MS.ID-IJPBS-8259
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2022) 12 (3): 33-37
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Cytotoxic Activity of *Ziziphus mauritiana (L.), Solanum lycopersicum* (*L.*), And Aerva *lanata (L.) Juss* Leaf Extract using Brine Shrimp Lethality Assay

T Pushpanathan[1]*, G Sumathi[1] and M Abhirami[2]
[1]Entomology Research Unit, Department of Zoology, St.Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.
[2]Department of Zoology, University of Madras, Chennai-600005, Tamil Nadu, India.

Received: 16 Mar 2022 / Accepted: 6 Apr 2022 / Published online: 1 Jul 2022
***Corresponding Author Email:** tpushpanathan@gmail.com

Abstract
Plants own huge diversity of bioactive compounds that have gained more attention in the field of Ethnopharmacology. Natural products present in plants, microbes, and animals have long been employed in traditional medicine due to their therapeutic values in preclinical and clinical trials. Besides the plant-derived natural products that have been utilized for new drug discovery. Nowadays, most of the drugs that have relied on in medical field are derived from natural products. Multidrug resistance (MDR) in cancer and bacteria has facing major barriers as they are getting resistant towards synthetic and natural drugs. To tackle and reverse the MDR, natural products can be valuable resources for the discovery of new bioactive compounds that have different mechanisms of action. The present study aims to determine the cytotoxic activity of methanolic and aqueous leaf extracts of *Ziziphus mauritiana*, *Aerva lanata* and *Solanum lycopersicum* against *Artemia salina L.* (Brine Shrimp Lethality Assay). The obtained results showed that *Z. mauritiana* had greatest cytotoxic activity that have LC_{50} value (285.27 µg/ml) is more than the methanolic extract of *S. lycopersicum* (580.32 µg/ml) and *A. lanata* (825.90 µg/ml) . Interestingly, both methanolic and aqueous extract showed excellent activity on *A.salina* except the aqueous extract of *S. lycopersicum*. Therefore, this study revealed the phytochemical compositions from the three plants are very beneficial for treatment of cancer and pathogens. Overall, *Z.mauritiana* exerted the strongest activity against *A.salina* as a potential drug.

Keywords
Artemia salina, Cytotoxicity, Plant extract, Brine shrimp lethality assay and *Ziziphus mauritiana*.

*****

INTRODUCTION
Medicinal plants have a wide range of bioactive molecules that have currently gained notability in the subject field of drug discovery [1]. Natural products have wide chemical diversity and unusual chemical structures are synthesized by terrestrial plants, animals, marine life, and microorganisms. About 10–15% of terrestrial plants are known for their medicinal values, and 88–95% of plant species remain unexplored. Plant synthesize secondary metabolites from plants are produced to protect them and have a role in defensive mechanisms which

are very species specific. Medicinal plants are well known for their therapeutic potency and have also been reported to have minimal side effects on humans and animals. Furthermore, numerous studies on plant extracts have been demonstrated as natural products that exhibit positive pharmacological effects in threatening microbial diseases and biomedical research. More than 30,000 human disease treatments depend upon drugs, which create a great socio-economic impact. So far, currently, available drugs are not effective against drug resistant pathogens and newly emerging infections. MDR on microbes and cancer chemotherapy toward synthetic and natural drugs has prompted researchers to look for new bioactive compounds from medicinally important plants [2].

In India, 130 pure compounds were isolated from 100 plant species that are being used by people worldwide [3]. The majority of the plant extracts, their fractionation, and pure compounds were unemployed for their pharmacological or biological activity by reason of the high cost of an assessment of those activities. Mammalian model organisms have long been used to find out the toxicological and pharmacological effects of plant extracts that are now restricted due to social, religious, and ethical norms. For all these reasons, the researchers were urged to search for better alternative animals that have the nature of short life span, inexpensive, easy to handle, and have a user-friendly nature. Due to the fact that artemia consider as alternative research experimental model [4].

