Research Journal of Biological Sciences 8 (1): 11-16, 2013
ISSN: 1815-8846

Comparison of the *Artemia Salina* and *Artemia Uramiana* Bioassays for Toxicity of 4 Iranian Medicinal Plants

Ali Mirzaei and Nooshin Mirzaei
Medicinal Plant Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

Abstract: For evaluation of 4 Iranian medicinal plant toxicity brine shrimp lethality assays *Artemia salina* and *Artemia uramiana* bioassay were used. *A. urmiana* and *A. salina* eggs were purchased and kept in a hatching chamber that containing artificial sea water for preparation of nauplii. The active nauplii were collected for study after 48 h. The *Plantago major*, *Artemisia maritime*, *Mentha piperita* and *Borago officinalis* were prepared in Yasuj, Iran and individually extracted with methanol, hexane and ethyl acetate by Soxhlet apparatus. The toxicity rate of extracts was estimated on the basis of the number of dead nauplii or mortality rate by *Artemia salina* and *Artemia uramiana*. LC_{50} values with 95% confidence intervals were determined by the probit analysis. All extracts, exception of *B. officinalis* displayed 100% mortality at 1000 μg mL^{-1} by *A. urmiana* and *A. salina*. Ethyl acetate extract was the most potent and presented the highest percentage of mortality with the lowest LC_{50} values by both assays too. After ethyl acetate, hexane extract showed the highest toxicity; however the methanol extract exhibited the lowest mortality. According to *A. urmiana* and *A. salina* toxicity results, trend of the extracts *P. major*>*A. maritima*>*M. piperita*>*B. officinalis* was reported. There was a positive correlation between the results from *A. urmiana* and *A. salina*, for detecting plants toxicity with a Pearson correlation of $R^2 = 0.989$. *A. uramiana* assay is valuable for the screening of plant extracts to detect of toxicity.

Key words: *Artemia salina*, *Artemia uramiana*, medicinal plant, toxicity, *Plantago major*

INTRODUCTION

Man has used herbs as medicine since the earlier stone age. Plants are rich in various bioactive compounds in the name of photochemical that include total phenols, flavonoids, alkaloids, steroids, terpenoids and tannins (Cowan, 1999; Rosangkima and Prasad, 2004).

The over consumption of medicinal plants can lead to excessive accumulation of herbs in the body which may cause toxicity. Recent studies indicate that a large number of people in industrial societies use medicinal herbs for their health requirements. Therefore, medicinal plants play a significant role in the healthcare system of a large percentage of the world's population (Akerele, 1988). Over 80% of the world's people is dependent on the herbal traditional system for their major health care (Madhuri and Pandey, 2008; Sivalokanathan *et al*., 2005). Although plants have valuable effects, they can be toxic to humans. Therefore, numerous research studies have recently focused on the pharmacology and toxicity of medicinal plants that used by human (Parra *et al*., 2001). Recent studies indicate that although, numerous plants are used as food sources and some of them may have mutagenic or genotoxic potential (Fekadu *et al*., 1996; Celik and Aslanturk, 2007).

For toxicity evaluation of medicinal plants in this study Brine Shrimp Lethality Test (BSLT) with 2 Artemia species was designated: *Artemia salina* (*A. salina*) and *Artemia uramiana* (*A. uramiana*). Artemias (Artemiidae) are a type of salt-water shrimp invertebrate which are found in almost 500 salty lakes (Sorgeloos *et al*., 1996). *A. uramiana* is found in Urmia lake which is one of the largest natural environments of *Artemia* sp. and is the only source of *A. uramiana* in the world (Agh and Sorgeloos, 2004). Most plant toxicity studies and research have been carried out by *A. salina* but this is the first plant toxicity study in *A. uramiana* system.

The BSLT is used for screening of chemical and natural products toxicity and isolation of active compounds in herbal extracts (Sadighara *et al*., 2010). The BSLT is also useful animal method for evaluating toxicology studies because, their eggs or cysts are abundant, follow a simple transformation from hatching into the larvae cycle and are easily maintaining a population in laboratory environment (Anubha, 2007). The BSLT is simple, portable, reliable, rapidly conducted and its results significantly correlate with the state of toxicity and therefore, BSLT is a reliable answer to routine requests for toxicity screening (McLaughlin *et al*., 1993).

