

USE OF LETTUCE SEEDS TO DETECT THE PRESENCE OF BIOMARKERS IN PLANTS OF THERAPEUTIC VALUE

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ABSTRACT

Lettuce seed germination Bioassay was conducted to detect and confirm the biological cytotoxicity of methanolic extract and its fractions of selected plants, viz; *Aegle marmelos*, *Gymnema sylvestre* and *Tinospora cordifolia*. The experiment works on the principle of the inhibition on the process of seed germination shown by active components of plant extracts. ED₅₀ value for the plant extract on seed germination is calculated using Finney's computer program. The result indicates that among the plants screened, butanol fraction of methanolic extract of *A. marmelos* has more cytotoxicity with ED₅₀ value as 575.82 g/ml on the seed germination process of lettuce when compared to all other fractions of the all the extracts. The report indicates that Lettuce seed germination test can be used to detect biomarkers present in plant extracts and so serve as an index of bioactivity.

KEYWORDS:Lettuce seeds germination, bioactivity, selected plants, therapeutic value

Biomarker is the constituent of any biological system due to which a specific biological activity is exhibited. The detection of the presence of such biomarkers could be of use in screening a particular bioactivity of interest. Medicinal plants with exciting biological activity can offer an immense resource of novel natural compounds. Since plant extract is a mixture of complex organic substances, the bioactivity of such extract could be due to various constituents where there are fair chances of interaction among the constituents in showing bioactivity. Hence based on Prof. Labedie's concept of biomarker (Vlietinck, 1999), analysis of plant components (biomarkers) should begin with bioactivity directed screening technique. Bioassays offer a special advantage in the standardization and quality control of heterogeneous compounds found in crude plant extracts having therapeutic value (McLaughlin et al., 1998). Determining the effect of substances present in environment or in plant extract on the living organism is accomplished by a bioassay. A substance found in environment or expected to be present in plant extract is tested at various concentrations with a living organism to what concentrations are beneficial or harmful to the organism. Assays used for these purposes should be simple to perform, rapid, inexpensive and have valid statistical correlation with desired bioactivities (Sam, 1993). Lettuce seed germination test is one such simple,

inexpensive and rapid screening test. One standard measurement of toxicity of a substance is the LD₅₀, the lethal doses that causes death of fifty percent of the organisms exposed. The most common LD₅₀ is the acute oral toxicity, that is, single internal dosage of a material necessary to kill half the test organisms. It's a known fact that pharmacology is toxicology at lower dose, in LSGT toxic effect of plant extract was observed in germination of seeds which is used as an index for the presence of bioactive components in the extract.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa*) belongs to family Asteraceae and the seeds of this plant were used to test the presence of cytotoxic components in the selected plant extract. The plants selected for screening were based on availability and also based on literature survey. Plant materials (sun dried) Fruit of *Aegle marmelos*, leaves of *Gymnema sylvestre* and bark of the stem of *Tinospora cordifolia*. Successive methanolic and aqueous extracts of different plant materials were prepared by reflux method (Houghton and Amla, 1993). The active compound were separated in the form various fractions (Rastogi and Mehrotra, 1991) using solvents like butanol, ethyl acetate and water since different solvents dissolve various fractions of methanolic extract.

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The extracts so obtained were concentrated to get pasty or powdery form and stored in refrigerated condition. A stock solution of extract used for bioassay was prepared in water at the concentration of 1 gram / 100 ml (0.01 g / ml). The stock solutions so prepared of various extract were used to prepare required concentration in bioassay setup. Lettuce seeds are soaked for few hours before the test is conducted. Moist filter paper is taken and spread in both the lid and the dish of a petri plate, few drops of water is sprinkled over the filter paper. A cover slip is placed in the center of the petri-dish. Previously soaked lettuce seeds are counted (15) and placed around the centrally placed cover slip in the petri dish. 5l of plant extract of known concentration is placed on the cover slip. The lid is closed and the set up is kept for incubation for three days at 20C. The set up is kept in triplicate. The number of seeds germinated are counted after the incubation period to find out the numbers not germinated. The control for the same was also set in triplicate using deionized water instead of extract. The observation is recorded in the table and the values are fed in the Finney's computer program (Finney,1976) to check the statistical validity of the results obtained in terms ED₅₀ for methanolic extract and its fractions of specific plant.

