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RESEARCH ARTICLE

ASSESSMENT OF CYTOTOXIC EFFECTS OF *Aegle marmelos* LEAF EXTRACTS USING BRINE SHRIMP (*Artemia salina*) LETHALITY ASSAY

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ABSTRACT

Objective: To determine the cytotoxicity of *Aegle marmelos* leaf extracts using the brine shrimp test (BST) method for antitumour study.
Methods: Test tubes containing different concentrations of leaf extracts were introduced with ten brine shrimp larvae (10 nauplii) and were maintained at room temperature for 24 hours. The numbers of surviving and dead shrimps were counted. Percentage mortality was determined. LC_{50} was calculated using graph and Finney Method.
Results: Among the three leaf extracts tested, aqueous extract showed significant toxicity to brine shrimps in a dose-dependent manner with low LC50 value of 15.28 µg/mL.
Conclusion: From the results it can be concluded that the significant lethality of aquoues extract of *Aegle marmelos* leaves to brine shrimp is indicative of the presence in this plant of a potent cytotoxic component which warrants further investigation.

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INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). The plant-derived compounds have always been an important source of medicines for various diseases and have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Gonzales and Valerio, 2006, Hemalatha *et al*., 2013). These bioactive compounds may include more active agents, such as vitamins, minerals, alkylating agents, anti-metabolites, antineoplastic agents, immune stimulators and anti-oxidants. Previous studies demonstrate that certain phytochemicals (alkaloids, phenylpropanoids, flavonoids, phenolic compounds and terpenoids) present in medicinal plants exert anti-tumor activity (Park *et al*., 2008; Wang *et al*., 2009; Turk *et al*., 2011). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Nearly 50% of drugs used in medicine are of plant origin, and only a small fraction of plants with medicinal activity has been assayed. There is therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them.Available ethnomedical literatures reveal that *Aegle marmelos* leaf, fruit, stem bark, root and essential oil of fruits are used in various diseases. The brine shrimps lethality assay of fresh leaves of this plant is yet to be determined. Brine shrimp assay is an indicator used in determining the cytotoxicity and insecticidal properties of compound and it is very useful preliminary tool for isolation of bioactive compounds from plant (Sam, 1993). Since, there was no detailed pharmacological study on the cytotoxicity of this plant. The objectives of this study were to evaluate the cytotoxic effects of the *Aegle marmelos* leaf extract of this plant using the brine shrimp test (BST) method as a broad measure of anti-tumour activity.

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MATERIALS AND METHODS

Leaf collection and identification

The leaf specimens were collected from Chidambaram, Tamil Nudu,India and were authenticated by Department of Botany, Annamalai university. After a thorough investigation leaves were checked for any pathological disorders and contamination of other plants and were washed with distilled water.

Preparation of extracts

The fresh leaves (300 grams) were grounded into paste and were extracted with water for 12 h at room temperature. This process was repeated successively with chloroform and acetone petroleum ether for 72 h at room temperature until the color of the extract becomes pale. The extracts obtained were filtered separately using Whatmann No. 1 filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and dried on water bath until the constant weight with dry mass was obtained for solvent extracts. The residual extracts were stored in refrigerator at 4°C in small and sterile glass bottles

Brine shrimp cytotoxicity study

Brine shrimp lethality bioassay was performed to investigate the cytotoxicity of crude *Aegle marmelos* leaf extracts. Brine shrimp (*Artemia salina*) eggs were hatched using conical shaped vessel (capacity, 1L) containing filtered seawater. They were kept for 48 hours with continuous aeration. After hatching, active nauplii free from egg shells, were collected from brighter portion of the hatching chamber. Dimethyl sulfoxide (DMSO, 1.0 ml) was used for the preparation of different concentrations (10, 20, 30, 40. 50, 60 and 70 µg/mL) of sample extracts, in triplicates. After evaporation of vehicle solvent, each test tube was introduced with ten brine shrimp larvae (10 nauplii). All test tubes were maintained at room temperature for 24 hours. The numbers of surviving and dead shrimps were counted.

Percentage mortality was determined. LC_{50} were calculated using graph and Finney Method (Statplus software v, 2007).

RESULTS

The aqueous, acetone and chloroform extracts of the leaves of *Aegle marmelos* were studied for their cytotoxicity activity by means of Brine shrimp assay. The results showed that the leaf extracts showed significant toxicity to brine shrimps in a dose-dependent manner (Table 1-3). At 50 µg/mL, 100% mortality was recorded for aqueous extract. LC_{50} was obtained from the best-fit line slope (Fig.1-3) and found to be 15.28, 34.73 and 42.60 µg/mL for aqueous, acetone and chloroform extracts (Fig. 4) respectively. This significant lethality of *Aegle marmelos* leaf extracts to brine shrimp is indicative of the presence in this plant of a potent cytotoxic component which warrants further investigation.