Brine shrimp, commonly referred to as a sea monkey, is a tiny marine crustacean that belongs to Genus Artemia and lives in salt lakes and brackish water. It can grow up to 8-12 mm long [5], feed on microalgae, and be used as feed for fish. The Genus Artemia includes six important species, namely, *A. franciscana, A. salina, A. sinica, A. urmiana, A. persimili, and A. tibetiana* [4]. Generally, *A. franciscana, A. salina,* and *A. urmaina* are used for biological activity studies. About 90% of brine shrimp assays have been performed on *A.salina* as a model organism. Among all animal models, the Genus Artemia has a genetic amenity, simple anatomy, and primitive organ systems [6].

Ziziphus mauritiana belongs to the family Rhamnaceae, commonly referred to as the Indian jujube fruit shrub in tropical and subtropical regions [7]. Traditionally, the leaves were used for treatment of typhoid and as an astringent. Ashraf *et al* (2015) [8] reported that the Phytochemical studies of *Z. mauritiana* leaves have many pharmacological activities such as antioxidants, antitumor, and anticancer activities due to the presence of high phenolic compounds. The high content of flavonoids presents in methanolic extract of *Z. mauritiana* that has the strongest DPPH scavenging and antimicrobial activity. *Aerva lanata* is a perennial herb and a common weed that grows in India and Bangladesh. Several studies on the plant *A. lanata* found that it has properties of antihelminthic, demulcent, diuretic, expectorant, hepatoprotective, nephroprotective, anti-diabetic, anti-inflammatory, and antimicrobial properties [9, 10]. *Solanum lycopersicum* is an important edible plant that *belongs to* the Solanaceae family. The *S. lycopersicum* leaves are reported to possess phytochemicals such as flavonoids, saponins, terpenoids, essential oils, carbohydrates, and steroids in notable quantities [11] and are used for the treatment of ulcers, bacterial infections, and inflammations [12 and 13].

METHOD AND MATERIAL

PLANT MATERIAL COLLECTION

The leaves of three plants, namely, *Z. mauritiana, A. lanata,* and *S. lycopersicum,* were collected from Palayamkottai, Tamil Nadu, and Southern Region of India and brought to the laboratory in polythene bags. The plants were identified by Dr V Chelladurai, Former Research Officer, and Central Council for Research in Ayurveda and Siddha, Government of India. The leaves were washed with tap water and shed dried for 15 days at room temperature.

PREPARATION OF AQUEOUS AND METHANOL EXTRACT

Plant parts were extracted at room temperature with methanol and distilled water, which were removed by vacuum rotary evaporator to yield the crude extracts. The Methanolic leaf extract of three plants were prepared by using of a soxhlet apparatus and hot extraction were followed for aqueous extract. The dried leaves were powdered with the help of an electric grinder. One hundred gram of leaves was packed in a Whatman Filter Paper No. 1 for soxhlet extraction and was extracted with 400.0 ml of methanol. The process was run till getting a clear solution. For aqueous extraction, fifty gram of leaf powder was soaked in 100.0 mL of distilled water and was boiled in the conical flask for 30 minutes. Then, the plant extract was filtered through Whatman Filter Paper No.1. The filtered extract was concentrated with the help of vacuum rotary evaporator; the crude was used for bioassay.

BRINE SHRIMP TOXICITY BIOASSAY

Brine Shrimp Lethality Assay (BSLA) for the chosen plants was determined using the procedure of Meyer *et al,* 1982 [14]. 50.0 mg of brine shrimp cysts (*Artemia salina,* Artemiidae) were incubated in

plastic container that has artificial sea water which was prepared by dissolving of 40-gram sea salt in one liter of tab water. After the 48 hours of incubation, the active nauplii were attracted towards light beam and was collected using of a light lamp. The stock solution was prepared by dissolving 20.0 mg crude extract in 2.0 mL of DMSO solution (Dimethylsulfoxide) to fill 5 mL total volume with sea water. The control was treated with DMSO. Ten nauplii were introduced into each test tubes using of a micropipette. All the concentrations were replicated for thrice. The percentages of mortality were calculated after 24 hours of treatment.

