

**“CYTOTOXICITY ASSAY OF ETHANOLIC EXTRACTS OF  
AMALAKYADI CHURNA AND ITS INGREDIENTS USING  
ARTEMIA SALINA”**

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**Summary**

Amalakyadi churna is an Ayurvedic formulation, which is extensively used in traditional system of Indian medicine and by folk practitioner for treating all types of fever, and other common ailments. In this study, an equal concentration of ethanolic extracts of Amalakyadi churna and its ingredients (50µg/ml of artificial sea water) were subjected to a bioscreening study to detect the cytotoxic activity by the brine shrimp (*Artemia salina*) lethality bioassay. The results obtained from the crude ethanolic leaf extracts of *Phyllanthus emblica* L (73%), *Terminalia chebula* Retz (87%), *Piper longum* L (90%), *Plumbago zeylanica* L (93%), Amalakyadi churna (98%) were exhibited promising brine shrimp lethality. The present study supports that the brine shrimp bioassay is simple, reliable and convenient method for assessment of bioactivity of these medicinal plants and lends support for their use in traditional medicine.

**Key Words:** *Artemia salina*, brine shrimp lethality test, ethanolic extract, Amalakyadi churna, cytotoxicity

### Introduction

The brine shrimp lethality assay was proposed by Michael *et al.*, (1956) (1). It is based on the ability to kill laboratory cultured *Artemia salina* nauplii (brine shrimp). The assay is considered to be a useful tool for preliminary assessment of toxicity (2) and it has been used for the detection of fungal toxins (3), plant extract toxicity (4), heavy metals (5), cyanobacteria toxins (6), pesticides (7) and cytotoxicity testing of dental materials (8). The brine shrimp lethality test was used as a surrogate tool to evaluate the toxicities, and also to identify their potential for other biological activities.

Several reports suggested that, screening of cytotoxic effects on brine shrimp confirms the presence of bioactive, in particularly antitumor compounds in plant species. Before screening for biologically active plant constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the investigation. Plants used in traditional medicine are more likely to yield pharmacologically active compounds.

Considering the importance of the assay in possessing one or the other mechanism over active metamorphosis state of brine shrimp naupalii, it is necessitated to explore the cytotoxic assay of ethanolic extracts of individual plant ingredients and synergistic potential of all these above useful plants (Amalakyadi churna) to get total therapeutic disease targets.

### Materials and Methods

**Collection of the materials:** The plant materials of *Phyllanthus emblica* L. (Phyllanthaceae), *Terminalia chebula* Retz. (Combretaceae), *Plumbago zeylanica* L. (Plumbaginaceae) from Sandur, India. *Piper longum* L. (Piperaceae) from the Agricultural University, GKVK, Bangalore, India in the month of October-November and identity of these plants were confirmed by Flora of Karnataka, Hassan and Plants that heal (9-11). The rock salt was purchased from the local Ayurvedic shop, Gulbarga, India. Brine shrimp eggs was obtained from Sanders<sup>TM</sup> Great Salt Lake, Brine Shrimp Company L.C., U.S.A., NaCl, NaHCO<sub>3</sub>, ethyl alcohol from Ranbaxy fine chemicals, New Delhi, India..

**Preparation of Amalakyadi churna:** The pre-cleaned, dried powders of fruits of *Phyllanthus emblica*, *Piper longum*, *Terminalia chebula*, roots of *Plumbago zeylanica* and rock salt were taken in equal proportions and mixed well. This Amalakyadi churna is stored in an airtight container for further processing (12).

**Preparation of the Extracts:** The 100g of Amalakyadi churna and its plant ingredients were extracted with 90% alcohol at 50 - 60°C in a soxhlet apparatus. The extract was concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) and note down the yield of crude extract.

**Preparation of extracts:** The plant materials were dried under shade and ground to a coarse powder. The powdered plant materials (each 25g) were individually extracted with ethanol (200 ml) using soxhlet extractor and then filtered. Filtrates were concentrated individually, dried under vacuum and used for screening the brine shrimp lethality.

