WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 1182-1189. Research Article ISSN 2277– 7105

EVALUATION OF CYTOTOXIC ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF ANOGEISSUS LATIFOLIA WITH BRINE SHRIMP LETHALITY ASSAY

Manasi Arjun Bagayatkar*[1] and Vaibhavi N. Garge[2]

[1]Department of Pharmacology, Bharati Vidyapeeth's College of Pharmacy, Sector 8, C.B.D Belapur, Navi Mumbai, Maharashtra, India.

[2]Assistanct Professor, Bharati Vidyapeeth's College of Pharmacy.

Article Received on
28 Feb. 2018,

Revised on 21 March 2018,
Accepted on 11 April 2018,

DOI: 10.20959/wjpr20188-11918

***Corresponding Author**
Manasi Arjun Bagayatkar
Department of Pharmacology, Bharati Vidyapeeth's College of Pharmacy, Sector 8, C.B.D Belapur, Navi Mumbai, Maharashtra, India.

ABSTRACT

Cancer is a disease which involves abnormal growth of cells which can proliferate in an uncontrolled way. It can sometimes metastasize to various parts of the body. Natural sources having cytotoxicity can be used to treat cancer. The conventional chemotherapy for malignant tumor is unfavorable because it can lead to various side effects and also is the cause of growing resistance towards various anticancer agents. Therefore, focus has now been shifted from conventional therapy to natural sources to treat various malignancies. *Anogeissus latifolia* is a plant with potent antioxidant activity. It is also reported to have immune-modulatory, anti-inflammatory, microbicidal, antiulcer, and hepato protective activity, which makes it an ideal natural medicinal source which can be developed as an anticancer agent. Its cytotoxicity has been tested in various human cancer cell lines. The Cytotoxic activity of hydro alcoholic extract of *Anogeissus latifolia leaves* was investigated for its anticancer potential *in vitro* using Brine shrimp lethality assay (BSLA). BSLA is a simple, preliminary screening model for bioactive chemicals. It is based on lethality of the test compounds on brine shrimp (*Artemia salina*). The percent lethality of the brine shrimp in various concentration variations was plotted on a graph, and LC_{50} was calculated.

KEYWORDS: Cancer, Brine shrimp lethality test, anti-inflammatory.

INTRODUCTION

There are various plants that are discovered every year for treatment of cancer. These plants may have various valuable properties. It is also reported that various plants although used as food sources may have mutagenic or genotoxic potential.[1] Cytotoxicity of the plant depends on the various chemical compounds and contaminants that are found to be present in the different parts of the plant. Various assays are developed to assess the potential toxicity of herbal extracts different biological models. Various medicinal plants like *Isatis tinctoria* and *Sophora flavescent*, have been proved to have anticancer activity.[2]

Anogeissus latifolia belonging to family combretaceae is a deciduous tree almost about medium in size commonly known as Dhava or Ghatti.[3] Bark of the plant is smooth and pale to dark grey in color. Leaves are opposite or sub opposite, distictious, simple, entire with greyish yellow or whitish hairs below.[4] Flowers are sessile, in dense heads, fruits small, compressed, and winged with beak, seed ovoid. The tree usually flowers and fruits in the month of September – march.[5] *Anogeissus latifolia* is native to India. The plant is common in dry deciduous forests. It is found in sub Himalayan tract from Ravi to Nepal, and in most of south India.[6]

Anogeissus latifolia showed cytotoxicity for ethanolic extracts in 9 cancer cell lines by inhibiting cell proliferation. Cytotoxicity was seen in a concentration dependant manner in cancers of lung (A549), prostrate (PC-3), breast (T47D and MCF -7), Colon (HCF-16 and COLO – 205) and leukaemia (THP, HL-60 and K562 by using SRB and MTT assays.[7]

This plant is of extensive ethno-botanic significance. Various secondary metabolites derived from *A. Latifolia* have been reported which include polysaccharides, lectins, peptides, flavonoids and tannins. The phytochemicals found include tri-terpenoids like 3- β- hydroxyl-28- acetyl taraxen and β- sitosterol, leucocyanidin, gallotannins, and many others. *A. latifolia* is found to have hypolipidemic diuretic, anti-microbial and hepato protective activity, anti-ulcer and wound healing activity.[8-16]

The Methanolic extract of leaf was found to have Anti-Angiogenic activity by CAM assay. The CAM assay is a simple model to monitor the invasion of cancer cell lines. Jurkat cell lines were also in line with chick embryo studies.[17]