DATA ANALYSIS

The mean results of mortality were plotted against the logarithms of concentrations using Probit Analysis developed by SPSS software Version 22. From the above, median lethal concentrations (LC_{50}) at 95% confidence intervals (CI) were calculated, according to the method of Finney, 1971 [15]. Biological activity using the Brine shrimp bioassay was recorded as a lethal concentration when 50% of the larvae were killed within 24 hours of contact with the extracts.

RESULTS

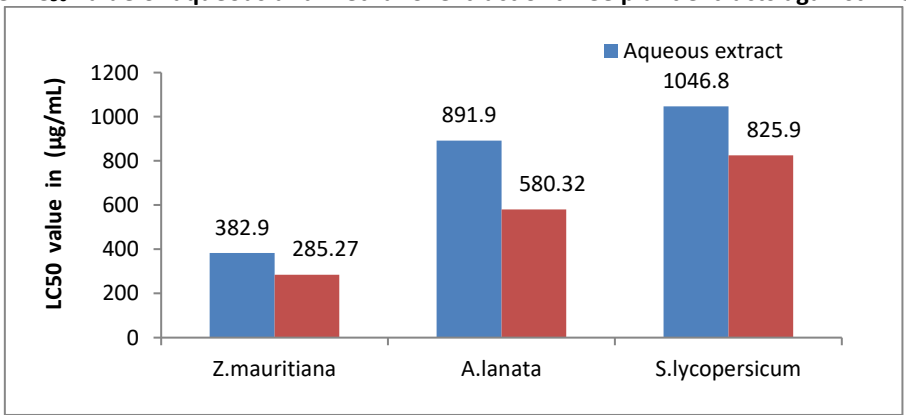
The mortality rate and LC_{50} values for *A. salina* were shown in Table 1 after the acute exposure of these three plant extracts. Methanolic and Aqueous extract of *Z.mauritiana* increased the mortality rate of *A. salina* by 100 % (LC_{50} value is 285 µg/ml and 32.90 µg/ml) at highest dose of 1000 µg/ml. Minimum mortality of 0.0 and 7.0 % were shown by methanolic and aqueous extract of *S. lyopersicum* at 50 µg/ml. Methanolic extract showed excellent cytotoxic activity compared with the aqueous extract for the selected three plants. The results of three plants suggested that *A. salina* mortality increased as extract concentration increased. So, it is evident that the methanolic plant extracts have great cytotoxic activity on *A. salina* as well as biologically active.

Table 1: Cytotoxic effect of Aqueous and methanolic leaf extract of three plants against *A.salina*

Plants	Extracts	% of mortality (µg/ml)				LC_{50} 24hr (µg/ml)	95% confidence Interval (LCL-UCL)
		50.0	100.0	500.0	1000.0		
Z.mauritiana	Aqueous	13.0	23.0	56.0	100.0	382.90	(295.35 - 486.94)
	Methanol	17.0	33.0	73.0	100.0	285.27	(201.78 - 379-82)
A.lanata	Aqueous	7.0	13.0	27.0	57.0	891.90	(706.59 - 1247.26)
	Methanol	7.0	17.0	43.0	83.0	580.32	(467.68 - 720.84)
S.lycopersicum	Aqueous	0.0	7.0	23.0	43.0	1046.80	(846.35 - 1467.06)
	Methanol	0	6.0	33.0	60.0	825.90	(689.74 -1032.43)

a) LC_{50} – Lethal concentration that kills 50% of the exposed larvae, b) LCL - Lower confidence limit, c) UCL - Upper confidence limit.

Figure: LC_{50} value of aqueous and methanol extract of three plant extracts against *A. salina*



DISCUSSION

Since natural products are widely used for the treatment of cancer and infectious disease, notably, 25% of cytotoxic drugs are primarily isolated from natural resources. Natural products are used as chemotherapeutic agents at the very beginning of cancer treatment. For instance, Taxol and Camptothecin were the most successful antineoplastic drugs still now, are derived from plants [16]. Multidrug resistance (MDR) remains a major barrier for treatment of cancer and infectious disease as they become resistance caused by natural and synthetic drugs. A study by Kars *et al* [17] reveals that plant derived MDR modulator such as capsanthin and zeaxanthin can inhibit cell proliferation and reverse the drug resistance in MDR

Human Mammary Carcinoma (MCF-7) when it was coadministration with cytotoxic drugs. Hence, pharmacotherapy has shed light on natural products that one may tackle and reverse the MDR. BSLA is a general bioassay that was very effective, economical, and rapid result as a used for the screening of general toxicity of medicinal plant, microorganism, and fungi [4].

