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Short Communication

CYTOTOXIC SCREENING OF THREE EGYPTIAN PLANTS USING BRINE SHRIMP LETHALITY TEST

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ABSTRACT

Objective: Brine shrimp (*Artemia salina* L.) bioassay is considered as a preliminary screening for the presence of antitumor compounds and used to determine the toxicity of plant extracts. Different extracts of three Egyptian plants, *philodendron selloum* (Araceae), *Bougainvillea alba* (Nyctaginaceae) and *Ulmus parvifolia* (Ulmaceae) were screened for their preliminary cytotoxicity.

Methods: The dry powdered leaves of the three plants under investigation (500 g from each plant) were extracted with 85% methanol; then undergo successive fractionation via petroleum ether, chloroform and ethyl acetate. The brine shrimp lethality test (BSLT) was carried out via taking 50 mg of *Artemia salina* Leach eggs in a hatching chamber and serial concentrations from each extract were used.

Results: The chloroform extract of *Philodendron selloum* showed the most potent cytotoxic activity at sub-lethal concentration (LC_{50}=16 µg/ml) followed by its methanol extract (LC_{50}=26 µg/ml). The methanol, chloroform and petroleum ether extracts of *Bougainvillea alba* plant showed the same cytotoxic effect at LC_{50}= 40 µg/ml. *Ulmus parvifolia* showed the least cytotoxic effect at LC_{50} =380 & 300 µg/ml for methanol and ethyl acetate extracts respectively.

Conclusion: It may be concluded that the tested plants possess strong cytotoxic activies; also this work suggests that plants extracts could possibly be used locally as naturally occurring cytotoxic agents.

Keywords: Brine Shrimp, *Artemia salina*, *Philodendron selloum, Bougainvillea alba*, *Ulmus parvifolia,* cytotoxicity.

The brine shrimp (*Artemia salina* L.) bioassay developed by Vanhaecke *et al.*, [1] as a useful tool for preliminary biological and pharmacological activity, it used to determine the toxicity of a wide variety of natural or synthetic products [2-4]. It is a crustacean belonging to subclass Branchiopoda, order Anostraca, occurring in brackish and marine water, adaptable to large range of salinity (5-250 g/l) and temperature (6-35 °C)[5]. The brine shrimp lethality test (BSLT) represents a rapid, inexpensive and simple bioassay for testing plant extracts lethality which in most cases correlated well with cytotoxic and antitumor properties [6]. Philodendron is a large genus of flowering plants in the Araceae family, consisting of close to 900 or more Philodendron species can be found in many diverse habitats in the tropical Americas and the West Indies; many are grown as ornamental and indoor plants. The chemical composition of the ethanol extract of the leaves and roots from *Philodendron imbe* Schott was investigated. The main constituents isolated from the leaves were; β-sitosterol, polyprenoid hexaprenol, and 6β-hydroxy-stigmast-4-en-3-one. A mixture of constituents namely; ethyl myristoleate, β-bisabolol, ethyl isopalmitate, 3-octadecenyl-phenol and ethyl palmitate were isolated from the roots [7]. Hydroxy cinnamic acid amides, amine derivatives and aromatic amines are abundant in philodendron species [8]. Bougainvillea is a genus of flowering plants native to South America from Brazil west to Peru and South to Southern Argentina. *B. glabra* bracts accumulated large amounts of flavonols (kaempferol and quercetin+conjugates) [9]. Momordin (quinoside-D)-[β-D-glucopyranosyl-3-*O*-[β-D-xylo pyranosyl-(1→3)-*O*-(β-D-glucopyranosyluronic acid)] oleanolate, quercetin-3-*O*-α-L-(rhamnopyranosyl) (1→6)-[α-L-rhamno pyranosyl (1→2)]-β-D-galactopyranoside and quercetin 3-*O*-α-L-(4-caffeoylrhamnopyranosyl) (1→6)-[α-L-rhamno pyranosyl (1→2)]-β-D-galactopyranoside were also isolated from *B. glabra* [10]. Ulmus species (Ulmaceae) are deciduous and semi-deciduous trees. It was cultivated as an ornamental plant by virtue of their rapid growth and variety of foliage and forms. Ulmus species are rich with triterpene [11-13], sesquiterpenes [14, 15] and flavonoids [16, 17]. *Ulmus davidiana* showed antimicrobial activity [18]. *Ulmus pumila* was used in traditional medicine to treat edema, mastitis, gastric cancer and inflammation [19-21]. To our knowledge, there is a little information about the reported chemical constituents and biological activities of the selected species. On the light of these words; the preliminary cytotoxic activity of three plants will be evaluated using BSLT for the first time upon such species, so this work suggests that plants extracts could possibly be used locally as naturally occurring cytotoxic agents.