Brine shrimp lethality assay

Newly developed bioassays should be reliable and should detect a broad spectrum of pharmacological activities in a wide range of plants. These assays should be cost effective and applicable in phytochemical screening of medicinal plants. Cytotoxic in vivo lethality observed in a zoologic organism can be used to monitor the preclinical preliminary screening of a bioactive natural compound. Biological assays have been developed which include various species of Artemisia like *A. Salina, A. franciscana, A. armiana* and *Thammocephalus platyrus*. These toxicity tests can be employed as a useful tool for preliminary assay of toxicity.[18,19,20]

Brine shrimp assay is used to detect cytotoxicity. It is simple in process as compared to other tedious and expensive in-vitro and in-vivo anti-tumor assays. [21] Advantages of brine shrimp are that it is rapid (required about 24 hours), inexpensive, simple. It required no sophisticated equipment and needs a relatively small quantity of the drug. Statistical validation is carried out utilizing a large number of organisms. Implementation of BSLA in several studies has demonstrated a good correlation between results between the lethal concentration that kills 50% of the exposed population (LC50) obtained with Brine Shrimp and acute oral toxicity assay in mice.[22,23]

The present study evaluates the cytotoxic activity of hydro-alcoholic (70: 30 Methanol: Water) extract of leaves of *Anogeissus latifolia* (combretaceae) on Brine Shrimp. Here, (70: 30 Methanol: Water) solvent was used to extract media as it is cheap, easily available and non-toxic.

MATERIALS AND METHODS

Procurement of Plant Material– Leaves of *Anogeissus latifolia* were collected. The collected leaves were thoroughly washed with distilled water. The leaves were sun-dried and powdered. The powder was authenticated by Department of Botany, Guru Nanak Khalsa.

College, Matunga, Mumbai – 400019. The voucher specimen (Specimen No. - # rcb p 1050448) has been preserved for future reference.

Preparation of Crude Extract

The powdered plant material of A. Latifolia was subjected to soxhlet extraction to prepare hydro alcoholic (70:30 – Methanol: Water) solvent for a larger period of time by,[19] and was

followed by filtration, evaporation and concentrated to dryness under reduce pressure using rotary evaporator.[20]

Hatching of Brine Shrimps

About 1gm of *Artemia Salina* (Linnaceae) cysts procured from local pet shops was aerated in 1-liter capacity hatching chamber containing salt water (30% NaCl solution, pH – 8.2). complete aeration was provided to the hatching chamber. The cysts were incubated at room temperature (24ºC - 28º) for 48 hours under continuous illumination. Newly\ hatched freely swimming pink colored nauplli were harvested. As the empty cyst capsules floated on the surface, the collection of the nauplii from the bottom of the hatching chamber ensured pure harvest. These nauplii were then used for the bioassay. Varying concentrations of the test sample (extract) was prepared by dissolving it in artificial sea water (30% NaCl solution, pH – 8.2) ranging from 1µg/ml - 1000 µg/ml. the vehicle used was 1% DMSO.[24] Artificial saltwater containing 1% DMSO was used as control.

The bioassay system was prepared with artificial sea water containing the varying concentration of the extract in a test tube. 1% yeast was added as a feed. The concentration of each dose were prepared in triplicates. 10 naupliis were transferred in each of the above test tube and the setup was kept for 24 hours under constant illumination. Number of surviving naupliis were counted with the help of hand lens after the period of 24 hours. LC50 values were then determined on the average of the percent mortality.[25,26]

OBSERVATIONS

1. % Mortality of the Brine shrimp as per the concentration of test drug.

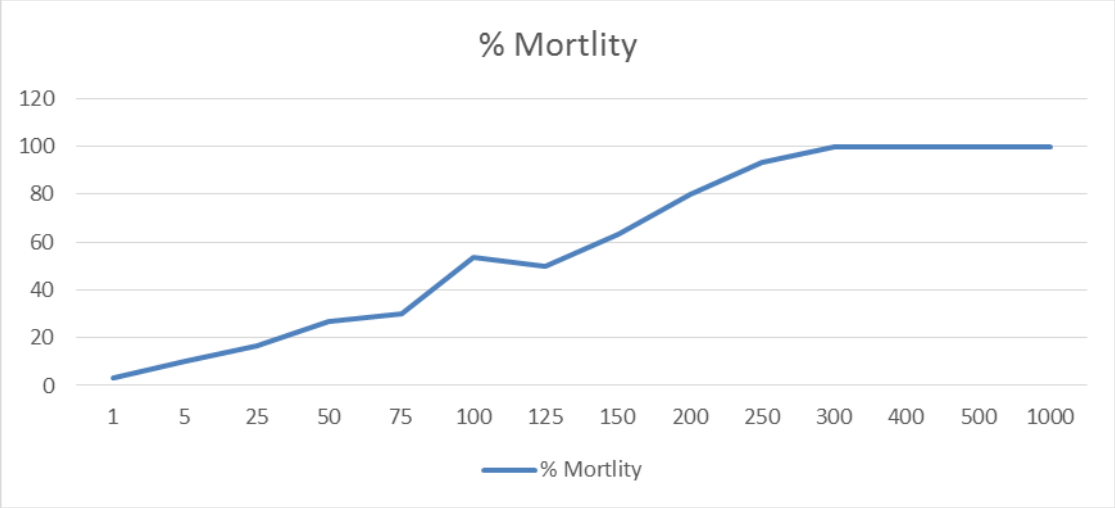
Conc. of Samples (µg/ml)	No. of alive shrimp				% Mortality	LC50
	Test 1	Test 2	Test 3	Total No of Survivors		
1	9	10	10	29	3.33	125µg/ml
5	8	9	10	27	10	
25	8	8	9	25	16.66	
50	7	8	7	22	26.66	
75	7	8	6	21	30	
100	5	6	5	16	53.55	
125	5	5	5	15	50	
150	5	4	2	11	63.33	
200	3	2	1	6	80	
250	1	1	0	2	93.33	
300	0	0	0	0	100	
400	0	0	0	0	100	

