 8.4<sup>22</sup>. In the other side of the tank, a light source was placed to attract the nauplii. After 24 hours of hatching period, the nauplii were ready for the experiment.

4 mg of each of the samples was dissolved in 200 µl of dimethylsulfoxide (DMSO) and the volume was adjusted to 5 ml using simulated seawater (pH was adjusted to 8.4). So the concentration of the stock solution was 800 µg/ml. A series of solutions of lower concentrations were prepared by serial dilution with seawater.

From each of these test solutions, 2.5 ml solution were added to pre-marked vials containing 2.5 ml of seawater and 10 shrimp nauplii. So, the final concentrations of samples in the vials were ranges from 400 µg/ml to 0.781 µg/ml. In the control vials, same volumes of DMSO (as in the sample vials) were taken as negative control and different concentration of potassium permanganate (KMN) was used as positive control. The vials were kept for 24 hours, thereafter the active nauplii were counted with the aid of a 3x magnifying glass and the percentage of mortality (rounded to nearest whole number) at each dose and control were determined by using the following formula (a):

% of mortality =

$$(\text{no. of dead nauplii} / \text{total number}) \times 100 \quad \text{-----}(a)$$

The mortality endpoint of this bioassay was defined as the absence of controlled forward motion during 30 sec of observation<sup>23</sup>. The experiment was carried out in triplicate and the average values were noted.

In case of 0 and 100 % of mortalities, the % of mortalities was then corrected by the following formulas (b & c) proposed by Ghosh<sup>24</sup>.

$$\text{For 0 \% mortality: } 100 \times (0.25 \times n) \quad \text{-----}(b)$$

$$\text{For 100 \% mortality: } 100 \times (n - 0.25/n) \quad \text{-----}(c)$$

Where, n= no. of test animal in each group.

Data must be corrected using Abbott's formula (d) if there is more than 10 % mortality in the control<sup>25</sup>.

Corrected % mortality =

$$\{(M_{\text{obs}} - M_{\text{control}}) / (100 - M_{\text{control}})\} \times 100 \quad \text{-----} (d)$$

Where,  $M_{\text{obs}}$  = observed % mortality;  $M_{\text{control}}$  = control % mortality

The corrected % of mortality for each concentration was then transformed to probit by using Finney's<sup>26</sup> probit table (**Table 1**). The purpose of the probit transformation is to straighten the line so that the  $LC_{50}$  can be estimated more easily.

The  $LC_{50}$  values (concentration of sample required to kill 50 % of brine shrimp) were calculated using Microsoft Excel 2007 by a plot of probit against the logarithm of the sample concentrations.

Mean and standard error of the mean (SEM) were calculated using the formulas = average(range);

and = (std. dev(range))/(sqrt(count(range))) respectively,

whereas confidence interval level of 95% was calculated using formula =

confidence (0.05, stdev(range), count(range)) in Microsoft Excel 2007.

TABLE 1: TRANSFORMATION OF PERCENTAGE OF MORTALITIES TO PROBITS

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
-	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

**RESULTS:** The data obtained during BSL bioassay of crude ethanol extract of leaves of *S. sisymbriifolium* and the steps of the calculation of LC<sub>50</sub> are presented in **table 2 and figure 1**. Besides the LC<sub>50</sub> value obtained for the crude extract, the LC<sub>50</sub> values of the fractions of the crude extracts and the positive control are also presented in **table 3**.

The LC<sub>50</sub> values of the crude extract and *n*-hexane, chloroform, carbon tetrachloride, aqueous fractions crude extract of *S. sisymbriifolium* leaves were found to be 52.50 µg/ml, 32.91 µg/ml, 10.10 µg/ml, 219.30 µg/ml and 321.35 µg/ml respectively (Table 3).

The crude extract and *n*-hexane, chloroform, carbon tetrachloride, aqueous fractions crude extract of *S. sisymbriifolium* flowers and fruits showed cytotoxicity with LC<sub>50</sub> values 23.84 µg/ml, 13.19 µg/ml, 80.16 µg/ml, 30.58 µg/ml and 70.83 µg/ml respectively (**Table 4**).

The LC<sub>50</sub> values of the crude extract and *n*-hexane, dichloromethane, carbon tetrachloride, aqueous fractions crude extract of *J. gossypifolia* leaves were found to be 17.19 µg/ml, 18.89 µg/ml, 50.03 µg/ml, 58.16 µg/ml and 98.19 µg/ml respectively (**Table 5**).