RESULTS

To detect cytotoxic property of the selected plant extract a bioassay was conducted taking lettuce seed

germination as the screening model. The lettuce seed germination inhibition is maximum with crude extract *A. marmelos* ME at the concentrations of 650 , 750 and 850 ppm with the ED₅₀ value as 801.0 g / ml when compared to ED₅₀ value as 942.65 with crude extract of *G. sylvestres* ME at the concentration of 750, 850 and 950 ppm and 874.21 with the crude extract of *T.cordifolia* ME at the concentration of 700, 800 and 900 ppm. (table, 1,2 and 3 and fig.1).

The similar experiment with lettuce seeds were conducted using various fractions of methanol extract of all three plant i.e. *A. marmelos* , *G. sylvestres* and *T.cordifolia*. Of various fractions used i.e. butanol fraction, ethyl acetate fraction and water fraction, Butanol fraction of *A.marmelos* seem to be most effective one with the ED₅₀ value as 575.82 g / ml on the seed germination process of lettuce when compared to all other fractions of the all the extracts as shown in table, 1,2 and 3 and fig., 2.The results obtained using various extract was compared with control set up.

DISCUSSION

The primary screening to search for the fraction with higher bioactivity is confirmed by the results of Lettuce seed germination assay. The results indicate that the crude extract of *A. marmelos* and its butanol fraction seem to be more effective (ED₅₀ as 801 and 575 .82 respectively)

Table 1:Effect of *Aegle marmelos* Extract and Fraction on Lettuce Seed germination

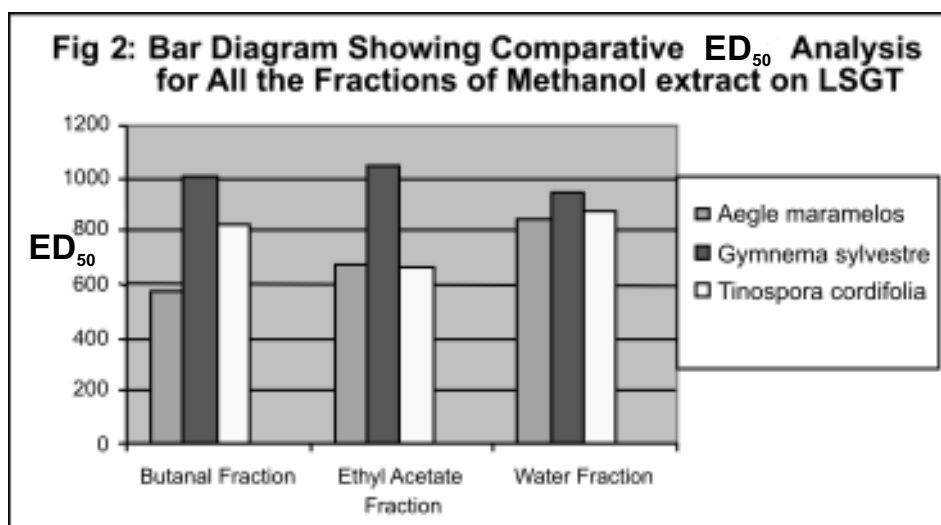
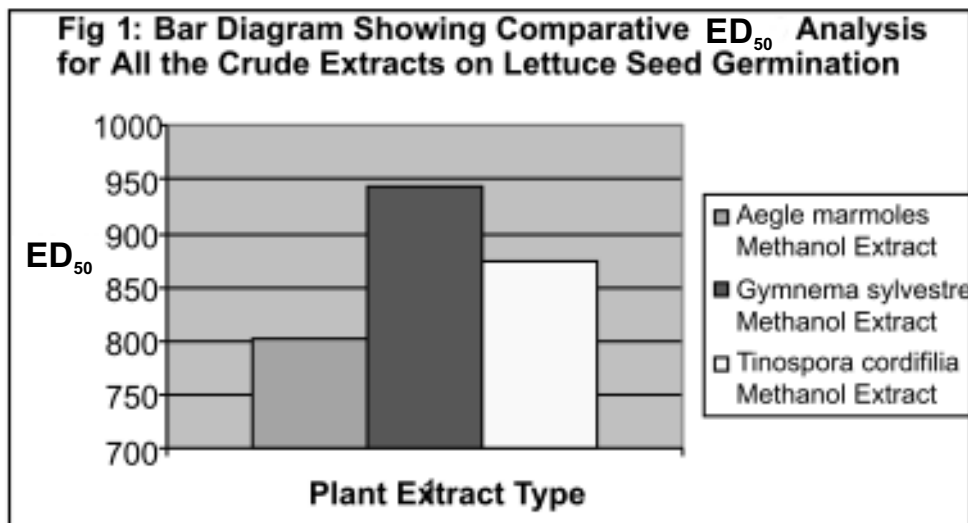
S. No.	Sample	Concentrations (In ppm)	Total No.of Seeds taken in Each Trial	Number of Seeds failed to germinate (Average of Three Trials)	ED ₅₀ Value
1.	Crude Methanol Extract	650ppm 750ppm 850ppm	15 15 15	02 06 09	801.0
2.	Methanol Extract Butanol Fraction	650ppm 750ppm 850ppm	15 15 15	09 10 12	575.821
3.	Methanol Extract Ethyl Acetate Fraction	650ppm 750ppm 850ppm	15 15 15	07 09 11	674.106
4.	(Methanol Extract) Water Fraction	650ppm 750ppm 850ppm	15 15 15	02 04 08	842.786