500	0	0	0	0	100	
1000	0	0	0	0	100	
Control	9	10	10	29	3.33	

Statistical analysis

The test tubes were inspected for living nauplis after a period of 24 hours. The control group had all the nauplii surviving throughout the period. Dose concentration group of 125µg/ml had 50% mortality and dose concentration of 250µg/ml had 90% mortality.

Hence, LC50 was found to be 125µg/ml and LC90 was found to be 250µg/ml.



RESULTS AND DISCUSSION

Anogeissus latifolia was tested for concentrations ranging from 1µg/ml - 1000 µg/ml to determine the brine shrimp lethality. Out of the various concentrations 125µg/ml was found to inhibit 50% of the Brine shrimp. The observed lethality of *A. Latifolia* extract to brine shrimps indicated the presence of potent cytotoxic and probably antitumor component of these plant.

Brine shrimp lethality assay determines cytotoxic activity by a simple method in an efficient, rapid and inexpensive way. It is an excellent choice for testing bioactivity of various plants. According to Meyer et al., crude plant extract is toxic (active) if it has an LC50 value of less than 1000 µg/mL while non-toxic (inactive) if it is greater than 1000 µg/mL.[27]

Anogeissus latifolia has significant anti-oxidant activity. Various oxidation procedures induced by ROS result in cell membrane disintegration, membrane protein damage and DNA mutation which can lead to initiation of development of cancer. Anti-oxidant drugs can

provide significant contribution in prevention and treatment of such diseases. *A. latifolia* also consists if β-sitosterol and triterpenoids which can be isolated and used as an anti-tumor agent. Hydroalcholic extract (70:30 – Methanol: Water) of *A. latifiolia* is consists of various phytoconstituents such as flavonoids, polyphenols, saponins, tannins which have therapeutic potential in treatment of malignancies,

A.latifolia is reported to be a good Hepato protective and Anti-ulcer agent. Anticancer cell line studies should be hence carried out in Gastric and liver cell lines. In-vivo studies should be carried out in various models like zebra fish.

CONCLUSION

From the present study it can be concluded that *Anogeissus latifolia* can be developed as a potential anticancer agent.

The ethno-pharmacological activity present in the above plant is due to presence of various bioactive compounds. Although BLSA, is inefficient in determining its mechanism of action, it is certainly useful in providing a preliminary screening model that can be supported by more studies. Various further research studies are required in this process. Further studies on the molecular level are required to assess its mechanism of action.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude the head of Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai for providing us the facility to conduct this experiment

REFERENCES

1. Tülay, A.Ç., Özlem, S.A., (Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts). Biologia, 2007; 3: 292–296.
2. Elham Safarzadeh, et al, (Herbal Medicine as Inducers of Apoptosis in Cancer Treatment). Adv Pharm Bull, 2014; 4(Suppl 1): 421–427.
3. P. V. Orwa, (Agroforestry database), 2006; 4.0: 1-5.
4. P. K.Warrier. (Indian medicinal plants: A compendium of 500 species), 1994; 4: 381.
5. S. R. Yadav, M. M. Sardesai. (Flora of Kolhapur District), 2002; 451.
6. R. N. Chopra, Nayar, I. C. Chopra, (Glossary of Indian medicinal plants). Council of scientific and Industrial research, New Delhi, 1956; 158.