Corresponding Author: Ali Mirzaei, Medicinal Plant Research Center, Yasuj Medical Sciences University, Yasuj, Iran

Among the herbal plants of the Iranian flora *Plantago major*, *Artemisia maritime*, *Mentha piperita* and *Borago officinalis* were collected from Yasuj, the Southern of Iran. These plants have potent pharmacological potentials and used broadly in the traditional medicine by the Iranian people.

The toxicological evaluation of medicinal plants performed routinely in mice which is costly and the animals suffering caused by these procedures. Therefore, there is recently a tendency to replace the utilize of laboratory animals in toxicological procedures (Parra *et al*., 2001). Therefore, in this research, BLST instead of animal toxicological assay for plant toxicity was designated.

Plantago major L. (English name: Larger plantain) is used as an anti-inflammatory, anthelmintic, antiviral, analgesic, antihistaminic, antitumor and hypotensive agent in traditional medicine (Kobeasy *et al*., 2011). *Artemisia maritima* (English name: Worm seed) is a medicinal plant with potent antioxidant, anthelmintic, antiseptic, antimalarial, febrifuge and tonic activities. It contains total phenols and flavonoids (Sati *et al*., 2010). *Mentha piperita* (English name: Peppermint) is a member of the lamiaceae family and used for the treatment of headaches, coughs, congestion and intestinal disorders. Its active compounds include flavonoids, tannins, caffeic acid, volatile oils and carotenes (Singh *et al*., 2011). *Borago officinalis* L. (English name: Borage) is a medicinally important plant that used as a diuretic, expectorant, bronchodilator, antispasmodic and vasodilator. Its chemical compounds contain total phenols, alkaloids, tannins and linolenic acid (Badi and Sorooshzadeh, 2011; Mayank and Swati, 2010).

In this study, the efficiency of *A. uramiana* for predicting the toxicity of plant extracts was evaluated by comparison with *A. salina* as the golden standard.

MATERIALS AND METHODS

Plant collection: Aerial plant parts were collected in 2010 in various areas of Yasuj, Iran. Botanical identification was conducted for each sample and the voucher samples were kept at the herbarium of the Medicinal Herb Research Center.

Plant extraction: The plants were dried in the shade and ground using a mill (Restsch Ultra Centrifugal Mill and Sieving Machine, Haan, Germany). The ground materials were individually extracted with methanol, hexane and ethyl acetate by Soxhlet apparatus for 6 h and filtered through Whatman No. 1 filter paper. The extracts were collected and concentrated using a rotary evaporator (Heidolph Laborota, Model 4000; Germany) and remained frozen prior to the study.

The hatching larvae: *A. urmiana* and *A. salina* eggs were purchased from the Artemia Research Center of Urmia, Iran. The Artemia brine shrimp eggs were kept in a special conical-shaped container known as a hatching chamber (1 L) filled with Artificial Sea Water (ASW) which was prepared by dissolving 30 g of sea salt in 1 L of distilled water at 27-29°C. Regular air flow with average pressure and proper light was supplied for 48 h. The pH of the environment was adjusted to 9.0 to prevent the risk of death to the Artemia nauplii due to a drop in the pH during development (Amara *et al*., 2008). After hatching, the active nauplii were collected with a plastic pipette for study.

The brine shrimp lethality test: All experiments were conducted in glass petri dishes (60 mm diameter and 12 mm height). The containers were filled with 0.5 mL herbal extract diluted with different concentrations (10-1000 μg mL^{-1}) of Dimethyl Sulfoxide (DMSO) and then 4.5 mL of the brine shrimp solution was added to the petri dishes. Ten brine shrimp larvae (Nauplii) from each Artemia species which had developed for 48 h were added to each petri dish. For each concentration of plant sample, one control group was conducted which included 0.5 mL (Vehicle treated, DMSO) with 4.5 mL of brine shrimp solution without extract.

