

# Brine Shrimp Lethality Assay of Some Medicinal Plants Using *Artemia franciscana* and *Artemia salina*

C. Kalai Selvi

Department of Zoology, Sivanthi Aditanar College, Pillayarapuram, Kanyakumari District, Tamilnadu, India -629501

**Abstract:** This is to determine whether *Artemia franciscana* can be used to test toxicity of plants extract. Brine shrimp lethality assay was performed with aqueous, methanol, hexane and chloroform extracts of 16 medicinal plants frequently used in the Sidha and Ayurveda systems of traditional medicine. Hexane extracts of these plants were most toxic to the Brine Shrimp nauplii, followed by methanol extract, chloroform extract and aqueous extract regarding the lethal dose  $LC_{50}$  of *Artemia*. All these extracts gave positive results in the brine shrimp lethality test and the shrimp mortality increased with the concentration of extract in the test samples. The  $LC_{50}$  values of *Andrographis paniculata*, *Andrographis lanceolata*, *Adhatoda vasica*, *Acalypha indica*, *Phyllanthus amarus*, *Vigna trilobata*, *Indigofera tinctoria*, *Leucas aspera*, *Ocimum basilicum*, *Evolvulus alsinoides* were greater than 1000 $\mu$ g/ml for *Artemia franciscana* and *A. salina*. The  $LC_{50}$  values of *Annona squamosa*, *Centella asiatica*, *Gymnema sylvestre*, *Tylophora indica* and *Ricinus communis* were between 500 $\mu$ g and 1000 $\mu$ g/ml while those of *Vinca rosea* and *Ricinus communis* were less than 500 $\mu$ g/ml but not less than 100 $\mu$ g/ml. Therefore, all these plants can be used in the preparation of medicines without fear of acute toxicity about these medicinal plants. The correlation coefficient between *A. franciscana* and *A. salina* was only  $\pm 0.02$  so that both species may be employed in the brine shrimp lethality test for plant extracts. This investigation recommends that *Artemia franciscana* found in Kanyakumari district is suitable animal for the screening of phytochemicals in plants for certain physiological activities and pharmacological potentials.

**Keywords:** *Artemia franciscana*, brine shrimp lethality, plant extract,  $LC_{50}$ , mortality.

## 1. Introduction

In recent years, there has been an alternative trend to use lower animals as substitutes for laboratory animals such as mice and rats in toxicological tests because of their high cost and sufferings from the impact of the tests. These alternative methods replace the experiments that use large laboratory animals, reduce the number of animals used in the tests and refine the existing techniques for reducing pain and stress, which are the three main goals of Animal Welfare and Ethics (Gadir, 2012). The brine shrimp *Artemia* is one of the simple marine organisms that can be bred easily under laboratory conditions (Michael, *et al.*, 1956) and used to test the toxicity conditions since it has effective range of tolerance in most toxicity testing (Hossain *et al.*, 2009).

The brine shrimp, *Artemia* is a crustacean closely related to shrimp belonging to the family Artemiidae of the phylum Arthropoda. Adult *Artemia* measures the average length of 8mm, but it can reach lengths up to 20mm under optimum environmental conditions and sufficient nutritional supply. The male *Artemia* possesses a paired penis in the posterior part of its trunk and a pair of claspers at the anterior end while the female *Artemia* has brood pouch in the posterior part of the trunk, but no claspers. This is the rule in all the sexually reproducing species of *Artemia*, but in parthenogenetic species all individuals are female and resemble the female *Artemia* of sexual species. Based upon a complex pattern of microspeciation, *A. franciscana*, *A. monica*, *A. persimilis*, *A. funicihana*, *A. salina* and *A. urmiana* are sexual species of *Artemia* found in different brackish water areas of the world, and all the strains of parthenogenetic species are together called *A. parthenogenetica* (Abreu-Grobois and Beardmore, 1980).

