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Brine Shrimp (*Artemia salina*) Toxicity Assay of selected medicinal plants in Tamil Nadu

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Abstract

The brine shrimp lethality bioassay is extensively used for the isolation of antitumour and cytotoxic agents from several medicinal plants. In the aim of the present study, to assess as cytotoxic activity of ethanolic extract of six species were *Cinnamomum wightii Meisner, Garcinia gummi-gutta (L.) Robs., Michelia nilagrica Zenk. Tylophora indica (Burm. f.) Merr., Hemidesmus indicus (L.) R. Br. and Gymnema sylvestre (Retz.) R. Br. ex Schult* using by brine shrimp lethality bioassay method. The results of the present study, maximum cytotoxic activity of both plants of *Gymnema sylvestre and Michelia nilagrica* ethanolic extracts were observed. The highest activity was found for ethanolic extract of *Gymnema sylvestre and Michelia nilagrica* with LC_{50} value of 0.56 and 0.89µg/ml respectively. These results suggest that the leaves of *Cinnamomum wightii Meisner, Garcinia gummi-gutta (L.) Robs., Michelia nilagrica Zenk. Tylophora indica (Burm. f.) Merr., Hemidesmus indicus (L.) R. Br. and Gymnema sylvestre (Retz.) R. Br. ex Schult* may be a promising source of novel anticancer agents.

Key words: Brine shrimp lethality, cytotoxic, medicinal plants

Introduction

The medicinal plants were constituting a source of raw materials for Ayurvedic, Chinese, Unani, Homeopathy, and Siddha and modern medicine. In India, estimated to be between 3,000 to 3,500 species are using medicinal purposed of higher plants. The list of medicinal plants around 3000 species is publishes in the books of Glossary of Indian Medicinal Plants (Asolkar *et al*., 1992; Chopra *et al*., 1956;1969). Jain, (1991) who publishes in the ethnomedicinal uses of 2500 plants have been reported. Mishra and Kumar, (2000) reported in the 560 plants listed in in Ayurvedic Materia Nighantu. Department of Indian System of Medicine, prepared by the Ayurvedic Drug prepared by 387 medicinal plants (Sarin, 1996). The Unani system of medicine describes 440 plants, out of which 360 are common to other systems practiced in the country (Said, 1969). Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over the counter drugs, and ingredients for the pharmaceutical industry. The number of plants having confirmed therapeutic properties or yielding a clinically useful chemical compound thus lies around 700 species. Now-a-days brine shrimp (*Artemia salina,* fairy shrimp or sea monkeys) lethality assay is commonly used to check the cytotoxic effect of bioactive chemicals. Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp of *Artemia salina* (Harwig and Scott,1971). This assay was first proposed by Michael *et al*., (1956) and further developed by several groups (Michael *et al*.,1956; Van Walbeek *et al*.,1971; Vanhaecke *et al*.,1981). It is a preliminary toxicity screening of plant extracts (Ghosh *et al*., 2015; Kibiti and Afolayan, 2016; Sufian and Haque, 2015; Syahmi *et al*., 2010). In this present study, ethanolic extracts of the six selected medicinal plants collected in Courtallam forest, Tirunelveli Hills were tested *in vivo* for their cytotoxic effect against the brine shrimp nauplii.

Materials and Methods

Collection of plants

Cinnamomum wightii Meisner, Garcinia gummi-gutta (L.) *Robs., Michelia nilagrica* Zenk. *Tylophora indica* (Burm. f.) *Merr., Hemidesmus indicus* (L.) R. Br. and *Gymnema sylvestre* (Retz.) R. Br. ex Schult was collected from Courtallam forest region of Tirunelveli District, Tamil Nadu.

Phytochemicals identification

The phytochemical identification of six medicinal plants of *Cinnamomum wightii* Meisner (Lauraceae), *Garcinia gummi-gutta* (L.) Robs. (Clusiaceae), *Michelia nilagrica* Zenk (Magnoliaceae). Tylophora indica (Burm.f.) Merr. (Asclepiadaceae), *Hemidesmus indicus* (L.) R. Br.(Asclepiadaceae) and *Gymnema sylvestre* (Retz.) R. Br. ex Schult (Asclepiadaceae) was collected and separated into leaves and air dried for two weeks. The air-dried plant materials were powdered and extracted with ethanol for 4 hrs in Soxhlet apparatus. The obtained extracts were concentrated in distillation for excess of solvents removed. The

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phytochemical constituents were identified standard method previously described (Harborne,1984).