This study was performed in 3 replicates for each concentration. The petri dishes were kept covered with their lids in the darkness at room temperature for 24 h. Feeding and air were not required during the study. In each plate, the numbers of dead and surviving nauplii were counted and the LC_{50} was calculated. Nauplii that did not show any movement within 10 sec were defined as dead. In this bioassay experiment, Thymol was used as a positive control (Sam, 1993).

The toxicity rate of extracts was estimated on the basis of the number of dead nauplii or the mortality rate that was estimated using the following equation:

$$\text{Mortality or death rate (\%)} = \frac{\text{d test} - \text{d control}}{\text{A control}} \times 100$$

Where:

d test = The average number of dead nauplii in the experimental groups

d control = The average number of dead nauplii in the control group

A control = The average number of living nauplii in the control group

Statistical analysis: The results are expressed as means. LC_{50} values with 95% confidence intervals were determined by the probit analysis method. For *A. urmiana* and *A. salina* comparison t-student analysis was managed.

RESULTS

The hatched nauplius of *Artemia uramiana* and 48 growth of nauplius was demonstrated in Fig. 1a, b. For each plant, 3 extracts were tested at 4 concentrations (10, 100, 500 and 1000 μg mL^{-1}). The LC_{50} value and 95% confidence intervals were recorded for each extract concentration by the BSLT (Table 1 and 2), increased extract concentrations were associated with increased mortality rates.

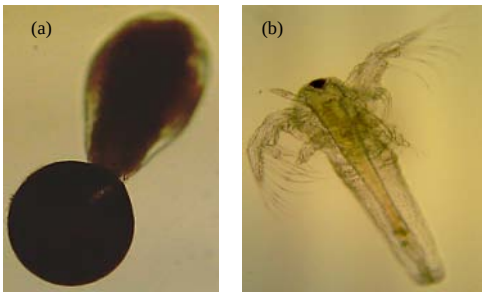

Fig. 1: Recently hatched nauplius of: a) *Artemia uramiana*; b) 48 growth

The bioactivities of different extracts against *A. urmiana* and *A. salina* are shown in Table 1 and 2, respectively. All extracts showed 100.0% mortality at 1000 μg mL^{-1} concentration by *A. urmiana* and *A. salina* with the exception of *B. officinalis* (Table 1 and 2). The other extract concentrations demonstrated a 10-72% mortality.

Ethyl acetate extract was the most potent and showed the highest percentage of mortality with the lowest LC_{50} values (187.6-373.5 and 216.5-545.6 for *A. urmiana* and *A. salina*, respectively). After ethyl acetate, hexane extract presented the highest toxicity with LC_{50} value of 236.4-977.5 and 290-1107.7 for *A. urmiana* and *A. salina*, respectively. The methanol extract showed the lowest mortality with the highest LC_{50} values (303.7-1027 and 321.6-1144.6 for *A. urmiana* and *A. salina*, respectively) at all concentrations.

According to the BSLT results, the rate of extracts toxicity were as follow: *P. major*>*A. maritima*> *M. piperita*>*B. officinalis* by both assay systems.

In present study, the average of LC_{50} by *A. urmiana* in extracts 14% was less than *A. salina*, this means that

Table 1: Cytotoxic results of plant extracts by *Artemia uramiana* a brime shrimp lethality assay

	Percentage mortality at different concentration (μg mL^{-1})					
Extracts	10	100	500	1000	LC_{50} 24 h (μg mL^{-1})	95% confidence interval
Plantago major						
Hexan	20	45	72	100	234.4	182.3-286.00
Ethyl acetate	26	49	76	100	187.6	135.4-239.70
Methanolic	21	32	63	100	303.7	251.6-355.70
Artemisia maritima						
Hexan	26	34	69	100	260.5	206.5-314.50
Ethyl acetate	32	48	65	100	207.9	154.4-261.50
Methanolic	20	26	64	100	326.2	271.6-380.80
Mentha piperata						
Hexan	16	24	66	100	333.2	283.8-382.60
Ethyl acetate	23	34	71	100	268.3	220.6-316.00
Methanolic	13	22	53	100	392.6	342.5-442.70
Borrago officianalis						
Hexan	11	28	31	38	977.5	829.2-1125.7
Ethyl acetate	19	32	48	100	373.5	258.5-488.00
Methanolic	10	25	31	36	1027.0	868.6-1175.0