The hatching rate of *Artemia* cysts after exposure to pesticides, petroleum products, carcinogens and other contaminants has been used to determine the toxicity level in samples (Anubha, 2007). *Artemia* Lethality Assay has also been applied to screen the toxicity of plant extracts (Meyer *et al.*, 1982; McLaughlin *et al.*, 1998a; Moshi *et al.*, 2010; Ogugu *et al.*, 2012; Gadir, 2012; Solanki and Selvanayagam, 2013; Sharma *et al.*, 2013), toxicity of heavy metals (Sleet and Brendel, 1985; Martínez *et al.*, 1999) and metal ions (Kokkali *et al.*, 2011), toxicity of cyanobacteria (Jaki *et al.*, 1999) and algae (Mayorga *et al.*, 2010), cytotoxicity of dental materials (Pelka *et al.*, 2000), toxicity of nanoparticles (Maurer- Jones *et al.*, 2013), and screening of some natural products (Carballo *et al.*, 2002). Although all stages in the lifecycle of *Artemia* are suitable for toxicity testing, nauplii after 48 hours of hatching are suitable for bioassay (Novakova *et al.*, 2008). Lethal concentration of toxic substances for 50% mortality of *Artemia* after 6 hours of exposure is considered to be the lethal dose (acute  $LD_{50}$ ) and this concentration is calculated for the total fluid content of humans to determine the  $LD_{50}$  value of the substance for humans (Novakova *et al.*, 2008).

*Andrographis paniculata* (Acanthaceae) is astringent, anodyne and alexipharmic, used in the treatment of cholera, diabetes, influenza, bronchitis, itches, piles, jaundice, and helminthes infestations (Farnsworth and Soejarto, 1991). More like this, *Andrographis lanceolata* is also employed in the treatment of cholera, diabetes, influenza, bronchitis, itches, piles, jaundice, and helminthes infestations. *Adhatoda vasica* (Acanthaceae) is rich in the active principle vasicine, which is useful in the treatment of bronchitis, diarrhea, hemorrhage, dysentery and glandular tumors (Kamala

Ambasta, 1992). *Acalypha indica* (Euphorbiaceae) is a laxative containing a cynogenetic glucoside and alkaloid acalyphine, which is used in the ailment of cough, gastro-intestinal irritations, bleeding and skin troubles of different kinds (Farnsworth and Soejarto, 1991). *Annona squamosa* (Annonaceae) is an abortifacient and insect repellent to expel mosquitoes. *Centella asiatica* (Apiaceae) is a diuretic herb that is used in the treatment of leprosy, jaundice, low memory, poor health and stamina, which are mainly due to the presence of the glucoside asiatoside. *Evolvulus alsinoides* (Convolvulaceae) is a good febrifuge and vermifuge included in many medicinal preparations being used to cure human diseases. *Gymnema sylvestre* (Asclepiadaceae) contains the alkaloid gymnemic which is the active principle curing diabetes, dysentery and stomach disorders. *Indigofera tinctoria* (Fabaceae) is used in the treatment of epilepsy, nervous disorders, sores, ulcers, urinary complaints, hepatitis and breeding. *Leucas aspera* (Lamiaceae) is given for psoriasis, skin eruptions, cough and colds (Kamala Ambasta, 1992). *Ocimum basilicum* (Lamiaceae) is found to have stomachic, alexipharmic, antipyretic, diaphoretic, expectorant, carminative, stimulant and anthelmintic properties, for which it is used in medicinal preparations for ringworms, sinuses, cough and respiratory problems. *Phyllanthus amarus* (Euphorbiaceae) has astringent, febrifuge, stomachic, diuretic and febrifuge properties, for which it is employed in the treatment of diarrhea, dysentery, colic, dropsy, sores, jaundice and diseases of urino-genital system. *Ricinus communis* (Euphorbiaceae) contains ricinoleic acid that has medicinal values in the treatment of boils and sores. *Tylophora indica* (Asclepiadaceae) is stimulant, emetic, cathartic, expectorant, stomachic and diaphoretic and hence useful in asthma, bronchitis, whooping cough, dysentery, diarrhea, rheumatism and gouty pains; leaves of this plant contains the alkaloids tylophorine and tylophorenine. *Vigna trilobata* (Fabaceae) is used in irregular fever (Kamala Ambasta, 1992). *Vinca rosea* has carminative, vomitive, hemostatic, depurative, hypotensive, astringent and diuretic properties, for which it is used in the treatment of hypertension, diarrhea, dysentery and phthisis, which are mainly due to vincamine alkaloid (Kamala Ambasta, 1992).