**Preparation of Brine solution:** Take sodium chloride (NaCl) 15gms and sodium bicarbonate (NaHCO<sub>3</sub>) 4.2gms were dissolved completely in distilled water and made the final volume to 1000ml with pH-8.2.

**Brine Shrimp Lethality Bioassay:** Brine shrimp lethality assay, was carried out to investigate the cytotoxicity of extracts of medicinal plants. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial sea water (prepared using sea salt 38 g l<sup>-1</sup> and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 hours. 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 experiment, 0.5ml of the plant extract was added to 0.45 ml of brine solution and maintained at room temperature for 24 hours under the light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), concentration (50 µg ml<sup>-1</sup>) of the test substances in a set of three tubes per dose (13).

**Statistical analysis:** The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. Results are expressed as mean ± standard deviation of mean (SD).

## Results

In the present study, a total extracts of Amalakyadi churna and its plant ingredients were used in the traditional systems of medicine to brine shrimp lethality was determined using the procedure of Meyer *et al* (13). The results of the present study clearly demonstrated that, the ethanolic extracts of Amalakyadi churna and its plant ingredients showed different mortality rates. The crude extract of Amalakyadi churna displayed the highest

activity, approximately about 98% mortality rate, similar observations were noticed with the extracts of *Piper longum*, *Terminalia chebula* and *Plumbago zeylanica* extracts and exhibited 90%, 87% and 93% respectively. Where as, the crude extract of *Phyllanthus emblica* showed activity but comparatively less i.e., 73% mortality (Table-1).

Table-1 Cytotoxicity assay of the ethanolic extracts of Amalakyadi churna and its ingredients

Sl. No	Plant name	Parts used	Traditional Uses	Percentage of Brine shrimp lethality (Mean $\pm$ SD)
1	Amalakyadi Churna	Mixture	All types of fever, carminative, digestive etc	98.00 $\pm$ 0.01
2	<i>Phyllanthus Emblica</i>	Fruits	Antioxidant, antitumour, hepatoprotective, antiageing, hypoglycemic	73.00 $\pm$ 0.07
3	<i>Piper Longum</i>	Fruits	Antifertility, anticancerous, antiviral, also beneficial for peptic ulcers, diabetes etc	90.00 $\pm$ 0.01
4	<i>Plumbago zeylanica</i>	Roots	Antifertility, antitumour, antimicrobial activities	93.50 $\pm$ 0.50
5	<i>Terminalia Chebula</i>	Fruits	Laxative, diuretic, cardiogenic, antimicrobial activity etc.	87.00 $\pm$ 1.73

### Discussion

Brine shrimp assay is considered as a reliable indicator for the preliminary assessment of cytotoxicity, which in most cases correlates reasonably well with cytotoxic and antitumor properties (14). Toxicity at its lower dosage can serve as pharmacology and it can be exploited for cell line toxicity and anti-tumour activity. This assay is widely employed in the screening process of botanical for the isolation of bioactive metabolites (15). Since cell death, apoptosis approach is one of the strategies in cancer chemotherapy, the general lethality effect over brine shrimp is considered as cytotoxic effect to cause cell death and organism death. Besides, there may be specific mode of action of plant drugs like topoisomerase-I and topoisomerase-II inhibition, inhibition of spindle fibre elongation, action over cyclin dependent kinases, action over check-points in cell cycle and several other anticancer properties at cellular and organism level. It is evidenced by many reports, which suggested the presence of antitumor activity in different ingredients of Amalakyadi churna. The antitumor activity of piperine and piperidine components of *Piper* species by their *in-vivo* studies on mice transplanted with sarcoma-180 tumor cell line (16). The antitumor effect of *Phyllanthus emblica* (17). Thus confirming the presence of highly biologically active compounds in these extracts.