Table 2: Cytotoxic results of plant extracts by *artemia salina* a brime shrimp lethality assay

	Percentage mortality at different concentration (μg mL^{-1})					
Extracts	10	100	500	1000	LC_{50} 24 h (μg mL^{-1})	95% confidence interval
Plantago major						
Hexan	16	40	65	100	290.0	233.1-346.90
Ethyl acetate	19	45	71	100	262.0	204.5-320.80
Methanolic	17	35	60	100	321.6	264.7-378.50
Artemisia maritima						
Hexan	28	30	72	100	262.7	211.0-314.50
Ethyl acetate	30	42	70	100	217.5	165.6-269.50
Methanolic	24	20	60	100	343.7	290.9-396.60
Mentha piperata						
Hexan	20	19	61	100	353.7	303.2-404.10
Ethyl acetate	20	31	65	100	301.2	251.6-350.70
Methanolic	15	20	55	100	388.0	337.0-439.00
Borrago officianalis						
Hexan	10	25	31	35	1107.7	922.5-1293.8
Ethyl acetate	23	30	45	83	545.6	415.8-675.40
Methanolic	7	20	40	30	1144.6	954.9-1334.6

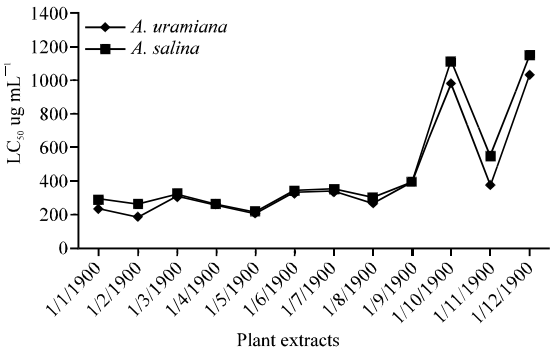

Fig. 2: Comparison of *A. salina* and *A. urmaiana* systems by LC_{50} of different plant extracts; 3 Arabic numbers in x-axis belong to each plan species: *Plantago major* (1-3), *Artemisia maritime* (4-6), Mentha piperata (7-9), Borrago officianalist (10-12); 1st-3rd number of each plant species belong to hexane, ethyl acetate and methanol extracts, respectively

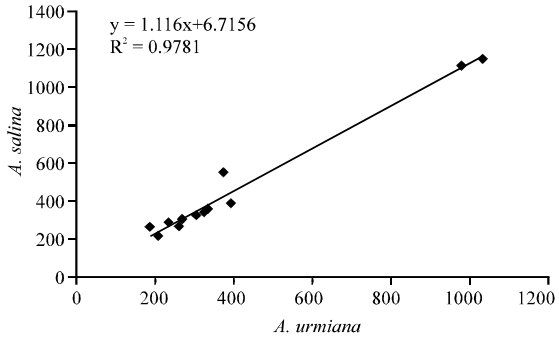

Fig. 3: Relationship between medium lethal concentration (LC_{50}) in *Artemia salina* and *Artemia uramiana* brime shrimp lethality assay in different plant extracts

plant toxicity rate was more in *A. urmiana* when compare with *A. salina*. Therefore, no significant difference was reported in LC_{50} of *A. urmiana* and *A. salina* in plant extracts (Fig. 2).

There was a positive correlation between the results of *A. urmiana* and *A. salina* for detecting toxic compounds in plant extracts with a Pearson correlation of $R^2 = 0.978$ (Fig. 3).