*Artemia salina* has been the potential animals for such assays but *Artemia franciscana* has only been used in the toxicity testing of radiations and some pollutants in the water bodies. The present study attempts to use *Artemia franciscana* to determine whether it is suitable to test the toxicity of Indian plants as *A. salina* can be used.

## 2. Materials and Methods

Leaves of test plants were collected from the local areas of Kanyakumari district of Tamilnadu during December 2017 and washed with clean water to remove dirt. The clean leaves were dried under shade at room temperature. The dried leaves were ground into a fine powder, the active principle of which was extracted from 25g powder using 200ml of water, chloroform, ethyl alcohol and hexane separately. The extract was filtered and the filtrate was concentrated under vacuum. 20mg of each extract was dissolved in 10 ml of pure dimethyl sulfoxide (DMSO) to get stock solutions of 2mg/ml. Experiments were conducted

along with control (DMSO) and different concentrations (10µg, 100µg, 1000µg, 2000µg, 3000µg, 4000µg, 5000µg, 6000µg, and 7000µg/ml of artificial seawater medium) of the test substances.

Cysts of *Artemia franciscana* were hatched in a conical flask (1L) filled with sterile artificial seawater prepared using sea salt 38 g/L (pH 8.5) under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each test tubes containing 5ml of test solutions. The tubes were maintained at room temperature for 24 hours under the light. The experiment was done in triplicate. The test tubes were inspected using a magnifying glass against a black background and the number of survived nauplii in each tube was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration and the observed mortality percentage was plotted against the concentration in a graph from which the median lethal concentration (LC<sub>50</sub>) was estimated. The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. LC<sub>50</sub> values were obtained by best-fit line method.

## 3. Results and Discussion

Hexane extracts of these plants were most toxic to the Brine Shrimp nauplii, followed by methanol extract, chloroform extract and aqueous extract regarding the lethal dose LC<sub>50</sub> of *Artemia* (Table 1 and 2). In the LC<sub>50</sub> bioassay, it is established that LC<sub>50</sub> values >1000µg/ml are non toxic, that LC<sub>50</sub> values ≥ 500 ≤ 1000 µg/ml are weakly toxic, and that LC<sub>50</sub> values < 500 are toxic (Deciga-Campos *et al.* 2007; Bastos *et al.*, 2009). Aqueous extracts of *Andrographis paniculata*, *Andrographis lanceolata*, *Adhatoda vasica*, *Acalypha indica*, *Phyllanthus amarus*, *Vigna trilobata*, *Indigofera tinctoria*, *Leucas aspera*, *Ocimum basilicum*. *Evolvulus alsinoides* had the LC<sub>50</sub> values greater than 1000µg/ml for *Artemia franciscana* and *A. salina*. On the other hand, extracts of *Annona squamosa*, *Centella asiatica*, *Gymnema sylvestre*, *Tylophora indica* and *Ricinus communis* had LC<sub>50</sub> values between 500µg and 1000µg/ml, which imply that these were weakly toxic to both *Artemia salina* and *A. franciscana*. Extracts of *Vinca rosea* and *Ricinus communis* had LC<sub>50</sub> values less than 500µg/ml for both these experimental animals and hence they are toxic materials which have to be used in low quantity in medicinal preparations. Methanol extracts of *Andrographis paniculata*, *Andrographis lanceolata*, *Adhatoda vasica*, *Acalypha indica*, *Phyllanthus amarus*, *Vigna trilobata*, *Indigofera tinctoria*, *Leucas aspera*, *Ocimum basilicum*. *Evolvulus alsinoides* had the LC<sub>50</sub> values greater than 1000µg/ml for *Artemia franciscana* and *A. salina*. Methanol extracts of *Annona squamosa* and *Centella asiatica* had LC<sub>50</sub> values between 500µg and 1000µg/ml, which imply that these were weakly toxic to both *Artemia salina* and *A. franciscana*. Extracts of *Vinca rosea*, *Gymnema sylvestre*, *Tylophora indica* and *Ricinus communis* and *Ricinus communis* had LC<sub>50</sub> values less than 500µg/ml for both these experimental animals.