### Conclusion

From the preliminary screening, it is clear that all the test samples exhibited considerable cytotoxicity by brine shrimp lethality assay might be due to the presence of secondary metabolites in their plants extracts. Here, the combination of all these plant materials in equal proportions making the Amalakyadi churna preparation as one of the rich source of phytoconstituents and interaction of all these chemicals might be resulted in synergistically enhanced the rate of brine shrimp lethality than its individual plant extracts, this lend pharmacological support to folkloric, ethno-medical and traditional uses of this churna for treatment of various diseases.

### References

1. Michael AS, Thompson CG, Abramovitz M. *Artemia salina* as a test organism for a bioassay. *Science* 1956; 123: 464.
2. Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. A microwell cytotoxicity assay using *Artemia salina*. *Plant Med* 1993; 59: 250 -252
3. Harwig J, Scott P. Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. *Appl Microbiol* 1971; 21 : 1011 -1016.
4. McLaughlin JL, Chang CJ, Smith DL. Bench top bioassay for the discovery of bioactive natural products: an update. In: *Studies in Natural Products Chemistry* (Ed.), Rahman AU., Elsevier, 1991: 383 -409
5. Martinez M, Delramo J, Torreblanca A, Diaz-Mayans J. Effect of cadmium exposure on zinc levels in the brine shrimp *Artemia partenogenetica*. *Aquaculture* 1998; 172: 315 – 325.
6. Jaki B, Orjala J, Burji HR, Sticher O. Biological screening of cyanobacteria for antimicrobial and molluscicidal activity, brine shrimp lethality and cytotoxicity. *Pharm Biol* 1999; 37: 138 -143.
7. Barahona MV, Sanchez-Fortun S. Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. *Environ Pollut* 1999; 104: 469 – 476

8. Pelka M, Danzl C, Distler W, Petschelt A. A new screening test toxicity testing of dental materials. *J. Dent* 2000; 28: 341 – 345.
9. Cecil Saldanha J. Flora of Karnataka. Vol-I and Vol-II. Oxford and IBH publishing company, New Delhi, 1984
10. Cecil Saldanha J, Dan Nicolson H. Flora of Hassan District. Amerind Publishing Company Private Limited, New Delhi, 1978
11. Kurian JC. Plants That Heal, Edition-2., Oriental Watchman Publishing House, Post box-1417, Salisbury park, Pune, India, 2001
12. Srikanta Murthy KR. Sarangadhara Samhita, Chaukhamba Orientalia, Post Box No. 1032, Varanasi, India. 1984
13. Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL.. Brine shrimp: a convenient general bioassays for active plant constituents. *Planta Medica* 1982; 45: 31 – 34.
14. McLaughlin JL, Chang CJ, Smith DL. Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant antitumour compounds. In: *Human Medicinal Agents from Plants*. Kinghorn AD and Balandrin MF. (Eds), ACS Symposium 534, American Chemical Society, Washington, 1993:112-137.
15. Aseer Manilal, Sujith S, Seghal Kiran G, Joseph Selvin, Chippu Shakir. Biopotentials of Mangroves collected from the Southwest coast of India. *Global J Biotech & Biochem* 2009; 4(1): 59-65.
16. Bezerra DP, Castro FO, Alves APNN *et al.* *In vivo* growth-inhibition of Sarcoma 180 by piplartine and piperine, two alkaloid amides from *Piper*. *Brazilian J Med and Biol Res* 2006; 39(6): 801-807
17. Khosit Pinmai, Sriharut Chunlaratthanabhorn, Chatri Ngamkitidechakul, Noppamas Soonthornchareon. Synergistic growth inhibitory effects of *Phyllanthus emblica* and *Terminalia bellerica* extracts with conventional cytotoxic agents: Doxorubicin and Cisplatin against hepatocellular carcinoma and lung cancer cells. *World J Gastroenterol* 2008; 14(10):1491-1497.