TABLE 2: RESULTS OF THE BSL BIOASSAY OF CRUDE ETHANOL EXTRACT OF LEAVES OF *S. SISYMBRIIFOLIUM*

Group	Dose (µg/ml)	Log dose	Nos. of dead shrimp	% of mortality	Corrected mortality	Probits
1	200	2.3	10	100	97.5	6.96
2	100	2.0	6	60	60	5.25
3	50	1.7	3	30	30	4.48
4	25	1.4	2	20	20	4.16
5	12.5	1.1	1	10	10	3.72
6	6.3	0.8	0	0	2.5	3.04

TABLE 3: CYTOTOXIC ACTIVITY OF LEAVES OF *S. SISYMBRIIFOLIUM*<sup>a</sup>

Plant	Part used	Tested materials	LC <sub>50</sub> (µg/ml, 24 h)	95% confidence limit (µg/ml)	Linear regression equation	R <sup>2</sup>
<i>Solanum sisymbriifolium</i>	Leaves	Crude extract	52.50 ± 1.04	50.46 - 54.54	y = 2.326x + 0.999	0.912
		<i>n</i> -hexane fraction	32.91 ± 1.73	29.51 - 36.31	y = 3.029x + 0.404	0.929
		CHCl <sub>3</sub> fraction	10.10 ± 1.34	7.47 - 12.73	y = 1.926x + 3.066	0.888
		CCl <sub>4</sub> fraction	219.30 ± 1.48	216.4 - 222.2	y = 1.513x + 1.458	0.978
		Aqueous fraction	321.35 ± 3.09	315.3 - 327.4	y = 1.720x + 0.688	0.981
		KMN <sup>b</sup>	11.27 ± 0.90	9.51 - 13.03	y = 3.022x + 1.821	0.790

CHCl<sub>3</sub>: Chloroform; CCl<sub>4</sub>: Carbon tetrachloride. <sup>a</sup> Values are represented as Mean ± SEM of three observations. <sup>b</sup> Reference standard.

TABLE 4: CYTOTOXIC ACTIVITY OF FLOWERS AND FRUITS OF *S. SISYMBRIIFOLIUM*<sup>a</sup>

Plant	Part used	Tested materials	LC <sub>50</sub> (µg/ml, 24 h)	95% confidence limit (µg/ml)	Linear regression equation	R <sup>2</sup>
<i>Solanum sisymbriifolium</i>	Flowers and fruits	Crude extract	23.84 ± 1.25	21.03 - 25.93	y = 3.056x + 0.791	0.979
		<i>n</i> -hexane fraction	13.19 ± 1.41	10.43 - 15.95	y = 1.682x + 3.116	0.951
		CHCl <sub>3</sub> fraction	80.16 ± 1.17	77.88 - 82.44	y = 2.364x + 0.499	0.950
		CCl <sub>4</sub> fraction	30.58 ± 1.31	28.01 - 33.15	y = 1.611x + 2.607	0.953
		Aqueous fraction	70.83 ± 2.05	66.81 - 74.85	y = 2.203x + 0.924	0.922

CHCl<sub>3</sub>: Chloroform; CCl<sub>4</sub>: Carbon tetrachloride. <sup>a</sup> Values are represented as Mean ± SEM of three observations.

TABLE 5: CYTOTOXIC ACTIVITY OF LEAVES OF *J. GOSSYPIFOLIA*<sup>a</sup>

Plant	Part used	Tested materials	LC <sub>50</sub> (µg/ml, 24 h)	95% confidence limit (µg/ml)	Linear regression equation	R <sup>2</sup>
<i>Jatropha gossypifolia</i>	Leaves	Crude extract	17.19 ± 1.82	13.61 - 20.77	y = 2.487x + 1.928	0.973
		<i>n</i> -hexane fraction	18.89 ± 0.59	17.74 - 20.04	y = 2.562x + 1.73	0.988
		DCM fraction	50.03 ± 1.13	47.82 - 52.24	y = 3.162x - 0.373	0.996
		CCl <sub>4</sub> fraction	58.16 ± 1.59	55.05 - 61.27	y = 2.473x + 0.636	0.894
		Aqueous fraction	98.19 ± 0.99	96.24 - 100.14	y = 2.644x - 0.267	0.938

DCM: Dichloromethane; CCl<sub>4</sub>: Carbon tetrachloride. <sup>a</sup> Values are represented as Mean ± SEM of three observations.