Table 2:Effect of *Gymnema sylvestre* Extract & Fraction on Lettuce Seed germination

S. No.	Sample	Concentrations (In ppm)	Total No.of Seeds taken in Each Trial	Number of Seeds failed to germinate (Average of Three Trials)	ED ₅₀ Value
1.	Crude Methanol Extract	650ppm 750ppm 850ppm	15 15 15	04 05 08	942.651
2.	Methanol Extract Butanol Fraction	650ppm 750ppm 850ppm	15 15 15	00 03 05	1001.994
3.	Methanol Extract Ethyl Acetate Fraction	650ppm 750ppm 850ppm	15 15 15	01 03 05	1041.518
4.	Methanol Extract Water Fraction	650ppm 750ppm 850ppm	15 15 15	02 04 08	942.522

Table 3:Effect of *Tinospora cordifolia* Extract & Fraction on Lettuce Seed germination

S. No.	Sample	Concentrations (In ppm)	Total No.of Seeds taken in Each Trial	Number of Seeds failed to germinate (Average of Three Trials)	ED ₅₀ Value
1.	Crude Methanol Extract	650ppm 750ppm 850ppm	15 15 15	04 06 08	874.218
2.	Methanol Extract Butanol Fraction	650ppm 750ppm 850ppm	15 15 15	05 07 09	821.740
3.	Methanol Extract Ethyl Acetate Fraction	650ppm 750ppm 850ppm	15 15 15	08 10 11	668.079
4.	Methanol Extract Water Fraction	650ppm 750ppm 850ppm	15 15 15	03 06 08	872.020



inhibitors for the germination of lettuce seeds when compare to other extracts. The results are similar to that of another bioassay called brine shrimp lethality test. Razavi et al.,(2009) also used Lettuce seed germination test as the screening method to select the plant extract having phytotoxic property and found significant reduction in lettuce seed germination and shoots and roots growth. In the similar manner allelopathic potential of the three grass species extract on the germination of lettuce seed using soil germination assay was studied by Rey and Monalie.,2012. Among the experimental groups, most lettuce seeds were induced to germinate in Chloris barbata extract while most

lettuce seeds are inhibited to germinate in Saccharum spontaneum extract. Based on the results obtained in the experiment it can be concluded that the Lettuce seed germination inhibition concept may be used as one of its own kind of test to detect the presence of active principle in medicinally important plant extract.

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