DISCUSSION

Approximately, 50% of clinically used drugs are derived from plant products which play a key role in the treatment of inflammation, cancer and infectious diseases (Koehn and Carter, 2005; Fabricant and Farnsworth, 2001). The medicinal plants used in this research were selected on the basis of their uses in traditional medicine. One of the easiest assays for screening plant toxicity is the BSLT. In this study, it used *A. urmiana* for the first time to detect toxicity in 4 medicinal plants.

According to recent results, Artemia nauplii from the different populations, *A. urmiana* and *A. salina* showed insignificantly different sensitivities to the same toxicant according to their LC_{50} values $p>0.05$. Plant extracts toxicity differed from each other ($p<0.001$) in *A. urmiana* and *A. salina* systems which may be due to differences in the amounts and types of cytotoxic compounds. These compounds contain total phenols, flavonoids, coumarins, triterpenoids and tannins which exist in the plant extracts (Peteros and Mylene, 2010). In this study, there was a direct correlation between mortality rate and concentration level. The maximum and minimum mortality dose rates were 1000 and 10 μg mL^{-1}, respectively.

Compounds and extracts with LC_{50} values <1000 μg mL^{-1} were considered toxic (Meyer *et al*., 1982). Therefore, most of the plant extracts in this study had biological potential or toxic properties.

The LC_{50} values of *A. salina* ranged from 11-1000 μg mL^{-1} in Nigerian traditional medicinal plants (Oyewale1 *et al*., 2004) and ranged from 15.35-374 μg mL^{-1} in Tanzanian traditional medicinal plants, suggesting the presence of cytotoxic compounds. However, the LC_{50} values for ethanol extracts of several plants in the latter study were >1000 μg mL^{-1} (Oyewale1 *et al*., 2004; Moshi *et al*., 2009). LC_{50} values in Philippine medicinal plants, in *A. salina* ranged from 37.7-89.5 μg mL^{-1} (Peteros and Mylene, 2010). LC_{50} values in the *Euphorbia kamerunica* Pax plant, for different concentrations and extracts ranged from 0 in a hexane extract to 13.87 in an aqueous extract. A low LC_{50} indicates that the extract is highly lethal and that the plant extract may be contains potent cytotoxic compounds (Ogunnusi and Dosumu, 2008).

The results of the first study to evaluate food additive toxicity by *A. urmiana* was not comparable to Gold Standard Method (Sadighara *et al*., 2010). However in present study, the results were comparable and parallel with *A. salina* for evaluation of toxicity. The results from the cytotoxic assays of the plants by *A. urmiana* and *A. salina* revealed that *M. piperita* and *A. maritima* had mild cytotoxic potential whereas *B. officinalis* did not have a cytotoxic effect.

The strongest cytotoxic extract in the present screening was the ethyl acetate extract of the *P. major* plant. The low LC_{50} levels obtained from the different extracts of *P. major* is associated with its traditional

medicinal antitumor, anthelmintic, antiviral and antirheumatic uses (Franca *et al*., 1996; Matev *et al*., 1982). These low LC_{50} values are related to toxic substances such as flavonoids, flavone and luteolin which are present in this plant (Kobeasy *et al*., 2011).

P. major has demonstrated growth inhibitory and cytotoxic properties on different malignant tumors (Galvez *et al*., 2003), acute myelocytic leukemia and mammary adenocarcinoma (Kobeasy *et al*., 2011). Furthermore, this plant has displayed antibacterial activity (Gomez *et al*., 2000).

The santonin drug is derived from the *A. maritima* plant and is useful for *Ascaris lumbricoides* treatment. The anti-helminthes activity suggests that a toxic substance is present in *A. maritima* which is consistent with its low LC_{50} in this study.

M. piperita has potent antioxidant and free radical scavenger activities, however its oil had a modest toxic effect in the rat cerebellum. Water extracts of *M. piperita* are safe for use in cosmetic preparations. These observations are compatible with the research results (Nair, 2001).

B. officinalis is a medicinally important plant that has been used against prostate and liver cancer cells. Furthermore, this plant is useful for decreasing blood pressure, anti-inflammatory effects and immunity control. This potential may be due to presence of total phenols linoleic acid and alkaloids derivative (Lin *et al*., 2002; El-Shazly *et al*., 1996).