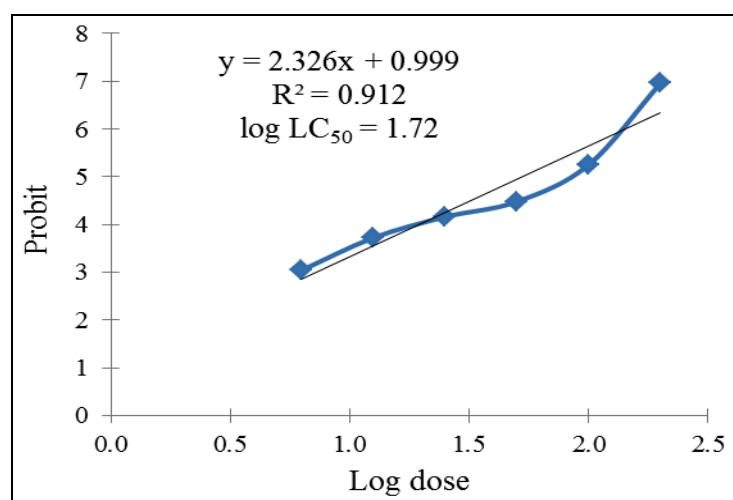


FIGURE 1: PLOT OF LOG DOSES VERSUS PROBITS FROM TABLE 2 FOR CALCULATION OF LC<sub>50</sub> OF CRUDE ETHANOL EXTRACT OF LEAVES OF *S. SISYMBRIIFOLIUM*.

**DISCUSSION:** In the present investigation, varying degree of lethality was observed with exposure to different dose levels of the test samples. The degree of lethality was found to be directly proportional to the concentration ranging from the lowest concentration to the highest concentration. In other words, mortality increased gradually with the increase in concentration of the test samples.

From the LC<sub>50</sub> values, it can be concluded that the flowers and fruits contain more potent cytotoxic compounds compared to leaves of *S. sisymbriifolium*. The LC<sub>50</sub> value of the crude extract of the leaves of *S. sisymbriifolium* was found to be 52.50 µg/ml whereas the value was 23.84 µg/ml for the flowers and fruits (Table 3, 4).

Among the fractions, carbon tetrachloride and aqueous fractions (Table 3) of crude extract of leaves of *S. sisymbriifolium* were virtually non-toxic on the shrimps as because they exhibited very low toxicity, giving LC<sub>50</sub> values greater than 100 µg/ml<sup>27</sup>. Lower LC<sub>50</sub> values of non-polar *n*-hexane (32.91 µg/ml) and chloroform (10.10 µg/ml) fractions of crude extract of leaves of *S. sisymbriifolium* indicate the presence of non-polar cytotoxic compounds. On the other hand, the fractions of crude extract of flowers and fruits of *S. sisymbriifolium* showed (Table 4) significant toxicity as their LC<sub>50</sub> values were ranges between 13.19 – 80.16 µg/ml.

The crude extract and all the fractions of crude extract of leaves of *J. gossypifolia* exhibited (Table 5) significant toxicity to brine shrimp as the LC<sub>50</sub> values were less than 100 µg/ml<sup>27</sup>. Cytotoxic activity decreased with the increase of polarity of the fractions. The *n*-hexane, non-polar fraction of the methanol extract showed the most cytotoxic activity, which indicates that the responsible cytotoxic agent could be non-polar in nature.

Among the twelve fractions, there are several highly active fractions of the crude extracts (LC<sub>50</sub>: around 20 µg/ml). Therefore, further isolation of the highly active fractions may lead to the discovery of new cytotoxic compounds. Several potent cytotoxic compounds such as apigenin, cyclogossine, jatropha-trione, jatrophone, jatropholone have already been isolated from different parts of *J. gossypifolia*<sup>19</sup>.

The methanol extract of flowers of *S. sisymbriifolium* was reported to be highly cytotoxic<sup>15</sup> but the cytotoxic activity of fruits and leaves of the plant have never been tested.

Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc.<sup>28</sup>. The plant extracts were found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the plants may contain antitumor, antibacterial or pesticidal compounds.

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#### REFERENCES:

1. Avendano C and Menendez JC: Medicinal Chemistry of Anticancer Drugs. Elsevier, First Edition 2008.
2. Colegate SM and Molyneux RJ: Bioactive Natural Products: Detection, Isolation and Structure Elucidation. CRC Press, Boca Raton, Edition 2, 1993:15-18.
3. Lieberman M: A brine shrimp bioassay for measuring toxicity and remediation of chemicals. Journal of Chemical Education 1999; 76(12): 1689-1691.
4. Michael AS, Thompson CG and Abramovitz M: *Artemia salina* as a test organism for a bioassay. Science 1956; 123: 464.
5. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 45: 31-34.
6. Solís PN, Wright CW, Anderson MM, Gupta MP and Phillipson JD: A microwell cytotoxicity assay using *Artemia salina*. Planta Medica 1993;59:250-252.
7. McLaughlin JL, Chang CJ and Smith DL: Frontiers in Natural Products Chemistry. Shamim Printing Press, First Edition 1990.
8. Ferro EA, Alvarenga NL, Ibarrola DA, Helliñon-Ibarrola MC and Ravelo AG: A new steroidal saponin from *Solanum sisymbriifolium* roots. Fitoterapia 2005; 76: 577-579.
9. Shilpi JA, Rouf R, Sarker MAM and Uddin SJ: Antinociceptive activity of methanolic extract of *Solanum sisymbriifolium* Lamk. Pakistan Journal of Biological Sciences 2005; 8: 1123-1125.
10. Chakravarty AK, Mukhopadhyay S, Saha S and Pakrashi SC: A neolignan and sterols in fruits of *Solanum sisymbriifolium*. Phytochemistry 1996; 41: 935-939.