**Table 1:** Concentration of plant extract ( $\mu\text{g}$ ) leading to 50% mortality of *Artemia franciscana*

Plant source	Concentration of plant extract ( $\mu\text{g}$ ) causing 50% mortality of <i>Artemia</i>			
	Aqueous extract	Methanol extract	Chloroform extract	Hexane extract
<i>Andrographis paniculata</i>	3620	2500	2700	2340
<i>Andrographis lanceolata</i>	3710	2621	2821	2420
<i>Adhatoda vasica</i>	5101	4201	4420	3800
<i>Annona squamosa</i>	827	525	614	445
<i>Centella asiatica</i>	828	525	745	365
<i>Vinca rosea</i>	325	185	200	163
<i>Gymnema sylvestre</i>	820	640	764	560
<i>Tylophora indica</i>	460	210	260	170
<i>Ricinus communis</i>	487	172	198	153
<i>Acalypha indica</i>	7500	5000	5700	4300
<i>Phyllanthus amarus</i>	3950	2900	3100	2600
<i>Vigna trilobata</i>	2340	1400	1500	1100
<i>Indigofera tinctoria</i>	5030	3500	3700	3000
<i>Leucas aspera</i>	2100	1900	2000	1600
<i>Ocimum basilicum</i>	3100	2200	2400	1800
<i>Evolvulus alsinoides</i>	5354	3254	3455	2754

Chloroform extracts of *Andrographis paniculata*, *Andrographis lanceolata*, *Adhatoda vasica*, *Acalypha indica*, *Phyllanthus amarus*, *Vigna trilobata*, *Indigofera tinctoria*, *Leucas aspera*, *Ocimum basilicum*, *Evolvulus alsinoides* had the  $\text{LC}_{50}$  values greater than  $1000\mu\text{g/ml}$  for *Artemia franciscana* and *A. salina*; extracts of *Annona squamosa* and *Centella asiatica* had  $\text{LC}_{50}$  values between  $500\mu\text{g}$  and  $1000\mu\text{g/ml}$ ; extracts of *Vinca rosea*, *Gymnema sylvestre*, *Tylophora indica* and *Ricinus communis* had  $\text{LC}_{50}$  values less than  $500\mu\text{g/ml}$  for both these experimental animals. Hexane extracts of *Andrographis paniculata*, *Andrographis lanceolata*, *Adhatoda vasica*, *Acalypha indica*, *Phyllanthus amarus*, *Vigna trilobata*, *Indigofera tinctoria*, *Leucas aspera*, *Ocimum basilicum*, *Evolvulus alsinoides* had the  $\text{LC}_{50}$  values greater than  $1000\mu\text{g/ml}$  for *Artemia franciscana* and *A. salina*; extracts of *Annona squamosa* and *Centella asiatica* had  $\text{LC}_{50}$  values between  $500\mu\text{g}$  and  $1000\mu\text{g/ml}$ ; the extracts of *Vinca rosea*, *Gymnema sylvestre*, *Tylophora indica* and *Ricinus communis* had  $\text{LC}_{50}$  values less than  $500\mu\text{g/ml}$  for both these experimental animals.