B. officinalis was the safest plant according to the studies presented herein, however it has reported anticancer activities. The differences in these results and those reported in the literature may be due to the type of extraction and the plant species. This is may be a limitation of detecting plant properties by Artemia toxicity.

The results suggest that the BLST is a useful screening system in medicinal plants for toxicity and may be discovering new bioactive compounds with various activities.

CONCLUSION

In this study, the brine shrimp toxicity results suggest that the extracts of the 4 medicinal plants do not have high toxicity compared to the Thymol standard with LC_{50} of 7.2 μg mL^{-1}. The present data suggest that the ethyl acetate extract obtained from *P. major* was toxic with both system assays and can use for further study. Furthermore, a positive correlation was reported between *A. salina* and *A. uramiana* for detecting toxic compounds in plants. Thus, the *A. uramiana* assay is valuable for the screening of plant extracts to detect of toxicity.

ACKNOWLEDGEMENTS

Researchers are grateful to the Biochemistry Department and the Iranian Medicinal Plant Research Center for making their facilities available for this investigation.

REFERENCES

Agh, N. and P. Sorgeloos, 2004. INCO-DEV project on Artemia biodiversity (ICA4-CT-2001-10020) Iran internationl workshop. Artemia and Aquatic Animals Research Center, Urmia University, Urmia, Iran.

Akerele, O., 1988. Medicinal plants and primary health care: An agenda for action. Fitoterapia, 59: 355-363.

Amara, A.A., M.H. El-Masry and H.H. Bogdady, 2008. Plant crude extracts could be the solution: Extracts showing *In vivo* antitumorigenic activity. Pak. J. Pharm. Sci., 21: 159-171.

Anubha, S.K., 2007. Brine shrimp *Artemia salina* -a marine animal for simple and rapid biological assays. J. Chin. Clin. Med., 4: 1-4.

Badi, H.N. and A. Sorooshzadeh, 2011. Evaluating potential of borage (*Borago officinalis* L.) in bioremediation of saline soil. Afr. J. of Biotechnol., 10: 146-153.

Celik, T.A. and O.S. Aslanturk, 2007. Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. Biologia, 3: 292-296.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

El-Shazly, A., T. Sargm, A. Ateya, A. Abdel Aziz, S. El-Dahmy and L. Witte, 1996. Pyrrolizidine alkaloids from *Echium setosum* and *Echium* valgare. Fitoterapia, 5: 466-468.

Fabricant, D.S. and N.R. Farnsworth, 2001. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect., 109: 69-75.

Fekadu, K., P. Wolfram, M. Stephen, J. Ian, L. Gunther and S. Gerhard, 1996. Genotoxic e?ects of crude juices from Brassica vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. Chem. Biol. Interact., 102: 1-16.

Franca, F., E.L. Lago and P.D. Marsden, 1996. Plants used in the treatment of leishmanial ulcers due to *Leishmania* (Vannia) braziliensis in an endemic area of Bahia, Brazil. Rev. Soc. Bras. Med. Trop., 29: 229-232.

Galvez, M., C. Martin-Cordero, M. Lopez-Lazaro, F. Cortes and M.J. Ayuso, 2003. Cytotoxic effect of *Plantago* spp. on cancer cell lines. J. Ethnopharmacol., 88: 125-130.

Gomez, F.R., C.L. Calderon, L.W. Scheibel, P. Tamez-Guerra, C. Rodriguez-Padilla and R. Tamez- Guerra, 2000. Immunoenhancing properties of *plantago major* leaf extract. Phytother. Res., 14: 617-622.

Kobeasy, M.I., O.M. Abdel-Fatah, S.M. Abd El-Salam, M.M. Zahrat and E.l. Ola, 2011. Biochemical studies on *Plantago major* L. and *Cyamopsis tetragonoloba* L. Int. J. Biodivers. Conserv., 3: 83-91.

Koehn, F.E. and G.T. Carter, 2005. The evolving role of natural products drug discovery. Nat. Rev., 4: 206-222.

