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CYTOTOXICITY OF CERTAIN SEED EXTRACTS AGAINST *ARTEMIA FRANSISCANA*

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Abstract: The chloroform and methanolic extracts of seed coat and seed kernel of *Mucuna pruriens, Terminalia. bellirica, Syzygium cumini and Myristica fragrans* were tested against the brine shrimp, *Artemia fransiscana* nauplii for mortality at 24h post exposure. All the test extracts were found to be effective. The toxicity of the chloroform extracts of seed coat could be arranged in the order: *T. bellirica* > *M. pruriens* > *S. cumini* > *M. fragrans*. In case of seed kernel extracts (chloroform) the results could be arranged in the following order: *S. cumini* > *M. fragrans* > *T. bellirica* > *M. pruriens*. For methanolic extracts of seed coat and seed kernel the results could be arranged in the following order: *M. fragrans* > *T. bellirica* > *S. cumini* > *M. pruriens*, and *T. bellirica* > *S. cumini* > *M. fragrans* > *M.* pruriens. The brine shrimp bioassay is a simple, reliable and convenient method for the assessment of bioactivity of medicinal plants and lends support for their use in traditional medicine.

Key words: Seed coat and kernel extracts, cytotoxicity, *Artemia fransiscana*.

INTRODUCTION

Plants are the natural chemical factories that synthesize innumerable compounds. The plant-derived compounds have been utilized by the humankind from time immemorial in different sectors of life, including public health and pest management. The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures (Farnsworth and Soejarto 1991). There is great support provided by bench-top bioassays in discovery of bioactive compounds from plants.

*****Deceased. Obituary noted in this volume.

The extracts of the plants, *Mucuna pruriens* (Papilionaceae), *Terminalia bellirica* (Combretaceae), *Syzygium cumini* (Myrtaceae) and *Myristica fragrans* (Myristicaceae) have widely been utilized for different medicinal purposes. The seeds of *M. pruriens* have much pharmaceutical value (Rastogi and Mehrotra 1991a, b, Singh *et al.* 1995, Alluri *et al.* 2005, Gouveia-Figueira *et al.* 2014). In case of *T. bellirica*, many pharmaceutical reports are available (Padam *et al.* 1996, Valsaraj *et al.* 1997, Bhatnagar *et al.* 2011). Many worker reported the medicinal value of *S. cumini* (Chopra *et al.* 1958, Mahapatra *et al.* 1985, Ghosh *et al.* 1985, Chaudhary *et al.*1990, Stanely and Menon 1997, Stanely *et al.* 1998 a, b).There are also much information on pharmacology of the seeds of *M. fragrans* (Nishat *et al.* 2006, Afify *et al.* 2011).

Various workers investigated these plants giving emphasis mostly on the chemical constituents and their medicinal profile but very few works have been done on their pesticidal importance. Toxicity of oily extracts of seed coats and seed kernels were determined by using brine shrimp lethality bioassay (Persoone 1980; Meyer *et al.* 1982). In this investigation, cytotoxic activity tests were carried out on the brine shrimp, *Artemia fransiscana* nauplii to evaluate the efficacy of the seed coat and seed extracts as possible sources of potential secondary metabolites to be used as environment friendly pest control agents.

MATERIAL AND METHODS

Plant collection and identification: Fresh seeds of *M. pruriens* (alkushi), *T. bellirica* (bahara), *S. cumini* (kalojam) and *M. fragrans* (jayfal) were collected from the Botanical Garden, Rajshahi University and the identification of voucher specimens were confirmed at the Taxonomical Section, Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extract: The seeds were chopped into small pieces, dried under internal shade and powdered using a hand grinder separately. The seed and seed coat powder were extracted with chloroform and methanol (BDH, Pooleg, England) using Soxhlet apparatus according to Feuerhake and Schmutterer (1982). The extracts obtained were stored in a refrigerator at – 20^0C with proper labeling.