**Table 2:** Concentration of plant extract ( $\mu\text{g}$ ) leading to 50% mortality of *Artemia salina*

Plant source	Concentration of plant extract ( $\mu\text{g}$ ) causing 50% mortality of <i>Artemia</i>			
	Aqueous extract	Methanol extract	Chloroform extract	Hexane extract
<i>Andrographis paniculata</i>	3510	2450	2620	2230
<i>Andrographis lanceolata</i>	3620	2531	2711	2320
<i>Adhatoda vasica</i>	5210	4300	4530	3950
<i>Annona squamosa</i>	815	510	600	430

<i>Centella asiatica</i>	840	540	765	380
<i>Vinca rosea</i>	310	170	180	143
<i>Gymnema sylvestre</i>	800	600	730	520
<i>Tylophora indica</i>	550	200	240	130
<i>Ricinus communis</i>	500	200	210	203
<i>Acalypha indica</i>	7500	5000	5700	4000
<i>Phyllanthus amarus</i>	3850	2800	3000	2500
<i>Vigna trilobata</i>	2240	1300	1400	1000
<i>Indigofera tinctoria</i>	4930	3450	3650	2500
<i>Leucas aspera</i>	2000	1800	1900	1500
<i>Ocimum basilicum</i>	3000	2100	2300	1700
<i>Evolvulus alsinoides</i>	5435	3364	3565	2874

The lethality of a test sample to the shrimp (*Artemia salina*) was utilized by Meyer *et al.* (1982) to demonstrate the toxicity of substances in the test samples, which has been a helpful tool to screen a wide range of chemical compounds for their various bioactivities. This bioassay correlates reasonably well with cytotoxic and other biological properties (McLaughlin *et al.*, 1991). The brine shrimp bioassay has been established as a safe, practical and economic method for determination of bioactivities of synthetic compound (Almeida *et al.*, 2002) as well as plant products (Meyer *et al.*, 1982). In toxicity evaluation of plant extracts by Brine shrimp lethality bioassay  $\text{LC}_{50}$  values lower than  $1000\mu\text{g/ml}$  are considered bioactive (Meyer *et al.*, 1982). Therefore, extracts of *A. squamosa*, *C. asiatica*, *G. sylvestre* and *T. indica* were found to have active principles which have medicinal values. Extracts of *R. communis* and *V. rosea* had some toxic principles that kill *Artemia*. Further, this bioassay also indicates antifungal effects, pesticidal effects, teratogenic effects, and toxicity to environment (Vanhaecke *et al.*, 1981). The mortality rate due to the extracts was found to be directly proportional to the concentration of the extracts (Hossain *et al.*, 2009).

The shrimp mortality was due to the presence of certain secondary metabolites in the plant extracts (Dhar *et al.*, 1973; Badami *et al.*, 2003; Krishnaraju *et al.*, 2005). The secondary metabolites are alkaloids, glycosides, lignin derivatives, sapanins, tannins, anthraquinones flavonoids, phenolics and iridoids (Shankar *et al.*, 2009; Yadav *et al.*, 2008; Tiwari *et al.*, 2008; David *et al.*, 2012). (The brine shrimp lethality was maximum if the  $\text{LC}_{50}$  value was less than  $100\mu\text{g/ml}$ . No one plant chosen for this test showed  $\text{LC}_{50} > 100\mu\text{g/ml}$ . The high toxicity of methanolic extract of leaf probably attributed to the alkaloid that is confirmed in phytochemical screening.

The correlation coefficients ( $r$ ) between the bioassay using *Artemia franciscana* and *A. salina* were not different significantly ( $p > 0.05$ ). The  $r$  values were  $+0.02$  for *Andrographis paniculata* and *Andrographis lanceolata*,  $+0.03$  for *Adhatoda vasica*, *Gymnema sylvestre* and *Ricinus communis*  $-0.02$  for *Acalypha indica* and *Phyllanthus amarus*,  $+0.01$  for *Vigna trilobata*, *Indigofera tinctoria*, *Leucas aspera*, *Ocimum basilicum* and *Evolvulus alsinoides* and  $-0.01$  for *Annona squamosa* and *Centella asiatica*. The correlation coefficients ( $r$ ) between these two species of