11. Chauhan K, Sheth N, Ranpariya V and Parmar S: Anticonvulsant activity of solasodine isolated from *Solanum sisymbriifolium* fruits in rodents. Pharmaceutical Biology 2010; 49(2): 194-199.
12. Ibarrola DA, Helliñon-Ibarrola MC, Montalbetti Y, Heinichen O, Alvarenga N, Figueredo A and Ferro EA: Isolation of hypotensive compounds from *Solanum sisymbriifolium* Lam. Journal of Ethnopharmacology 2000;70:301-307.
13. Ibarrola D, Helliñon-Ibarrola M, Alvarenga N, Ferro E, Hatakeyama N and Shibuya N: Cardiovascular action of nuatigenosido from *Solanum sisymbriifolium*. Pharmaceutical Biology 2006; 44(5): 378-381.
14. Silva TM, Batista MM, Camara CA and Agra MF: Molluscicidal activity of some Brazilian *Solanum* spp. (Solanaceae) against *Biomphalaria glabrata*. Annals of Tropical Medicine and Parasitology 2005; 99(4):419-425.
15. Mamone L, Di Venosa G, Valla JJ, Rodriguez L, Gándara L and Batlle A: Cytotoxic effects of Argentinean plant extracts on tumor and normal cell lines. Cellular and Molecular Biology 2011;57:1487-1499.
16. Dhale DA and Birari AR: Preliminary screening of antimicrobial and phytochemical studies of *Jatropha gossypifolia* Linn. Recent Research in Science and Technology 2010; 2(7): 24-28.
17. Parvathi VS, Jyothi BS, Lakshmi T, Babu PS and Karthikeyan R: Morpho-anatomical and physicochemical studies of *Jatropha gossypifolia* (L.). Der Pharmacia Lettre 2012; 4(1): 256-262.
18. Oduola T, Adeosun GO, Tesleem, Oduola A, Awwioro GO and Oyeniyi MA: Mechanism of action of *Jatropha gossypifolia* stems latex as a haemostatic agent. European Journal of General Medicine 2005; 2(4): 140-143.
19. Khumrungsee N, Bullangpoti V and Pluempanupat W: Efficiency of *Jatropha gossypifolia* L. (Euphorbiaceae) against *Spodoptera exigua* hübner (Lepidoptera: Noctuidae): toxicity and its detoxifying enzyme activities. KKU Science Journal 2009; 37: 50-55.
20. Bullangpoti V, Wajnberg AE, Audantb BP and Feyereisenb R: Antifeedant activity of *Jatropha gossypifolia* and *Melia azedarach* senescent leaf extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use as synergists. Pest Management Science 2012; 68(9): 1255-1264.
21. Wagenen BCV, Larsen R, Cardellina II JH, Ran dazzo D, Lidert ZC and Swithenbank C: Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. Journal of Organic Chemistry 1993; 58: 335-337.
22. Khan A, Rahman M and Islam MS: Antibacterial, antifungal and cytotoxic activities of amblyone isolated from *Amorphophallus campanulatus*. Indian Journal of Pharmacology 2008;40(1):41-44.
23. Middleton P, Stewart F, Al-Qahtani S, Egan P, O'Rourke C and Abdulrahman A: Antioxidant, antibacterial activities and general toxicity of *Alnus glutinosa*, *Fraxinus excelsior* and *Papaver rhoeas*. Iranian Journal of Pharmaceutical Research 2005;2:81-86.
24. Ghosh MN: Fundamentals of Experimental Pharmacology. Scientific Book Agency, Second Edition 1984.
25. Abbot WS: A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925;18:265-267.
26. Finney DJ: Probit Analysis. Cambridge University Press, Third Edition 1952.
27. Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC and Nondo RSO: Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. BMC Complementary and Alternative Medicine 2007; 7:9. doi:10.1186/1472-6882-7-9
28. Anderson JE, Chang CJ and McLaughlin JL: Bioactive components of *Allamanda nodiflora* Linn. Journal of Natural Products 1988; 51: 307-308.

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