Preparation of simulated seawater: The eggs of shrimps were collected from the Department of Pharmacy, University of Rajshahi, Bangladesh. Since the lethality test involves the culture of brine shrimp nauplii, i.e., the nauplii should be grown in water with salinity similar to that of seawater. Accordingly, a 3.8% sodium chloride solution was prepared by dissolving 38 gm sodium chloride in 1000 ml distilled water. The P^H of the brine water was maintained between 8.0 and 9.0 by using $NaHCO_3$.

*Hatching of the brine shrimp eggs***:** Brine water was taken in a small tank and shrimp eggs (1.5 gm/L) were added to one side of the perforated tank with a constant oxygen supply. A constant temperature (35 ±2ºC), sufficient light and air supply were maintained to give the sufficient aeration. After 48 hours, shrimp nauplii were collected and used for the experiment.

Cytotoxicity test: Preparation and application of doses on A. fransiscana: The chloroform and methanolic extracts of seed coat and kernel of the test plants were applied against the brine shrimp nauplii. For the seed coat and kernel of *M. pruriens, T. bellirica, S. cumini and M. fragrans* samples were initially dissolved in 200µl of pure dimethylsulfoxide (DMSO) to make them hydrophilic. Clean vials were taken for the 10 samples in five concentrations (two vials for each concentration) and 10 vials were also taken for control test. Five milliliter of seawater containing 10 brine shrimp nauplii was kept in each vial. With the help of a micropipette, specific volumes of samples were transferred from the stock solutions to the vials to get final concentrations of 5.0, 10.0, 20.0, 40.0 and 80.0 µg/5 ml of brine, because above this concentration cytotoxicity due to DMSO may arise. In the control vials the same volume of DMSO (as in the sample vials) and 5 ml of seawater were taken and 20µl DMSO was added to each vial.

Brine shrimp eggs were hatched in simulated seawater to get nauplii. Test samples were prepared by the addition of the requisite amounts of DMSO for obtaining desired concentrations of the test sample. The nauplii were counted by visual inspection and were taken in vials containing 5ml of brine water. Then samples of different concentrations were added to the pre-marked vials with the help of a micropipette. The vials were left for 24 hours and then the nauplii were counted again to find out the cytotoxicity of the test agents and compared to the results with positive control.

Collection and analysis of data for cytotoxicity: The test tubes containing the nauplii with the treated brine water were kept on a rack near the window in the laboratory. The recorded mortality was corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), and

P_c = Control mortality (%).

Mortality data were subjected to statistical analyses according to Finney (1947) and Busvine (1971) by using software developed at the Department of Agricultural and Environmental Science, University of Newcastle- upon -Tyne, U.K. The dose-mortality relationship was expressed as median lethal concentrations (LC_{50}).

RESULTS AND DISCUSSION

The results of dose-mortality assays of different seed extracts against *A. fransiscana* nauplii are presented in Tables 1-4. From this experiment, it is revealed that each of the test samples showed different mortality rates at different concentrations.

Table 1. Toxicity of seed coat (chloroform) extracts against *Artemia fransiscana* nauplii.

Test extract	Time exposed	LC_{50} value (µg/ml)	95% Confidence limits		Regression equation	χ^2 value
			Lower limit	Upper limit		
M. fragrans	24 h	95.58479	42.39236	215.511	Y=2.750502+1.135887X	1.77241
S. cumini	24 h	88.28874	42.93272	181.5609	Y=2.604101+1.231251X	1.53685
T. bellirica	24 h	64.79947	35.06378	119.7524	Y=2.816124+1.205515X	0.39005
M. pruriens	24 h	79.46446	42.78583	147.5863	Y=2.439498+1.34751X	0.61027

Table 2. Toxicity of seed coat (methanolic) extracts against *Artemia fransiscana* nauplii.

