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Short Communication

In-vitro cytotoxicity (LC_{50}) of extracts obtained from the seeds of *Zea mays*

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Abstract

Zea mays was tested for cytotoxicity (LC_{50}) efficacy against brine shrimp via extracts obtained from soxhlation using methanol, ethyl aectate, n-hexane, n-butanol solvent and one pure fraction. The *in-vitro* cytotoxicity (LC_{50}) activity was performed by brine shrimp lethality bioassay. Among these extracts, ethyl acetate, hexane and pure compounds have been found to be effective.

Keywords: Isolation and separation**,** brine shrimp lethality bioassay, Bangladesh.

Introduction

Plants have great potential for producing new drugs of benefit to mankind. There are many approaches in the search for new biologically active principles in higher plants [1]. Many efforts have been expended to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One such resource is folk medicine and systematic screening of these traditional herbs may result in the discovery of novel effective compounds [2, 3, 4].

Antibacterial properties of various plant parts like roots, stems, leaves, flowers, fruit and seeds have been well documented for some of the medicinal plants for the past two decades

[3]. Medicinal and aromatic plants and essences are rich in antibacterial compounds which can be an alternative to combat bacterial diseases [4-10]. In recent years antimicrobial properties of Bangladeshi medicinal plants have been increasingly reported [10, 11, 12].

Zea mays is a small tree, which grows in tropical and sub tropical Asian countries, America, and is currently cultivated in sub-tropical countries of the world and in warm climates. It is locally known as 'Bhutta'. Maize is a potential allergen source from early infancy as it is an ingredient in infant's diets in many parts of the world. A relatively low degree of relation is thought to exist between maize and the other cereals [13] although maize and rice antigens show similarities [14]. Recently it has been used as a diuretic in acute and chronic cystitis in the bladder irritation of uric acid and phosphatic gravel, gonorrhoea and hepato-biliary disease.

In the present study, the crude sample, the chloroform extract, the ethyl acetate extract, the n-hexane extract, the butanol extract and the ZM-1 fraction were tested for brine shrimp lethality bioassay.

Materials and Methods

Plant Material

The ripe seeds of ***Zea Mays*** were collected from the district Manikgonj of Bangladesh in June 2007. The plant was identified and voucher specimen number was deposited at the Department of Pharmacy, Rajshahi University, Rajshahi, Bangladesh with No. 10132**.**

Extraction

The dried and milled seeds (500 gm) were extracted with methanol (1L) in a Soxhlet extractor for 72 hours. The extract was evaporated in a rotating evaporator and dried under vacuum. The methanol extract (5 g) was suspended in water and extracted successively with petroleum spirit, hexane, chloroform and ethyl acetate to yield petroleum spirit (0.320 g), hexane (1.23 g), chloroform (1.50 g) and ethyl acetate-soluble (0.63 g) fractions, respectively. The petroleum spirit soluble fraction (1.26 g) was subjected to chromatography on silica gel (60-120 mesh, Merck) and eluted with dichloromethane-acetone (2:3) solvent system. The compound obtained from the column was further chromatography afforded one major fraction namely Fraction-1, 0.412 g and several other minor fractions. The major fraction was purified by using preparative TLC to give pure ZM-1. All the five plant extracts and one pure compound were tested against brine shrimp method.

Principle

Brine shrimp eggs were hatched in simulated sea water to obtain nauplii. Sample solutions were prepared by dissolving the test materials in a pre-calculated amount of DMSO (dimethyl sulphoxide). Ten nauplii were taken in vials containing 5 ml of simulated sea water. The samples of different concentrations were added to premarked vials with a micropipette. The assay was performed using three replicates. Survivors were counted after 24 hours. These data were processed in a simple program for probity analysis to estimate

LC_{50} values with 95% confidence intervals for statistically significant comparisons of potencies [15].

Preparation of sea water
38 gm of sodium chloride (NaCl) was weighed, dissolved in 1000 ml of distilled water and filtered to obtain a clear solution.

Hatching of brine shrimp
Seawater and shrimp eggs were placed in a tank and hatched for 1 day and matured as naupulii (larvae). The hatched shrimps were attracted to the lamp through the perforation in the dam. These nauplii were taken into bioassay.

