**Original Research Paper**

**Microbiology**

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

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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. asparagine 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 chemothterapeutics has recently been evidently (Newman and Cragg, 2007).

**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 Whatman 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, KH₃PO₄ – 1.52 gm, KCl – 0.52 gm, M₃S₄.7H₂O – 0.52 gm, C₅S₄.3H₂O-trace, ZnSO₄.7H₂O trace and F₂O₇H₂O-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 37°C. The reaction was stopped by the addition of 500 microlitres of 1.5 M trichloroacetic acid. After centrifugation at 1000 rpm at 4°C, 0.05 ml of the supernatant was diluted 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 37°C.

**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).

**Conformatory test for ammonia**

Brown colour and white colour fumes were observed by using Nessler’s reagent and showed it into fumes, till brown coloured fumes appear. Same procedure was repeated but filter paper was dipped in Nessler’s reagent and showed it into fumes, till brown coloured fumes appear. 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.

**Plate Assay**

**Sample 1: Annona mucicata (Table I)**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>10 L</th>
<th>25 L</th>
<th>50 L</th>
<th>100 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of incubation in hours</td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>24</td>
</tr>
<tr>
<td>Diameter of pink zone in millimeter</td>
<td>10</td>
<td>50</td>
<td>65</td>
<td>60</td>
</tr>
</tbody>
</table>

**Sample 2: Catharanthus roseus (Table II)**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>10 L</th>
<th>25 L</th>
<th>50 L</th>
<th>100 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of incubation in hours</td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>24</td>
</tr>
<tr>
<td>Diameter of pink zone in millimeter</td>
<td>45</td>
<td>55</td>
<td>67</td>
<td>60</td>
</tr>
</tbody>
</table>

**Sample 3: Simarouba glauca (Table III)**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>10 L</th>
<th>25 L</th>
<th>50 L</th>
<th>100 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of incubation in hours</td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>24</td>
</tr>
<tr>
<td>Diameter of pink zone in millimeter</td>
<td>45</td>
<td>55</td>
<td>67</td>
<td>60</td>
</tr>
</tbody>
</table>
Pink zone developed on C-zapek Dox by simarouba glauca

Volumetric analysis (Table IV)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Starting point</th>
<th>End point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annona muricata</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Catharanthus roseus</td>
<td>0</td>
<td>5.3</td>
</tr>
<tr>
<td>Simarouba glauca</td>
<td>0</td>
<td>4.2</td>
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</table>

Estimation of L. asparaginase enzyme activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Optical density of test in nm</th>
<th>Concentration of test in g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annona muricata</td>
<td>0.38</td>
<td>1.58</td>
</tr>
<tr>
<td>Catharanthus roseus</td>
<td>0.37</td>
<td>1.58</td>
</tr>
<tr>
<td>Simarouba glauca</td>
<td>0.42</td>
<td>2.0</td>
</tr>
</tbody>
</table>

FIGURES

Starting point and end point of simarouba glauca

BIBLIOGRAPHY

24. Verma, N., Kumar, K., Anand, S. L-asparaginase: a promising chemotherapeutic agent and its significance of having reduced glutaminase side activity for the better development Nat Prod; 1997; 60