Original Research Paper

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Microbiology

L. ASPARAGINASE ACTIVITY OF LEAF EXTRACTS OF ANNONA MURICATA, CATHARANTHUS ROSEUS AND SIMAROUBA GLAUCA.

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ABSTRACT

L. asparaginase is an enzyme that deaminates the free *L. asparagine* to yield aspartic acid and is used as an antileukemic agent. *L. asparaginase* producing different medicinal plant like *Annona muricata, Catharanthus roseus* and *Simarouba glauca* was checked by using different concentration of leaf extract. The product of this reaction was aspartic acid and ammonia. It also checked by titration and confirmatory test

KEYWORDS :

Introduction

L. asparaginase (*L. asparaginase* aminohydrolase, EC 3, 5-1.1) constitutes one of the most biotechnologically and bio medically important group of therapeutic enzymes accounting for about 40 percent of the total worldwide enzyme sales (Wrangler and Khobragad, 2010). The enzyme catalyzes the deamination of L. asparaginase to *L. aspartic acid* and ammonia (El-Bessoumy et al., 2001). Plants and microorganism have been the major sources of natural products throughout the centuries (Balunas and Kinghhem, 2008). The valuable contribution of nature as source of potential chemotherapeutics has recently been eivdently (Newman and Cragg, 2007). The first written records on the medicinal uses of plants appeared in about 260 BC from the Sumerians and Akkaidians (Samuelsson, 1999). The discovery of Simarauraba glaucea is another evidence of the success in natural products extract discovery. Annona muricata was reported to use in the Indian ayurvedic medicine.

Materials and Methods

Collection of plant materials

The test leaves Annona muricata, Catharanthus roseus and Simarouba glauca leaves were collected from a botanical garden in Malappuram, Kerala.

Preparation of plant extracts

Collected plant materials were washed with clean sterile distilled water and dried for 3 days in an oven at 60°C to reduce water content. Then the dried plant materials were crushed into fine powder using motor and pestle. 5 g of powder was dipped into 250 ml solvent (methanol) in a conical flask with rubber corks and left for two days on shaking water bath. Then filtration was done through Whatsman No.1 filter paper. The filtrate was taken into glass beaker and kept into water bath at 60°C for evaporation of excess solvent and stored at 4°C.

Primary screening

The rapid assay to screened *L. asparaginase* activity of plant extract was performed on the C. zapek Dox medium contains the constituents in (glucose 2 gm L. asparagine – 10 gm, KH_2PO_4 – 1.52 gm, KCl – 0.52 gm, $M_gS_0.4.7H_2O$ – 0.52 gm, $C_uS_0.4.3H^2O$-trace, ZnSO4.7H2O trace and $F_eSO_4.7H_2O$-trace) at pH 6.2 and 0.09% phenol used indicator to media. To determine the *L. asparaginase* activity of plant extract, C-zapek Dox agar plates were prepared for each sample, separately labelled with different concentrations of 10 micro litres, 25 micro litres, 50 and 100 micro litres by using agar well diffusion method. The plates were incubated at 37°C for 24 to 48 hours. The zone of growth inhibition was recorded in millimeters.

Secondary Screening

The rate of hydrolysis of *L. asparagine* was determined by measuring the released ammonia using Nessler's reagent (David and David, 1974). A mixture of 100 microlitre enzyme extract, 200 microlitre 0.05 M tris-HCl buffer (pH 8.6) and 1.7 ml of 0.01 M L-asparagine was incubated for 10 minutes at 37oC. The reaction was stopped by the addition of 500 microlitres of 1.5 M trichloroacetic acid. After centrifugation at 1000 rpm at 4oC, 0.05 ml of the supernatant was dilated to 7 ml with distilled H2O and adds 1 ml of Nessler's reagent. The colour reaction was allowed to develop for 10 minutes and the OD was checked at 425 nm using spectrophotometer. The ammonia liberated was entrapped from a curve derived with ammonia Sulphate as standard curve. One unit of L. asparagine was defined as amount of enzyme which liberate microlitre of ammonia under the assay condition pH 8.6 to 37oC.