In the present study, the methanolic leaf crude of *Z. mauritiana* had greatest cytotoxic activity on *A. salina*, was followed by *A. lanata* and S. *lycopersicum.* The methanolic extract of *Z. mauritiana* that have LC_{50} value (285.27 µg/mL) is less than 500.0 µg/mL which was considered as moderately toxic, followed by LC_{50} values 580.30 and 826.00 µg/mL for the Methanolic extracts of *A. lanata* and *S. lycopersicum*, respectively and were between 500.0 -1000.0 µg/mL was considered to be weakly toxic [18]. Maximum cytotoxicity was observed at 1000.0 µg/mL and minimum cytotoxicity was observed at 50 µg/mL (Table 1). The range of mortality was found to be directly proportional to the amount of extract exposed on animal.

A large number of studies on Genus Ziziphus plant showed that it possesses significant antitumor activity [19], anti-inflammatory [20], antimicrobial activity [21], antioxidative [22], and antileishmanial activity [23]. The fruit, seeds, and leaves of *Ziziphus lotus* and *Ziziphus mauritiana* have been studied for antioxidant and antimicrobial studies. The results showed that leaves of *Ziziphus lotus* were very efficient against four pathogenic bacteria (*Staphylococcus aureus, Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli*) significantly than other the two parts of these two plants. Overall, the 28 phenolic compounds were detected in LC-ESI-MS analysis of *Z. lotus* leaves which contain major bioactive compounds are qunic acid, p-coumaric acid, rutin and quercitin that were the most responsible for the inhibition of bacterial proliferation [21].

In a study by Ahmad et al [24], the *Ziziphus jujube* was screened for antifungal, cytotoxic, anti-termite, and insecticidal activity. The highest cytotoxic activity was observed in 1000.0 (µg/ml) with 73.33 % mortality while other test sample have low activity. The results showed that *Z. jujube* might be the better cytotoxic drug. Another study, which was carried out by Said *et al* [25] reported that the cytotoxic effect of five medicinal plants against brine shrimp. The stem and bark of *Z. jujube* were extracted using different solvents - hexane, chloroform and ethyl acetate. The LC_{50} value for chloroform extract and ethyl acetate were 93.60 and 145.80 µg/ml respectively. Ambrin *et al* [26] investigated the cytotoxic activity of two plants *Ziziphus mauritiana Var. Spontanea edgew. and Oenothera biennis L.* against brine shrimp. The results showed that maximum mortality found in ethanolic extract of *Z. mauritiana* (83.40 %) than ethyl acetate extract (73.40 %) and aqueous extract (50.0 %) at 1000.0 µg/ml and the minimum LD_{50} of ethanolic extract was 107.68 µg/ml.

In another study, carried out by Chowdhury *et al* [27], the Artemia was exposed to ethyl acetate, methanol and petroleum ether extract of *A. lanata.* Among all the extracts, petroleum ether extract was very effective that have LC_{50} value was 28.21 ppm, followed by 20.37 ppm and 22.41 ppm for ethanol and methanol extract. Bahar *et al* [28] explored the methanol extract of *A. lanata* was most effective than ethyl acetate and petroleum ether extract against *A. salina*. The LC_{50} value for methanol and petroleum extract were 49.99 and 40.85 ug/ml respectively.

Likewise, Silva *et al* [29] studied that the cytotoxic activity of 13 plant species belongs to the Solanaceae family using Brine Shrimp Lethality Assay. Among all plants, the fruit extracts of *Solanum aspericum* and *Solanum patudosum* had a greatest LC_{50} value is 420.50 and 548.00 µg/mL. Similarly, Dougnon *et al* [30] used the *Solanum macroparcon* for cytotoxic assay on *A.salina*. The crude extract of leaves and fruit was found to be non-toxic and LC_{50} values is 1.33 mg/mL and 1.51 mg/mL, and these studies reveal that LC_{50} values were greater than the upper limit of toxic. It concluded that Solanaceae family plant has no biological activity.