Three local plants were selected for this study; *Philodendron selloum, Bougainvillea alba* and *Ulmus parvifolia.* The leaves of *Philodendron selloum* were collected from many gardens in El-Giza Governorate. Leaves of *Bougainvillea alba* were collected from of Banha Agriculture University gardens. Leaves of *Ulmus pravifolia* (Ulmaceae) were collected from El-Orman garden, El-Giza Governorate. The plants were identified by Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Giza, Egypt and authenticated at herbarium of Medicinal Chemistry Department, Theodor Bilharze Research Institute. The plants were undergo shade drying at room temperature and finally powdered by electric mill. The dry powdered leaves (500 g from each plant) were extracted with 85% methanol. Then; the crude methanol extracts were successively extracted with petroleum ether, chloroform and ethyl acetate; all extracts were evaporated under reduced pressure using rotatory evaporator (Bushi, Rotavapour L114). The crude extracts stored in dry clean and dark glass bottles till used.

A solution of instant ocean sea salt (Aquarium System, Ohio) was made by dissolving 2.86 g in distilled water (75 ml). 50 mg of *Artemia salina* Leach eggs (Artemia, Inc., California) was added in a hatching chamber [22]. The hatching chamber was kept under an inflorescent bulb for 48 h for eggs to hatch into shrimp larvae. 20 mg of the tested extract was dissolved in 2 ml of methanol or solvent in which it was soluble and from this, 500, 400, 300, 200, 100, 50, 5 µl of each solution was transferred into vials corresponding to 1000, 800, 600, 400, 200,100, and 10 µg/ml respectively. Each dose was tested in triplicate. The vials and the control containing 500 µl of solvent were allowed to evaporate to dryness in about 48 h at room temperature. 4.5 ml of instant ocean sea solution were added to each vial and 10 larvae of *Artemia salina* (taken 48-72 h after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5 ml with sea salt solution

immediately after adding the shrimp. 24 h later the number of surviving shrimp at each dosage was counted and recorded. LC_{50} values were determined with 95% confidence intervals by analyzing the data. The data were analyzed and LC_{50} values were calculated and carried according to Reed-Muench method. The Reed-Muench method assumes that an animal that survived a given dose would also have survived any lower dose, and conversely, that an animal that died with a certain dose would have also died at any other higher dose. Thus, the information from anyone group can be added to that of the other groups in the range of dose tested [23].

Table 1: Mean % mortality of brine shrimp larvae after 24 h of exposure to different concentrations of *Philodendron selloum* extracts

Concentration	Mean % mortality of brine shrimp[1]			
Of plant extract	Petroleum ether	Methanol	Ethyl acetate	Chloroform
1000	98.2	97.7	97.2	95.6
800	96.6	93.7	88.7	91.3
600	92.7	91.3	79.8	88.7
400	84.7	86.7	69.9	85.5
200	75.8	78.6	58.5	78.4
100	59.4	70.3	40.6	65.7
10		30.6	18.5	48.2

[1]:Mean % mortality of brine shrimp larvae after 24 h (n=3)

Table 2: Mean % mortality of brine shrimp larvae after 24 h of exposure to different concentrations of *Bougainvellea alba* extracts

Concentration	Mean % mortality of brine shrimp[1]		
of plant extract	Methanol	Petroleum ether	Chloroform
1000	99	100	97
600	90	96	89
200	69	79	72
100	58	77	57
10	34	27	33

[1]:Mean % mortality of brine shrimp larvae after 24 h (n=3)