Brine Shrimp Toxicity Assay

Brine shrimp lethality bioassay (BSLB) followed by according to Meyer *et al*., (1982) method. BSLB was conducted for the determination of general toxicity of standardized ethanolic extract of six species. In brief, brine shrimp eggs were hatched in a glass beaker filled with artificial seawater on the day prior to the experiment. Stock solution of *C. wightii, G. gummi-gutta, M. nilagrica, T. indica, H. indicus and G. sylvestre* ethanolic extract at 10mg/ml was prepared by dissolving the extract in 5% DMSO solution. From the stock solution, different concentrations of *C. wightii, G. gummi-gutta, M. nilagrica, T. indica, H. indicus and G. sylvestre* ethanolic extract (0.5-10mg/ml) were added into separate test tubes containing artificial seawater. Subsequently, 10 newly hatched brine shrimps were added to each test tube and incubated at room temperature for 24h. The final volume of each test tube was 5 ml. The percentage of mortality was calculated after 24h. The LC_{50} values were calculated by graphics from drug concentration vs. lethality percentage using a probit scale adjust. Data analysis was performed with Origin 6.0 software. The control samples containing DMSO under the same conditions, do not cause significant brine shrimp mortality. All the experiments were performed in triplicate. After 24h incubation under light, the number of dead and survivor brine shrimps in each test tube was counted.

Table -1: Preliminary phytochemical analysis of six medicinal plants

Medicinal plants	Active Compounds				
	Alkaloids	Flavonoids	Terpenoids	Tannins	Saponins
Cinnamomum wightii	+	++	+++	+	+
Garcinia gummi-gutta	+++	+++	+++	+	+
Gymnema sylvestre	+++	++	++	+	+
Michelia nilagrica	+++	+++	+++	++	++
Hemidesmus indicus	++	+++	+++	++	++
Tylophora indica	+++	++	+	+	+

"+++"- Maximum; "++ Medium" ; "+" - Minimum

Table-2: Results of 24h brine shrimp toxicity activity of ethanolic extract of six species

Medicinal plants	No of Animals	LC_{50} values µg/lr
Cinnamomum wightii	10	2.45
Garcinia gummi-gutta	10	1.34
Gymnema sylvestre	10	0.89
Michelia nilagrica	10	0.56
Hemidesmus indicus	10	2.45
Tylophora indica	10	3.12

Results and Discussion

The results of the phytochemical constituents of six medicinal plants were represented in the table-1. The maximum quantity of alkaloids present in the four species were *Garcinia gummi-gutta, Gymnema sylvestre, Tylophora indica* and *Michelia nilagrica*. Tannins and saponins were observed by minimum quantity of the six-species seen in the table-1.

Brine shrimp toxicity

Brine shrimp lethality assay (BSLA) is an *in vivo* lethality test using simple zoologic organism, *Artemia salina* as to screen and monitor the toxicity level of bioactive natural products. The results of the cytotoxic activity of determining for lethal concentration (LC_{50}) values of the extracts values were represented in the table-2. Earlier studies, experimental study on the lethality of *Artemia salina* has been utilized and strongly described by Meyer *et al*., (1982). Our results observed that the brine shrimp lethality activity of the six medicinal plants extracts was found to be good cytotoxic activity. The observed lethality activity of the six plants extracts to brine shrimps indicated that the presence of potent cytotoxic and probably antitumor components of these plants. Crude extracts considered the activity LC_{50} values of less than 1mg/ml are significant and 1-4µg per ml above extract consider as moderate activity. Our results observed by LC_{50} values of six species of *Cinnamomum wightii, Garcinia gummi-gutta, Gymnema sylvestre, Hemidesmus indicus, Tylophora indica and Michelia nilagrica* ethanolic extract were recorded in Table -2. The maximum cytotoxic activity of both plants of *Gymnema sylvestre* and *Michelia nilagrica* was represented in the table -2. *Moderate activity of Cinnamomum wightii, Garcinia gummi-gutta, Hemidesmus*

indicus and Tylophora indica were observed. The methanolic extracts of 13 Specieis of the genus *Solanum* (Solanaceae) have been tested for bioactivity in *Artemia salina.* Ealrier studies, several plants such as *S. asperum, S. capsicoides, S. palinacantum, S. paludosum, S. paniculatum, S. paraibanum, S. sisymbriifolium, S. crinitum, S. diamantinense, S. megalonyx, S. torvum, S. asterophorum* and *S. stipulaceum* were reported in the cytotoxicity activities (Tania Maria *et al*.,2007). Ethanolic leaf extracts of *Lantana camara* and *Chromolaena odorata* and *Euphorbia hirta* exhibited cytotoxic activity was reported previously reported (Olowa and Nuñeza, 2013). *According to* Mclaughlin *et al*., (1991) described the lethality test is a very useful tool for screening a wide range of chemical compounds and for their various bioactivities. It has been demonstrated that BSLA correlates reasonably well with cytotoxic and other biological properties (Mclaughlin *et al*., 1991). The brine shrimp bioassay test is safe, practical and economic method for determination of bioactivities of pytochemicals (Meyer *et al*., 1982). This bioassay has a good correlation with cytotoxicity and genotoxicity, and has led to the discovery of the new classes of natural pesticides and active antitumor agents. The significant correlation between the Brine shrimp assay and *in vitro* growth inhibition of human solid tumor cell lines demonstrated by the national Cancer Institute (NCI, USA) is significant because it shows the value of this bioassay as a pre-screening tool for antitumor drug research (Anderson *et al*., 1991). The conclusion of the present study, leaf extracts of *Garcinia gummi-gutta, Gymnema sylvestre, Tylophora indica and Michelia nilagrica* exhibited cytotoxic activity against the brine shrimp and considered as containing active or potent components. Although, BSLA is inadequate in determining the mechanism of action of the bioactive substances in these plant, it is very useful by providing a preliminary screening method that can be supported by a more specific bioassay. Further studies, the active of compounds could be isolated from these plants.