Confirmatory test for Ammonia

Added 1 ml of NaOH in a 1 ml of sample in a test tube burned the tubes till fumes will appear, dipped a glass rod in concentrated HCl and showed it into the fumes till dense white colour appears.

Same procedure was repeated but filter paper was dipped in Nessler's reagent and showed it into fumes, till brown coloured fumes appear.

Confirmatory test for Aspartic acid

20 ml of enzyme extracts with phenol used as indicator was pipetted into conical flask. This was titrated against iodine solution taken in the burette till pink colour is formed.

Result

Primary screening of plant extract

The development of pink colour is positive for production of L. asparaginase by a rapid plate assay (Figure 1). Diameter of pink zone of each plant extract is different concentration depicted in (Table I, II and III).

Secondary screening of plant extract

The maximum zone forming concentration of plant extract further subjected to secondary screening by using Nessler's reagent and the result was depicted (Table IV) and the graphical representation was represented in Figure II.

Volumetric analysis of plant extract

Starting and end point for confirmatory test for aspartic acid depicted in (Table V) and Figure (III).

Confirmatory test for ammonia

Brown colour and white colour fumes were observed by using Nessler's reagent and HCl to confirm ammonia presence.

Plate Assay

Sample 1: *Annona munricata* (Table I)

Concentration	10 L			25 L			50 L			100 L		
Time of incubation in hours	24	48	72	24	48	72	24	48	72	24	48	72
Diameter of pink zone in millimeter	10	50	65	40	60	87	35	53	76	43	70	87

Sample 2: *Catharanthus roseus* (Table II)

Concentration	10 L			25 L			50 L			100 L		
Time of incubation in hours	24	48	72	24	48	72	24	48	72	24	48	72
Diameter of pink zone in millimetre	45	55	67	60	68	87	63	76	87	70	87	87

Sample 3: *Simarouba glauca* (Table III)

Concentration	10 L	25 L	50 L	100 L

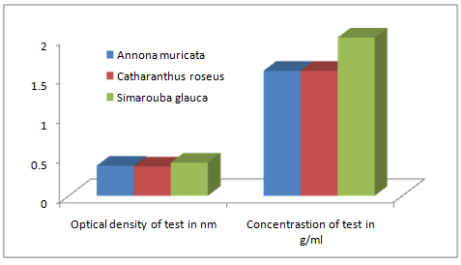
Time of incubation in hours	24	48	72	24	48	72	24	48	72	24	48	72
Diameter of pink zone in millimetre	48	65	77	65	80	87	76	87	87	83	87	86

Volumetric analysis (Table IV)

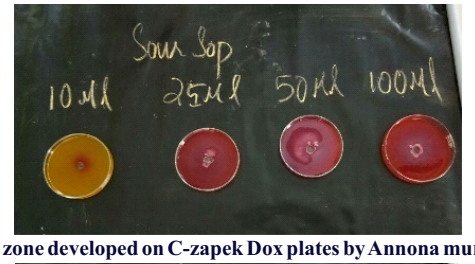
Sample	Starting point	End point
Annona muricata	0	5
Catharanthus roseus	0	5.3
Simarouba glauca	0	4.2

Estimation of L. asparaginase enzyme activity

Sample	Optical density of test in nm	Concentration of test in g/ml
Annona muricata	0.38	1.58
Catharanthus roseus	0.37	1.58
Simarouba glauca	0.42	2.0



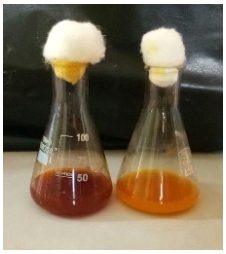
FIGURES



Pink zone developed on C-zapek Dox plates by Annona muricata

Pink zone developed on C-zapek Dox plates by Catharanthus roseus

Pink zone developed on C-zapek Dox by simarouba glauca



Starting point end point of *Annona muricata*

Starting point and end point of *Catharanthus roseus*

Starting point and end point of *simarouba glauca*

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