INTRODUCTION

Actiniopteris radiata Linn more commonly known as Nemaliadugu in telugu which belongs to the family Actiniopteridaceae, is a fern widely distributed throughout Africa and adjacent Islands, Madagascar, Arabia, Iran, Afghanistan, Nepal, India, Burma and Australia\textsuperscript{1-4}. The plant is claimed to possess anti-histaminic activity, anti-cholinergic, anti-microbial activity, anti-inflammatory activity, anti-helmenthic activity, analgesic activity and used as styptic\textsuperscript{5}. The aim of our study was to investigate the Antidiabetic activity of Ethanolic extract of Actiniopteris radiata Linn by using chromogenic DNSA method\textsuperscript{6-9}.
MATERIALS AND METHOD

Plant Materials
The whole plant of Actiniopteris radiata Linn were collected from Tirumala Hills, Tirupati and Chittoor district of Andhra Pradesh in the month of July - October and identified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V.University and Tirupati.

Preparation of Extract
The powder of whole plant of Actiniopteris radiata Linn was extracted with n-Hexane, Chloroform, Ethyl acetate and Ethanol successively by Soxhlation method and concentrated over water bath and evaporated under reduced pressure. The Ethanolic extract was chosen for Anti diabetic activity.

Chemicals
n-Hexane, Chloroform, Ethanol, Dinitro salicylic acid, Sodium potassium buffer, Amylase, Starch.

EXPERIMENTAL PROCEDURE
The inhibition assay was performed using the chromogenic DNSA method (Miller, 1959). The total assay mixture composed of 1400 µl of 0.05 M sodium phosphate buffer (pH 6.9), 50 µl of amylase (Diastase procured from HiMedia, Mumbai, Cat No. RM 638) and extracts at concentration 100, 250 and 500 µg were incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature and the absorbance measured at 540 nm. The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula:

\[
\text{Conc. of Maltose Liberated} = \frac{\text{Acidity} \times \text{ml of enzyme used}}{\text{Mol.wt of maltose} \times \text{incubation Time (min)} \times \text{Dilution factor}}
\]

One unit of enzyme activity is defined as the amount of enzyme required release one micromole of maltose from starch per min under the assay conditions.

The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula:

\[
\text{% Inhibition/induction} = \frac{\text{Activity in presence of compound}}{\text{Control Activity}} \times 100
\]

Phytochemical analysis
The n-Hexane, Chloroform and Ethanol extracts of Actiniopteris radiata Linn were subjected to Thin Layer Chromatography using TLC plates (0.1 mm thick silica gel) eluted with n-hexane: Ethyl acetate (8:2) and Chloroform: Benzene (6:1) respectively. The spots were identified under long UV light by using UV cabinet.

RESULTS AND DISCUSSION
Traditionally medicinal plants have been used in folk medicine throughout the world to treat various diseases; especially tuberculosis. We evaluated preventive effect of Ethanolic extracts of using Microplate Alamar Blue assay method. The standard inhibitor acarbose was assayed according to Sudha et al., (2011). The 2ml amylase assay reaction mixture containing different concentrations of acarbose (1-36 µg/ml) were assayed as explained before and activity calculated. The inhibitions were noted in percent (Table No.1 and 2).

Acarbose inhibition assay
The standard inhibitor acarbose was assayed according to Sudha et al., (2011). The 2ml amylase assay reaction mixture containing different concentrations of acarbose (1-36 µg/ml) were assayed as explained before and activity calculated. The inhibitions were noted in percent (Table No.1 and 2).