*Artemia* were only within the range of 0.01 to 0.03, which implies that both these species can be used in the brine shrimp lethality assay of plant extracts. Works of Carbello *et al* (2002), Veni and Pushpanathan (2004), Gosh *et al* (2015), and Quazi Sahely Sarah *et al* (2017) also agree with the present finding that both *A. franciscana* and *A. salina* behave differently in the brine shrimp lethality assays but the difference was so little as to show only minor difference in the results because of the genomic difference among the species. Zapata *et al.* (1990) and Agh *et al.* (2008) also concluded that the heterogeneity of *Artemia* species is a critical factor in determining the survival even in the same salinity condition of a natural habitat. Since *A. franciscana* and *A. salina* were quite different depending upon their difference in genetic composition, the genetic effect in controlling survival rate may be the reasons for difference in the tolerance limit of *Artemia* species (Michael Babu,1999; Immanuel *et al.*, 2002).

#### 4. Conclusion

From these results it is clear that, more like *A. salina* being used in the brine shrimp lethality assay, *A. franciscana* can be used to test the toxicity of plant extracts, and that the lethality of shrimp is due to cytotoxicity of the active principles in the plant extracts. The LC50 value between 100µg and 1000µg showed the presence of bioactive compounds which serve as active principles in the medicinal plants and hence this test would be a useful tool to screen a wide range of chemical compounds for their various bioactivities. This assay has been utilized to screen physiologically active plant extracts having some cytotoxic and other biological properties. This work further confirms that brine shrimp bioassay is a safe, practical and economic method for determination of bioactivities of plant –based medicinal formulations. All these plants may be used in the preparation of medicines without fear of acute toxicity about these medicinal plants because of their LC<sub>50</sub> values above 100µg/ml. If this assay is standardized as it should be, it may be adopted to test the toxicity of all the plants in the world.