Lin, L.T., L.T. Liu, L.C. Chiang and C.C. Lin, 2002. *In vitro* anti-hepatoma activity of fifteen natural medicines from Canada. Phytother. Res., 16: 440-444.

Madhuri, S. and G. Pandey, 2008. Some dietary agricultural plants with anticancer properties. Plant Arch., 8: 13-16.

Matev, M., I. Angelova, A. Koichev, M. Leseva and, G. Stefanov, 1982. Clinical trial of a *Plantago major* preparation in the treatment of chronic bronchitis. Vitr. Boles., 21: 133-137.

Mayank, G. and S. Swati, 2010. *Borago officinalis* linn. an important medicinal plant of mediterranean region. Int. J. Pharm. Sci. Rev Res., 5: 27-34.

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: Review of Recent Progress. In: Human Medicinal Agents from Plants, Kinghorn, A.D. and M.F. Balandrin (Eds.). American Chemical Society Publication, Washington, DC., USA., ISBN-13: 9780841227057, pp: 112-137.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med., 45: 31-34.

Moshi, M.J., E. Innocent, P.J. Masimba, D.F. Otieno and A. Weisheit *et al*., 2009. Antimicrobial and brine shrimp toxicity of some plants used in traditional medicine in Bukoba District, north-western Tanzania. Tanzania J. Health Resh., 11: 23-28.

Nair, B., 2001. Final report on the safety assessment of *Mentha piperita* (Peppermint) oil, *Mentha piperita* (Peppermint) leaf extract, *Mentha piperita* (Peppermint) leaf and *Mentha piperita* (Peppermint) leaf water. Int. J. Toxicol., 20: 61-73.

Ogunnusi, T.A. and O.O. Dosumu, 2008. Bioactivity of crude extracts of *Euphorbia kamerunica* Pax using brine shrimp (*Artemia salina*) lethality assay. J. Med. Plants Res., 2: 370-373.

Oyewale1, A.O., O.T. Audul, R.G. Ayo and J.O. Amupitan, 2004. Cytotoxic correlation of some traditional medicinal plants using brine shrimp lethality test. Chemclass J., 1: 110-112.

Parra, A.L., R.S. Yhebra, I.G. Sardinas and L.I. Buela, 2001. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Phytomedical, 8: 395-400.

Peteros, N.P. and M.U. Mylene, 2010. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. J. Med. Plants Res., 4: 407-414.

Rosangkima, G. and S.B. Prasad, 2004. Antitumour activity of some plants from Meghalaya and Mizoram against murine ascites Dalton's lymphoma. Indian J. Exp. Biol., 42: 981-988.

Sadighara, P., J. Salar-Amoli, S. Asadinejad and E. Zadehhashem, 2010. Using *Artemia urmiana* in detecting of the cytotoxicity of some selected food additives. Biharean. Biologist., 4: 81-82.

Sam, T.W., 1993. Toxicity Testing Using the Brine Shrimp: *Artemia salina*. In: Bioactive Natural Products Detection, Isolation, and Structural Determination, Colegate, S.M. and R.J. Molyneux (Eds.). CRC Press, Boca Raton, FL., pp: 442-456.

Sati, S.C., N. Sati, U. Rawat and O.P. Sati, 2010. Medicinal plants as a source of antioxidants. Res. J. Phytochem., 4: 213-224.

Singh, R., M.A.M. Shushni and A. Belkheir, 2011. Antibacterial and antioxidant activities of *Mentha piperita* L. Arabian J. Chem., 10.1016/ j. arabjc. 2011.01.019

Sivalokanathan, S., M. Ilayaraja and M.P. Balasubramanian, 2005. Efficacy of *Terminalia arjuna* (Roxb.) on N-nitrosodiethylamine induced hepatocellular carcinoma in rats. Indian J. Exp. Biol., 43: 264-267.

Sorgeloos, P., P. Lavens, P. Leger, W. Tackaert and D. Versichele, 1996. Manual for the Culture and use of Brine Shrimp *Artemia*in Aquaculture. State University of Ghent, Belgium-Faculty of Agriculture, Belgum, pp: 319.