DISCUSSION

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties (McLauglin *et al*., 1993; Krishnaraju *et al*., 2005). Findings of present study have shown that among the three extracts tested, aqueous extract is highly toxic to brine shrimps with low LC_{50} value (15.28 µg/mL). Some brine shrimp results that are already available (Moshi *et al*., 2004; 2006) provide a circumstantial evidence that plant extracts with LC_{50} values below 40 µg/mL have a likelihood of yielding anticancer compounds. This corroboration is demonstrated by *Bridelia cathartica* (Moshi *et al*., 2004), *Croton macrostachys* (Moshi *et al*., 2004), Maytenus *putterlickioides* (Shibuta, 1984), *Psorospermum febrifugum (*Moshi *et al*., 2006), *Phyllanthus engleri* (Moshi *et al.*, 2006*;* Ratnayake *et al*., 2009) and *Ximenia americana* (Moshi *et al.*, 2004). It is, thus stated that the plant has good

Table 1. Probit Analysis of aqueous extract - Finney Method [Lognormal Distribution]

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent(%)	N	R	E(R)	Difference	Chi-square
1	0.3	0.264907	10	3	2.64907	0.35093	0.046489
1.30103	0.7	0.655285	10	7	6.552849	0.447151	0.030513
1.477121	0.7	0.841568	10	7	8.415678	-1.41568	0.238144
1.60206	0.9	0.923289	10	9	9.232888	-0.23289	0.005874
1.69897	0.975	0.960666	10	9.75	9.606656	0.143344	0.002139
1.778151	0.975	0.978763	10	9.75	9.787628	-0.03763	0.000145
1.845098	0.975	0.98801	10	9.75	9.880098	-0.1301	0.001713

*where N = Total no of fish in a group, R=Calculated probit value and E(R)=Expected probit value

Table 2. Probit Analysis of acetone extract - Finney Method [Lognormal Distribution]

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent(%)	N	R	E(R)	Difference	Chi-square
1	0.025	0.001768	10	0.25	0.01768	0.23232	3.052845
1.30103	0.2	0.098017	10	2	0.98017	1.01983	1.061095
1.477121	0.3	0.3658	10	3	3.657995	-0.658	0.118359
1.60206	0.5	0.629674	10	5	6.296739	-1.29674	0.267048
1.69897	0.7	0.803386	10	7	8.033857	-1.03386	0.133044
1.778151	0.975	0.89989	10	9.75	8.998899	0.751101	0.062691
1.845098	0.975	0.949713	10	9.75	9.497126	0.252874	0.006733

*where N = Total no of fish in a group, R=Calculated probit value and E(R)=Expected probit value

Table 3. Probit Analysis of chloroform extract - Finney Method [Lognormal Distribution]

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent(%)	N	R	E(R)	Difference	Chi-square
1	0.025	0.000217	10	0.25	0.002166	0.247834	28.35439
1.30103	0.025	0.033201	10	0.25	0.332005	-0.08201	0.020255
1.477121	0.3	0.197375	10	3	1.97375	1.02625	0.533598
1.60206	0.5	0.439436	10	5	4.39436	0.60564	0.083471
1.69897	0.5	0.651548	10	5	6.515484	-1.51548	0.352498
1.778151	0.7	0.797372	10	7	7.973723	-0.97372	0.118908
1.845098	0.975	0.886212	10	9.75	8.862119	0.887881	0.088955

*where N = Total no of fish in a group, R=Calculated probit value and E(R)=Expected probit value

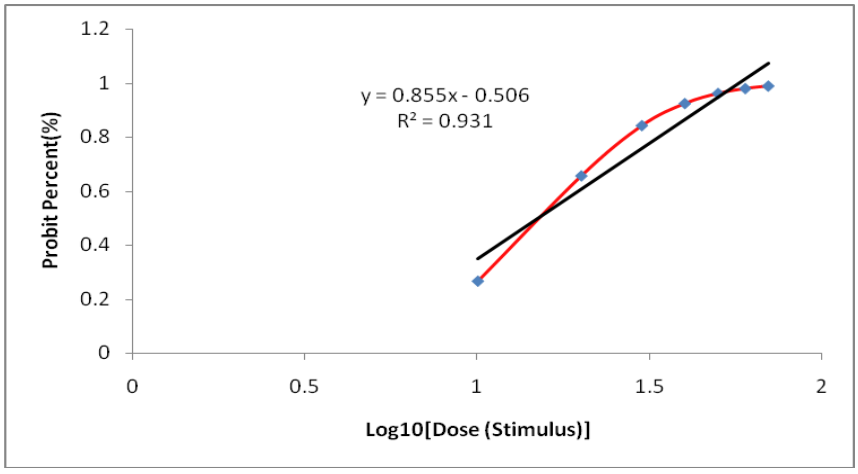

Fig. 1. Regression line of aqueous extract for probit %, plotted with a 95% confidence interval