#### References

- [1] Abreu-Grobois, F.A. and J.A. Beardmore (1980) International Study on Artemia-II. Genetic Characterization of *Artemia* Populations-An Electrophoretic Approach. In: The Brine Shrimp Artemia, Vol.I. Morphology, Genetics, Radiobiology, Toxicology. Persoone.G., P. Sorgeloos, O. Roels and E. Jaspers (Eds.), Universa Press, Wetteren, Belgium, pp.133-146.
- [2] Agh, N., G. Van Stappen, P. Bossier, A. Mohammad Yari, H. Rahimian and P.Sorgeloos (2008) Life Cycle Characteristics of Six *Artemia* Populations from Iran. *Pak. J. Biol. Sci.* 11(6):854-861.
- [3] Almeida, N.T., V. D'Almeida and M. Pustiglione (2004) The effect of fluorine and homeopathic medicines in rats fed cariogenic diet. *Homeopathy*, Vol.93 (3): 138-143.
- [4] Anubha S. K. (2007) Brine Shrimp (*Artemia salina*)-A Marine Animal for Simple and Rapid Biological Assays. *Journ. Chinese. Clin. Med.* Vol2(4):236-240.
- [5] Bastos, M.L.A., M. R. F. Lima, L.M. Conserva, V.S. Andrade, E.M.M Rocha and R.P.L. Lemos. (2009). *Ann of Clin Microbiol Antimicrob.* **2009**; 8(16): 1-6.
- [6] Carballo, J. L., Z.L. Hernández-Inda, P. Pérez, and M.D. García-Grávalos, (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotech.* 2, p.17.
- [7] David, P., T. Angamuthu, A. Karuppanan and N.S. Sreenivasapuram. (2012). Potent *in vitro* cytotoxic effect of *Gmelina arborea* Roxb. (Verbenaceae) on three human cancer cell lines. *Int J Pharm Sci Res* 2012; 3(4): 357-363.
- [8] Deciga-Campos, M., I. Rivero-Cruz, M. Arriaga-Alba, G. Castaneda- Corral, G.E. Angeles- Lopez and A. Navarrete. (2007). Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. *J Ethnopharmacol.* 110: 334-342.
- [9] Dhar, M. L., M.N. Dhar and B.N. Dhawan.(1973). Screening of Indian medicinal plants for biological activity. *Ind. J. Expt. Biol.*, 11: 43- 45.
- [10] Farnsworth, N. R. and D.D. Soejarto, (1991). Global importance of medicinal plants. In: Akerele, O., Heywood, V., and Syngé, H. (Eds.), *The Conservation of Medicinal Plants*. Cambridge University Press, Cambridge: 25-51.
- [11] Gadir, S.A. (2012). Assessment of bioactivity of some Sudanese medicinal plants using Brine Shrimp (*Artemia salina*) Lethality Assay. *J Chem Pharm Res.* 4, 5145-5148.
- [12] Ghosh, A., S. Banik and M. Islam. (2015). *In vitro* thrombolytic, anthelmintic, anti-oxidant and cytotoxic activity with phytochemical screening of methanolic extract of *Xanthium indicum* leaves. *Bangladesh J Pharmacol*; 10: 854-59.
- [13] Hossain, M.A., T. Ferdous, S.M. Salehuddin and A.K. Das. (2009) Brine Shrimp Lethality test for Certain Plant Products, *As. J. Food Ag-Ind.* **2009**; 2(3): 336-41.
- [14] Immanuel, G., T. Citarasu, V. Sivaram, V. Selva Shankar and A. Palavesam (2007) Bioencapsulation strategy and highly unsaturated fatty acids (HUFA) enrichment in *Artemia franciscana* nauplii by using marine trash fish *Odonus niger* liver oil. *African Journal of Biotechnology*, Vol. 6 (17), pp. 2043-2053.
- [15] Jaki, B., J. Orjala and O. Sticher, (1999). A novel extracellular diterpenoid with antibacterial activity from the cyanobacterium *Nostoc commune*. *J Nat Prod.* 62, 502-503.
- [16] Kamala Ambasta, (1992). (Eds.) Useful Plants of India, Publication and Information Directorate, New Delhi. Pp.918.
- [17] Kokkali, V., I. Katramados and J.D. Newman, (2011). Monitoring the effect of metal ions on the mobility of *Artemia salina* nauplii. *Biosensors.* 1, 36-45.
- [18] Krishnaraju, A. V., Tayi V. N. Rao, Dodda Sundararaju, Mulabagal Vanisree, Hsin-Sheng Tsay, and Gottumukkala V. Subbaraju, 2005. Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (*Artemia salina*) Lethality Assay, *International Journal of Applied Science and Engineering*. Vol. 3, 2: 125-134.
- [19] Martínez, M., J. Del Ramo, A. Torreblanca, and J. Díaz-Mayans, (1999). Effect of cadmium exposure on