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References

Asolkar, L.V., Kakkar, K.K. and Chakra, O. J. 1992. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles Part-1 (A-K) 1965-1981. NISC, CSIR, New Delhi, India, pp: 265-266.

Chopra, R.N., Chopra, I.C. and Verma, B.S. 1969. Supplement to Glossary of Indian Medicinal Plants. Publication and Information Directorate (CSIR), New Delhi.

Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, India, pp: 170.

Jain, S.K., 1991. Dictionary of Indian Folk Medicine and Ethnobotany. Deep Publications, New Delhi.

Mishra, A. and Kumar, A. 2000. Ayurvedic Medicinal plants for Skin disease. *Int. J. Mendel,* 17: 91-92.

Sarin, Y.K.1996. Illustrated Manual of Herbal Drugs Used in Ayurveda (CSIR/ICMR), NISCOM, New Delhi.

Said, M. 1969. Hamdard Pharmacopeia of Eastern Medicine, The Times Press, Karachi, Pakistan.

Harwig, J. and Scott, P. M. 1971. Brine Shrimp (*Artemia salina* L.) Larvae as a screening system for fungal toxins. *Appl. Microbiol*., 21(6): 1011-1016.

Michael, A.S., Thompson, C.G. and Abramovitz, M.1956. *Artemia salina* as a test organism for Bioassay. *Science*,6:123(3194):464-464.

Harborne, J.B. 1973. Phytochemical Methods, Chapman and Hall, Ltd., London, pp. 49-188.

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

Harwig, J. and Scott, P.M.1971. Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. *Appl. Microbiol*., 21:1011-6.

Michael, A.S., Thompson, C.G. and Abramovitz, M.1956. *Artemia salina* as a test organism for bioassay. *Science*,123:464.

Van Walbeek, W., Moodie, C.A., Scott, P.M., Harwig, J. and Grice, H.C.1971. Toxicity and excretion of ochratoxin A in rats intubated with pure ochratoxin A or fed cultures of Penicillium viridicatum. *Toxicol. Appl. Pharmacol*., 20:4239-41.

Vanhaecke, P., Persoone, G., Claus, C., Sorgeloos, P.1981. Proposal for a short-term toxicity test with *Artemia nauplii*. *Ecotoxicol. Environ. Saf*., 1981;5:382-7.

Ghosh, A., Banik, S. and Islam, M.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.

Kibiti, C. and Afolayan, A.2016. Antifungal activity and brine shrimp toxicity assessment of *Bulbine abyssinica* used in the folk medicine in the Eastern Cape Province, South Africa. *Bangladesh J. Pharmacol*., 11: 469-77.

Sufian, M. and Haque, M.2015. Cytotoxic, thrombolytic, membrane stabilizing and anti-oxidant activities of *Hygrophila schulli*. *Bangladesh J. Pharmacol*.,10: 692-96.

Syahmi, A.R.M., Vijayaratna, S., Sasidharan, S., Latha, L.Y., Kwan, Y.P., Lau, Y.L., Shin, L.N. and Chen, Y. 2010. Acute

oral toxicity and brine shrimp lethality of *Elaeis guinennsis* Jacq., (oil palm leaf) methanol extract. *Molecules,* 15: 8111-21.

Tania Maria, S., Silva, Roberto Jefferson, B., Nascimento, Michelle, M., Batista, Maria, F., Agra, Celso, A., and Camara, 2007. Brine shrimp bioassay of some species of *Solanum* from North Estern Brazil. *Brazilian Journal of Pharmacognosy* 17(1): 35-38.

McLaughlin, J.L, Chang, M. C.J. and Smith, D.L. 1991. Bench-top" bioassays for the discovery of bioactive natural products: an update. In: Rahman A, ed., *Studies in Natural Product Chemistry 9*, Elsevier, Amsterdam, pp. 383-409.

Anderson, J.E., Goetz, C.M., McLaughlin, J.L. and Suffness, M. 1991. A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor prescreens. *Phytochem Analysis*, 2: 107-111.

Olowa,L.F and Nuñeza,O.M.2013. Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines. *International Research Journal of Biological Sciences*, 2(11): 74-77.

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