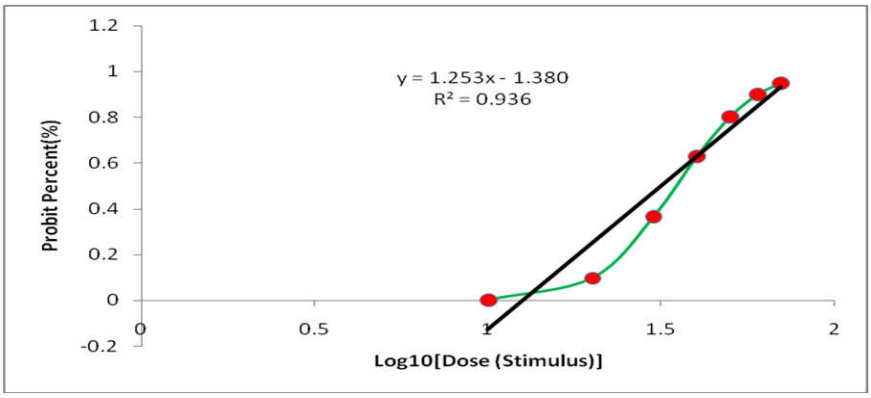

Fig. 2. Regression line of acetone extract for probit %, plotted with a 95% confidence interval

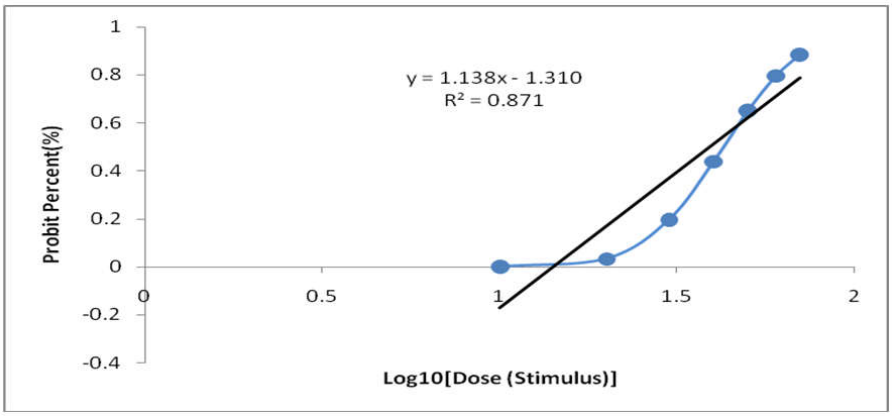

Fig. 3. Regression line of chloroform extract for probit %, plotted with a 95% confidence interval

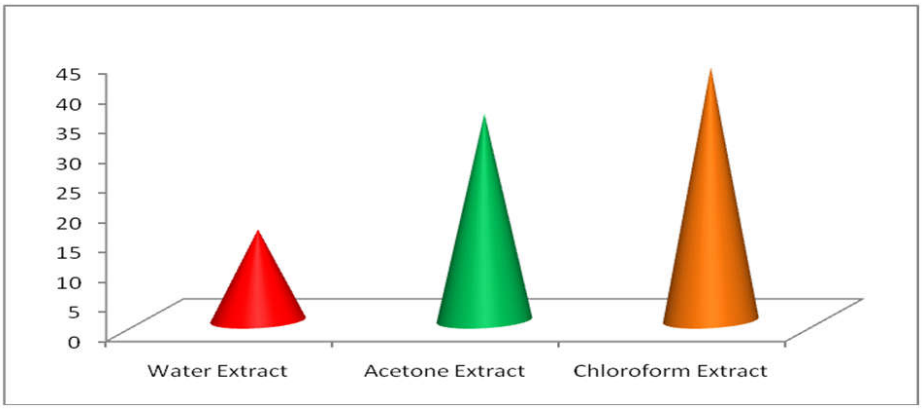

Fig. 4. LC_{50} values of of *Aegle marmelos* leaf extracts against *Artemia salina*

cytotoxic effect and, therefore, may be a source of anticancer constituents as there is positive correlation between the brine shrimp toxicity and human nasopharyngeal carcinoma (Rehman, *et al*., 2009; Ali *et al*., 2011). Thus the brine shrimp cytotoxicity implies for presence of cytotoxic constituents as well. From this experiment, it can be concluded that *Aegle marmelos* has got the very good potential as a candidate for future antitumor drugs. This is only a preliminary study and to make final comment the extract should thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

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