Table 3: Mean % mortality of brine shrimp larvae after 24 h of exposure to different concentrations of *Ulmus parvifolia* extracts

Concentration	Mean % mortality of brine shrimp[1]	
Of plant extract	Ethyl acetate	Methanol
1000	100	100
600	91	78
400	57	55.8
200	15.4	36
100	5	24.2
10	3.8	6.1

[1]:Mean % mortality of brine shrimp larvae after 24 h (n=3)

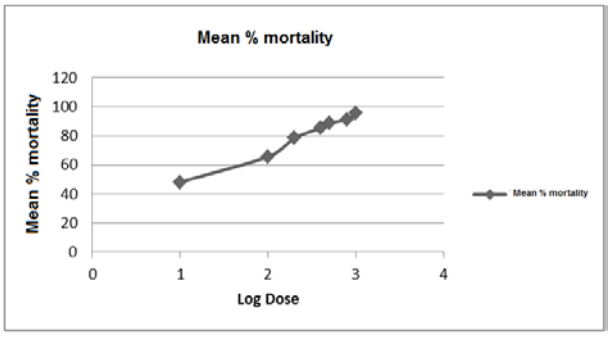

Fig. 1: Estimation of LC_{50} by plot mean % mortality of brine shrimp larvae against different dosage of *Philodendron selloum* chloroform extract

Three plants; *Philodendron selloum, Bougainvillea alba* and *Ulmus parvifolia* were randomly collected from different gardens where they are cultivated as ornamental plants and tested for their toxicity using brine shrimp test. Criterion of toxicity for fraction, compound or plant extract was established according to Deciga-Campos *et al.*, [24]as LC_{50} values above 1000 µg/ml are non toxic, between 500 & 1000 µg/ml are weak toxic, and that below 500 µg/ml are toxic. The chloroform extract of *P. selloum* showed the most potent toxic effect at LC_{50}= 16 µg/ml followed by its methanol extract which showed cytotoxicity at LC_{50}= 26 µg/ml, petroleum ether and ethyl acetate extracts showed a significant cytotoxic effect at LC_{50}= 70 & 160 µg/ml respectively (tables 1, 4 and fig. 1); which may indicate the different nature of the chemical constituents for each extract.

Table 4: Cytotoxic activity of different extracts of the selected plants

Plant/extract	(LC_{50}+SD/SEM)[1] (CL)[2]
Bougainvellea alba:	
Methanol	40+0.56/0.40 (46.4–33.6)
Chloroform	40+1.20/0.40 (46.4–33.6)
Petroleum ether	40+0.85/0.41 (46.7–33.3)
Philodendron selloum:	
Methanol	26+0.55/0.15 (28–24)
Ethyl acetate	160+0.75/0.44 (168–152)
Chloroform	16+1.45/0.43 (23.5–8.5)
Petroleum ether	70+1.25/0.23 (73–67)
Ulmus pravifolia:	
Methanol	380+1.90/0.56 (313–287)
Ethyl acetate	300+0.95/0.20 (382.5–377.5)

[1]Results are expressed as Means±Standard deviation/Standard Error Mean (SD/SEM) (n=3)., [2]95% confidence limits (CL) in parentheses

The methanol, petroleum ether and ethyl acetate extracts of *B. alba* showed equal potent cytotoxic effect at LC_{50}= 40 µg/ml (Tables 2, 4); which indicate high similarities in nature of the chemical constituents for the three extracts. *Ulmus pravifolia* showed the least activity at LC_{50}= 380 & 300 µg/ml for methanol and ethyl acetate extracts (tables 3, 4). According Deciga-Campos criteria all the extracts showed significant cytotoxic activity; suggesting a need to isolate and evaluate their constituents.

CONCLUSION

The current study showed the cytotoxic activity of 85% methanolic extracts of three Egyptian plants, *philodendron selloum*, *Bougainvillea alba* and *Ulmus parvifolia* via BSLT. *P. selloum* showed the most potent toxic effect followed by *B. alba* and *U. pravifolia.* It was concluded that such plants can be used as a source of naturally occurring cytotoxic agents.

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CONFLICT OF INTERESTS

There is no conflict of interest associated with the authors of this paper.

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