7. AE Diab*, Santosh Kumar Guru, Shashi Bhushan , Ajit K Saxena, (In Vitro Anticancer Activities of *Anogeissus latifolia*, *Terminalia bellerica*, *Acacia catechu* and *Moringa oleiferna* Indian Plants). Kawthar Asian Pac J Cancer Prev, 2015; 16(15): 6423-6428.
8. Jagtap SD, Deokule SS, Pawar P K, (Traditional Ethnomedicinal knowledge confined to Pawara tribes of Satpura hills), Maharashtra, India. Ethnobotanical leaflet, 2009; 13: 98-115.
9. Pradeep Hulikare, Ananth, Saleemulla Khan, Raghu Chandrasekhar, Mohammed Ibrahim. (Development of Validated HPTLC method for Simultaneous Quantification of Rutin and Quercetin from Bark of *Anogeissus latifolia*). International Journal of Pharmacology, 2012; 2(1): 33-38.
10. Akhilesh V. Singh, Anudwipa Singh, Nath LK, Ghosh TK. (*Anogeissus latifolia*: A recent update on its Chemistry and Pharmacological Application). Pharmacology online. 2010; 2: 446-449.
11. Arutla Rajender, Suresh A, Suresh V, Saker G, Varaprasad N. (Evaluation of anti-ulcer activity of methanolic extract of *Anogeissus latifolia* leaves in albino rats). International Journal of Research in Ayurvada and Pharmacy, 2011; 2(2): 677-680.
12. Hemamalini K, Om Prasad Naik K, Peddi Ashok. (Study of Phytochemical Diuretic Potential of Methanol and Aqueous Extracts of Leaf Parts of *Anogeissus latifolia*). International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011; 2(1): 136-139.
13. Parvathi KMM, Ramesh C K, Krishna V. (Hypolipidemic activity of gum ghatti of *Anogeissus latifolia*). Pharmacognosy Magazine, 2009; 5: 11-14.
14. Govindrajan R, Vijaykumar M, Singh M. (Antiulcer and antimicrobial activity of *Anogeissus latifolia*). Journal of Ethnopharmacology, 2006; 106(1): 57-61.
15. Yerra Rajeshwar, Thota Sreekanth, Getu Kahsay. (Hepatoprotective Activity of Anogeissuslatifolia bark in ethanol intoxicated rats). The American Journal of Science and Medical Research, 2016; 1(2): 196-201.
16. Agnihotri Vineet. (Anti-inflammatory potentials of *Anogeissus latifolia*: Assessment through effect on oxidative stress). IL-6 and TNF level. Inventi Journal, 2015; 3: 80-84.
17. Narayanan Nikhitha et al. (Evaluation of Anti-Angiogenic Activity of Anogeissus latifolia). Indo American Journal of Pharmaceutical Research, 2016: 6(09).
18. Carballo, J.L., Hernández-Inda, Z.L., Pérez, P., García-Grávalos, M.D., (A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products). BMC Biotech, 2002; 2: 17.

19. Veni, T., Pushpanathan, T., (Comparison of the *Artemia salina* and *Artemia fransiscana* bioassays for toxicity of Indian medicinal plants). J Coastal Life Med, 2014; 2: 453-457.
20. Mayorga, P., Pérez, K.R., Cruz, S.M., Cáceres, A., Comparison of bioassays using the anostracan crustaceans *Artemia salina* and *Thamnocephalus platyurus* for plant extract toxicity screening. Bras J Pharm, 2010; 20: 897-903.
21. McLaughin J L, Rogers L L. (The use of biological assays to evaluate botanicals). Drug information Journal, 1998; 32: 513-524.
22. Parra, L.A., Yhebra, R.S., Sardiñas, I.G., Buela, L.I., (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). Phytomed, 2001; 8: 395-400.
23. Arslanyolu, M., Erdemgil, F. Z., (Evaluation of the antibacterial activity and toxicity of isolated arctiin from the seeds of *Centaureas clerolepis)*. J Fac Pharm, 2006; 35: 103-109.
24. Camila Vidotto, (Brine shrimp lethality test as a biological model for preliminary selection of pediculicidal components from natural source). Biosci. J., Uberlândia, 2013; 29(1): 255-263.
25. Ghosh Ajoy And Chatterjee Padma, Brine Shrimp Cytotoxic Activity Of 50% Alcoholic Extract Of Croton *Bonplandianum Baill*. *Asian J Pharm Clin Res, 2013; 6(3): 40-41*.
26. Mentor R. Hamidi, Blagica Jovanova, Tatjana Kadifkova Panovska, Toxicological evaluation of the plant products using Brine Shrimp (*Artemias alina* L.) model, *Macedonian pharmaceutical bulletin*, 2014: 60(1): 9 – 18.
27. Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., (Brine Shrimp: A convenient general bioassay for active plant constituents). Planta Medica, 1982; 45: 31-34.