Test extract	Time exposed	LC_{50} value (µg/ml)	95% Confidence limits		Regression equation	χ^2 value
			Lower limit	Upper limit		
M. fragrans	24 h	34.23474	24.08017	48.6715	Y=2.617475+1.552673X	1.17796
S. cumini	24 h	41.12985	28.25775	59.86555	Y=2.466711+1.569419X	5.03311
T. bellirica	24 h	34.28775	24.38909	48.20393	Y=2.508102+1.62324X	0.32606
M. pruriens	24 h	47.93552	32.12584	71.52542	Y=2.331575+1.587727X	0.92116

It is evident that different plant seed extracts were found to be lethal to brine shrimp nauplii indicating that the extracts are biologically active. The methanolic extract was more active with lower LC_{50} values whereas the chloroform extract was less active with higher LC_{50} values. The Brine shrimp lethality bioassay is a recent development in the bioassay for the bioactive compounds (Chaterjee 1975, Meyer *et al.* 1982, Alkofahi *et al.* 1989, Pelcjar *et al.* 1986, McLaughlin 1993 and Persoone 1980). The brine shrimp assay has advantages of being rapid (24h), inexpensive and simple. It easily utilizes a huge number of organisms for statistical validation and requires no special equipment and needs relatively small amounts of sample. Most of the test extracts showed remarkable dose-mortality effects against the 1- day old nauplii of *A. fransiscana*. According to the degrees of activity of the extracts against the brine shrimp nauplii could be arranged in the order: methanolic extract >chloroform extract.

Our results are in agreement with Similar works with *T. chebula*. Other two species of genus *Terminalia viz. T. arjuna* and *T. bellerica* showed promising toxicity profile with 47 and 59% mortality of *A. fransiscana* at 100 µg/ml. The brine shrimp lethality bioassay indicates the cytotoxicity as well as wide range of

pharmacological activities, e.g. anticancer and antiviral activities of oily extract of *Sida rbombifolia* (Islam *et al.* 2000). LC_{50} values of petroleum ether, chloroform and methanol extracts on *A. fransiscana.* Leach were recorded as 1.14, 1.1, and 54.9mg/l respectively. Chemical analysis revealed the presence of fatty acids, steroids, triterpenoids, alkaloids, phenols, and phenyl propanoids, tannin, and mucilage in the extracts (Uyub *et al.* 2010).

Table 3. Toxicity of seed kernel (chloroform) extracts against *Artemia fransiscana* nauplii.

Test extract	Time exposed	LC_{50} value (µg/ml)	95% Confidence limits		Regression equation	χ^2 Value
			Lower limit	Upper limit		
M. fragrans	24 h	45.00616	29.06815	69.68294	Y=2.683566+1.401121X	0.32692
S. cumini	24 h	41.92609	27.55824	63.78479	Y=2.701551+1.416623X	0.59163
T. bellirica	24 h	58.82652	32.51715	106.4225	Y=2.910908+1.180563X	0.89138
M. pruriens	24 h	71.83673	36.79804	140.2389	Y=2.831675+1.168061X	0.28685

Table 4. Toxicity of seed kernel (methanolic) extracts against *Artemia fransiscana* nauplii.

Test extract	Time exposed	LC_{50} value (µg/ml)	95% Confidence limits		Regression equation	χ^2 Value
			Lower limit	Upper limit		
M. fragrans	24 h	30.21676	21.61476	42.24208	Y=2.671838+1.572819X	0.13132
S. cumini	24 h	28.72247	20.76573	39.72799	Y=2.634581+1.622125X	2.10562
T. bellirica	24 h	26.51602	19.22125	36.57927	Y=2.723973+1.598886X	1.45988
M. pruriens	24 h	33.80515	23.37058	48.89861	Y=2.747884+1.472951X	0.15647

A study on seasonal variation in cytotoxic and antioxidant activities of *T. bellerica* was demonstrated by Bhatnagar *et al.* (2011). Cytotoxicity screening of selected Indian medicinal plants using brine-shrimp lethality bioassay has been done by Chaitali *et al.* (2010).

This significant lethality of several plant extracts to brine shrimp is an indicative of the presence of potent cytotoxic components which warrants further investigation. A perusal of the data shows that all test extracts produced significant mortalities against *A. fransiscana* nauplii. However, more comprehensive studies are needed in this line.

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