CONCLUSION

The objective of this work was to find out the potential cytotoxic activity of aqueous and methanol crude extract of *Z. mauritiana, A. lanata* and *S. lycopersicum* leaves could be recommended as potential drugs against anticancer drugs. As a result, more research is needed to learn more about its cytotoxic components and the circumstances that cause this event. Therefore, brine shrimp lethality assay can be used to study of cytotoxicity of plant extracts that have advantage such as easy handle, simple procedure, and rapid testing. Natural products from these three plants can offer new kind of cytotoxic drugs. These three plants can be also suggested to inhibit microbial infections.

REFERENCE

1. Appendino G, Fontana G, and Pollastro F. 3.08—Natural products drug discovery. *Comprehensive Natural Products II Elsevier Oxford*, UK: 205-236, (2010).
2. Wangchuk P. Therapeutic applications of natural products in herbal medicines, bio discovery

programs, and biomedicine. *J Biol Act Prod Nat*, *8*(1): 1-20, (2018).
3. Maridass M and De Britto AJ. Origins of plant derived medicines. *Ethnobot leafl*, (1): 44, (2008).
4. Ntungwe NE, Dominguez-Martin, EM, Roberto, A, Tavares, J, Isca, V, Pereira, P, Rijo, P. *Artemia* species: An important tool to screen general toxicity samples. *Curr Pharm Des*, *26*(24): 2892-2908, *(2020)*.
5. Ogello, EO, Kembenya E, Githukia CM, Betty M, Munguti JM. The occurrence of the brine shrimp, *Artemia franciscana* (Kellog 1906) in Kenya and the potential economic impacts among Kenyan coastal communities. *Int j fish Aquat*, 1(5): 151-6, (2014).
6. Meyer, BN, Ferrigni, NR, Putnam, JE, Jacobsen, LB, Nichols, DEJ, McLaughlin, JL. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica,* 45(5): 31-4, (2007).
7. Abdallah EM, Elsharkawy ER, and Ed-dra A. Biological activities of methanolic leaf extract of *Ziziphus mauritiana*. *Pharm Commun Biosci Biotech Res Comm Thomson Reuters ISI ESC Crossref Index. J. NAAS J. Score*, 9(4): 605-614, (2016).
8. Ashraf A, Sarfraz RA, Anwar F, Shahid SA, and Alkharfy KM. Chemical composition and biological activities of leaves of *Ziziphus mauritiana* L. native to Pakistan. Pak J Bot, *47*(1): 367-376, (2015).
9. Raihan, O, Brishti, A, Bahar, E, Islam, F, Rahman, M, Tareq, SM, Hossain, M. Antioxidant and anticancer effect of methanolic extract of *Aerva lanata* Linn. against Ehrlich Ascites Carcinoma (EAC) in vivo. *Orient Pharm Exp Med*, 12(3): 219-225, (2012).
10. Mariswamy Y, Gnaraj WE and Antonisamy JM., Chromatographic fingerprint analysis on flavonoids constituents of the medicinally important plant *Aerva lanata L*. by HPTLC technique. Asian Pac J Trop Biomed, *1*(1): S8-S12, (2011).
11. Perveen, R, Suleria, HAR, Anjum, FM, Butt, MS, Pasha, I, Ahmad, S. Tomato (*Solanum lycopersicum*) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims-A comprehensive review. *Crit Rev Food Sci Nutr*, *55*(7): 919-929, (2015).
12. Sajet AL-Oqaili RM, and Salman, BBMMA. In vitro antibacterial activity of *Solanum lycopersicum* extract against some pathogenic bacteria. *In Vitro*, *27,* (2014).
13. Li, H, Deng, Z, Liu, R, Loewen, S, Tsao R. Bioaccessibility, in vitro antioxidant activities and in vivo anti-inflammatory activities of a purple tomato (*Solanum lycopersicum* L.). *Food Chem*, *159:* 353-360, (2014).
14. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. J., & McLaughlin, J. L. (1Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, 45: 31-34, (1982).
15. Finney, DJ. Probit Analysis, 3rd ed. *Cambridge University Press*, UK (1971).
16. Huang, M, Lu, JJ, and Ding, J. Natural products in cancer therapy: Past, present and future. *Nat Prod Bioprospecting*, 1-9, (2021).
17. Kars, MD, Iseri, OD, Gunduz, U, Molnar, J. Reversal of multidrug resistance by synthetic and natural compounds in drug-resistant MCF-7 cell lines. *Chemotherapy*, *54* (3): 194-200, *(2008)*.
18. Nguta, JM, Mbaria, JM, Gakuya, DW, Gathumbi, PK, Kabasa, JD, Kiama, SG. Evaluation of acute toxicity of crude plant extracts from Kenyan biodiversity using brine shrimp, *Artemia salina* L. (Artemiidae). *Open Conf Proc J,* 3: 30–34, (2012).
19. Mesmar, J, Fardoun, MM, Abdallah, R, Al Dhaheri, Y, Yassine, HM, Iratni, R, Baydoun, E. *Ziziphus nummularia* attenuates the Malignant Phenotype of Human Pancreatic Cancer Cells: Role of ROS. *Molecules*, *26*(14), 4295, (2021).
20. Kumar, S, Ganachari, MS, and Nagoor, VS. Anti-inflammatory activity of *Ziziphus jujuba* Lam leaves extract in rats. *J Nat Remedies*, *4*(2), 183-185, (2004*)*.
21. Yahia, Y, Benabderrahim, MA, Tlili, N, Bagues, M, Nagaz, K. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus* Mill. Species. PloS one, *15* (5), e0232599, *(2020)*.
22. Damiano, S, Forino, M, De, A, Vitali, LA, Lupidi, G, Taglialatela-Scafati, O. Antioxidant and antibiofilm activities of secondary metabolites from *Ziziphus jujuba* leaves used for infusion preparation. *Food Chem*, *230*: 24-29, (2017).
23. Albalawi, AE. Antileishmanial Activity of *Ziziphus spina-christi* Leaves Extract and Its Possible Cellular Mechanisms. *Microorganisms, 9*(10): 2113, *(2021)*.
24. Ahmad, B, Khan, I, Bashir, S, Azam, S Ali, N. The antifungal, cytotoxic, antitermite and insecticidal activities of *Ziziphus jujube*. *Pak J Pharm Sci*., *24*(4): 489-493, (2011).
25. Said, SA, Al-Saadi, SHA, Al-Abri, AR, Akhtar, MS, Weli, AM, Al-Riyami, Q. Cytotoxic properties of some herbal plants in Oman. *J Taibah Univ Sci*, *8*(2): 71-74, (2014).
26. Ambrin, GD, Bakhi, J, Adil, M. Phytotoxic, insecticidal and cytotoxic activities of *Ziziphus mauritiana* var. Spontanea edgew. and *Oenothera biennis* L. *Pak J* Bot*,* 52(6): 2191-2195, (2020).
27. Chowdhury, D, Sayeed, A, Islam, A, Bhuiyan, MSA, Khan, GAM. Antimicrobial activity and cytotoxicity of *Aerva lanata*. *Fitoterapia, 73*(1): 92-94, (2002).
28. Bahar E, Ara J, Hossain M, Nath B, and Runi N. Cytotoxic (In-Vitro) effect of methanol & petroleum ether extracts of the *Aerva lanata*. *J pharmacogn Phytochem*, *2*(1), (2013).
29. Silva, TMS, Nascimento, RJB, Batista, MM, Agra, MF, Camara, CA. Brine shrimp bioassay of some species of *Solanum* from Northestern Brazil. *Rev bras farmacogn, 17*: 35-38, *(2007)*.
30. Dougnon, TV, Bankolé, HS, Johnson, RC, Klotoé, JR, Dougnon, G, Gbaguidi, F, Edorh, AP. Phytochemical screening, nutritional and toxicological analyses of leaves and fruits of *Solanum macrocarpon Linn (Solanaceae) in* Cotonou (Benin) (2012).