Preparation of sample solution
Four grams of different extract samples (crude extract, chloroform extract, ethyl acetate extract, n-hexane extract, butanol extract and pure fraction ZM-1) was placed in a vial and dissolved in 100 µl of dimethyl sulfoxide (DMSO). A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 50 µl were added to pre-marked test tubes containing 5 ml of sea water and 10 nauplii. So the final concentration of the samples in the test tubes were 0.3906, 0.7812, 1.5625, 3.125, 6.250, 25, 50, 400 µg/ml for eleven dilutions.

Application of test solution and nauplii to the test tubes
Five millilitres of seawater was added to each test tube containing 10 brine shrimp nauplii. A magnifying glass was used for counting nauplii. Different concentrations of the test sample were applied to the test tubes containing nauplii. Exact count of 10 nauplii may not be possible. So 10±2 was considered.

Preparation of control
50 µl of solvent (DMSO) was placed in a test tube containing 5 ml of sea water and 10 brine shrimp. Three controls were taken.

Counting of nauplii
After 24 hours, the test tubes were observed and the number of surviving nauplii in each test tube were counted using magnifying glass and recorded. From the record, percentage of lethality of brine shrimps was calculated for each concentration of sample.

Results and Discussion

The brine shrimp lethality bioassay is used as a screening tool for the determination of bioactivity of different extracts, fractions and pure compounds. This test is an indication of cytotoxicity, anticancer, antiviral, pesticidal, antimicrobial and other different pharmacological activities. There are many procedures for cytotoxicity testing, however, brine shrimp lethality bioassay stands superior to other procedures. This is a rapid method utilizing only 24 hours, inexpensive and needs no special equipment. It is so simple that no aseptic technique is required. It utilizes a large number of organisms for validation and a

relatively small amount of sample. It does not require animal serum as needed for other methods of cytotoxicity testing.

In the present study, the crude sample, the chloroform extract, the ethyl acetate extract, the n-hexane extract, the butanol extract and the ZM-1 fraction were tested for brine shrimp lethality bioassay.

Table 1 gives the results of the brine shrimp lethality after 24 hours exposure to all the samples and the positive control, vincristine sulphate. The positive control, compared with the negative control (sea water) was lethal, giving significant mortality to the shrimp.

The lethal concentration LC_{50} of the test samples after 24 hours was obtained by a plot of percentage of the shrimp killed against the logarithm of the sample concentration (toxicant concentration) and the best fit was obtained from the curve data by means of regression analysis.

The degree of lethality was directly proportional to the extract ranging from significant with the lowest concentration (0.3906 µg/ml) to highly significant with the highest concentration (400 µg/ml). Maximum mortalities took place at a concentration of 400 µg/ml, whereas the least mortalities took place at 0.3906 µg/ml concentration. In other words, mortality increased gradually with the increase in concentration of the test samples.

LC_{50} was obtained from the crude sample (methanol extract), chloroform, ethyl acetate, n-hexane, butanol extract and the pure ZM-1 fraction respectively. In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by the ZM-1 fraction, chloroform extract, ethyl acetate extract and n-hexane extract were the most promising.

Table 1. Results of the test samples of *Zea mays* against brine shrimp.

Sample	LC_{50} (µg/ml)	Regression equation	R^2
Vincristine sulphate (Std.)	0.3227	Y= 29.799x + 64.624	0.9994
Crude extract	8.7	y = 17.498x + 33.543	0.9873
Chloroform extract	2.11	y = 25.961x + 41.537	0.9699
Ethyl acetate extract	2.5	y = 24.748x + 40.14	0.9834
n-hexane extract	3.42	y = 27.465x + 35.343	0.9998
Butanol extract	6.87	y = 21.115x + 32.305	0.9967
ZM-1 fraction	1.88	y = 23.233x + 43.619	0.9897

The brine lethality bioassay, the crude extract, chloroform extract, ethyl acetate extract, n-hexane extract, butanol extract and the ZM-1 fraction were found to show LC_{50} (median

lethal concentration obtained from graph) values of 8.7, 2.11, 2.5, 3.42, 6.87 and 1.88 µg/ml respectively (Table 1).

This indicated that the crude extract (8.7µg/ml) and the butanol extract (6.87 µg/ml) showed poor activity. However, the chloroform extract (2.11 µg/ml) and the ethyl acetate extract (2.5 µg/ml) showed significant cytotoxicity and the n-hexane extract (3.42 µg/ml) also showed moderate cytotoxicity. The pure fraction ZM-1 demonstrated strong anticancer activity with LC_{50} value of 1.88 µg/ml. The control drug vincristine sulphate demonstrated an LC_{50} value of 0.3µg/ml.

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