- zinc levels in the brine shrimp *Artemia parthenogenetica*. *Aquaculture*. 172, 315- 325.
- [20] Maurer-Jones, M.A., S.A. Love, S. Meierhofer, B. J. Marquis, Z. Liu and C.L. Haynes, (2013). Toxicity of nanoparticles to brine shrimp: An introduction to nanotoxicity and interdisciplinary science. *J Chem Edu*. 90, 475-478.
- [21] Mayorga, P., K.R. Pérez, S.M., Cruz and A. Cáceres, (2010). Comparison of bioassays using the anostracan crustaceans *Artemia salina* and *Thamnocephalus platyurus* for plant extract toxicity screening. *Bras J Pharm*. 20, 897-903.
- [22] McLaughlin, J. L., C.J. Chang and D.L. Smith, (1993). Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant antitumour compounds. In: *Human Medicinal Agents from Plants*. Kinghorn, A. D. and Balandrin, M. F. (Eds.), *ACS Symposium 534*, American Chemical Society, Washington, D. C.: 112-137.
- [23] Meyer, B. N., N.R. Ferrigni, J. E. Putnam, L. B. Jacobson, D. E. Nichols and J.L. McLaughlin, (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, 45: 31-34.
- [24] Michael Babu, M., V. Sivaram, G. Immanuel, T. Citarasu and S.M.J. Punitha (2008) Effects of Herbal Enriched *Artemia* Supplementation over the Reproductive Performance and Larval Quality in Spent Spawners of the Tiger Shrimp (*Penaeus monodon*), *Turkish Journal of Fisheries and Aquatic Sciences* 8: 301-30.
- [25] Michael, A. S., C.G. Thompson and M. Abramovitz, (1956). *Artemia salina* as a test organism for a bioassay. *Science*, 123: 464..
- [26] Moshi, M.J., E. Innocent, J. J. Magadula, D.F. Otieno, A. Weisheit, P. K. Mbabazi and R.S.O. Nondo, (2010). Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania. *Tanz J H Res*. 12, 63-67.
- [27] Ogugu, S.E., A.J. Kehinde, B.I., James and D.K. Paul (2012). Assessment of cytotoxic effects of methanol extract of *Calliandra portoricensis* using Brine Shrimp (*Artemia salina*) Lethality Bioassay. *Glob J Bio-Sci Biotech*. 2, 257- 260.
- [28] Pelka, M., C. Danzl, W. Distler and A. Petschelt (2000). A new screening test for toxicity testing of dental materials. *J Dent*. 28, 341-345.
- [29] Quazi Sahely Sarah, Fatema Chowdhury Anny and Mir Misbahuddin (2017). Brine Shrimp Lethality Assay, *Bangladesh J Pharmacol*. 2017; 12: 186-189.
- [30] Shankar, S.R., R. Girish, N. Karthik, R. Rajendran and V.S. Mahendran (2009). Allelopathic effects of phenolics and terpenoids extracted from *Gmelina arborea* on germination of Black gram (*Vigna mungo*) and Green gram (*Vigna radiata*). *Allelopathy J*; 2009; 23: 323-331.
- [31] Sharma, N., P.C. Gupta, A. Singh and C. V. Rao, (2013). Brine shrimp Bioassay of *Pentapetes phoenicea* Linn. and *Ipomoea carnea* jacq. leaves. *Der Pharm Lett*. 5, 162-167.
- [32] Sleet, R.B. and K. Brendel, (1985). Homogeneous populations of *Artemia nauplii* and their potential use for *in vitro* testing in developmental toxicology. *Teratog Carcinog Mutagen*. 5, 41-44.
- [33] Solanki, S.S. and M. Selvanayagam, (2013). Phytochemical screening and study of predictive toxicity of certain medicinal plants and extracts using brine shrimp. *J Herb Sci Tech*. 10, 1-4.
- [34] Tiwari, N., A.K. Yadav, P. Srivastava, K. Shanker, R.K. Verma and M.M. Gupta (2008). Iridoid glycosides from *Gmelina arborea*. *Phytochemistry* 2008; 69: 2387-2390.
- [35] Vanhaecke, P., G. Persoone, C. Claus and P. Sorgeloos, (1981). Proposal for a short-term toxicity test with *Artemia nauplii*. *Ecotoxicology and Environmental Safety*, 5: 382-387.
- [36] Veni, T and T. Pushpanathan, (2014). Comparison of the *Artemia salina* and *Artemia franciscana* bioassays for toxicity of Indian medicinal plants, *Journal of Coastal Life Medicine* 2014; 2(6): 453-457
- [37] Yadav, A.K., N. Tiwari, P. Srivastava, S.C. Singh, K. Shanker and R.K. Verma. (2008). Iridoid glycoside-based quantitative chromatographic fingerprint analysis: a rational approach for quality assessment of Indian medicinal plant Gambhari (*Gmelina arborea*). *J Pharm Biomed Anal*, 2008; 47: 841-846.
- [38] Zapata, C., G.M. Gajarado and J.A. Beardmore (1990) Multilocus Heterozygosity and Sexual Selection in the Brine Shrimp *Artemia franciscana*. *Mar. Ecol. Prog. Ser.*, 62:211-217.