Inference
Among both samples AR-II at 500 µg has displayed significant inhibition of amylase activity. Other concentrations tested moderately inhibited the activity. The solvents tested as control were ineffective against enzyme activity (Figure No.1-3).
## Table No.1: Acarbose inhibition assay

<table>
<thead>
<tr>
<th>S.No</th>
<th>Samples</th>
<th>OD at 540 nm</th>
<th>Concentration of Maltose liberated (µg)</th>
<th>Activity (µmoles/ml/min)</th>
<th>% activity</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2.23</td>
<td>178</td>
<td>0.0494</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>AR-I (100 µg)</td>
<td>1.89</td>
<td>152</td>
<td>0.0422</td>
<td>85.40</td>
<td>14.60</td>
</tr>
<tr>
<td>3</td>
<td>AR-I (250 µg)</td>
<td>1.75</td>
<td>140</td>
<td>0.0389</td>
<td>78.65</td>
<td>21.35</td>
</tr>
<tr>
<td>4</td>
<td>AR-I (500 µg)</td>
<td>1.74</td>
<td>139</td>
<td>0.0386</td>
<td>78.09</td>
<td>21.91</td>
</tr>
<tr>
<td>5</td>
<td>AR-II (100 µg)</td>
<td>1.81</td>
<td>145</td>
<td>0.0402</td>
<td>81.46</td>
<td>18.54</td>
</tr>
<tr>
<td>6</td>
<td>AR-II (250 µg)</td>
<td>1.78</td>
<td>143</td>
<td>0.0397</td>
<td>80.34</td>
<td>19.66</td>
</tr>
<tr>
<td>7</td>
<td>AR-II (500 µg)</td>
<td>1.35</td>
<td>108</td>
<td>0.0300</td>
<td>60.68</td>
<td>39.32</td>
</tr>
<tr>
<td>8</td>
<td>Pet. Ether (10 µl)</td>
<td>2.23</td>
<td>178</td>
<td>0.0494</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>Pet. Ether (25 µl)</td>
<td>2.23</td>
<td>178</td>
<td>0.0494</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>Pet. Ether (50 µl)</td>
<td>2.23</td>
<td>178</td>
<td>0.0494</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>11</td>
<td>Ethanol (10 µl)</td>
<td>2.22</td>
<td>177</td>
<td>0.0491</td>
<td>99.44</td>
<td>0.56</td>
</tr>
<tr>
<td>12</td>
<td>Ethanol (25 µl)</td>
<td>2.22</td>
<td>177</td>
<td>0.0491</td>
<td>99.44</td>
<td>0.56</td>
</tr>
<tr>
<td>13</td>
<td>Ethanol (50 µl)</td>
<td>2.22</td>
<td>177</td>
<td>0.0491</td>
<td>99.44</td>
<td>0.56</td>
</tr>
</tbody>
</table>

## Table No.2: Showing the data of acarbose inhibition analysis

<table>
<thead>
<tr>
<th>S.No</th>
<th>Samples</th>
<th>OD at 540 nm</th>
<th>Concentration of Maltose liberated (µg)</th>
<th>Activity (µmoles/ml/min)</th>
<th>% activity</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1.98</td>
<td>167</td>
<td>0.0463</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>1 µg</td>
<td>1.92</td>
<td>154</td>
<td>0.0427</td>
<td>92.31</td>
<td>7.69</td>
</tr>
<tr>
<td>3</td>
<td>2 µg</td>
<td>1.78</td>
<td>143</td>
<td>0.0397</td>
<td>85.72</td>
<td>14.28</td>
</tr>
<tr>
<td>4</td>
<td>4 µg</td>
<td>1.34</td>
<td>107</td>
<td>0.0297</td>
<td>64.14</td>
<td>35.86</td>
</tr>
<tr>
<td>5</td>
<td>8 µg</td>
<td>1.06</td>
<td>85</td>
<td>0.0236</td>
<td>50.95</td>
<td>49.05</td>
</tr>
<tr>
<td>6</td>
<td>16 µg</td>
<td>0.54</td>
<td>44</td>
<td>0.0122</td>
<td>26.38</td>
<td>73.62</td>
</tr>
<tr>
<td>7</td>
<td>32 µg</td>
<td>0</td>
<td>0</td>
<td>0.0000</td>
<td>0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Figure No.1: Standard Maltose Curve

Figure No.2: Graph showing the comparative analysis
CONCLUSION
This study reveals significant Antitubercular effect of n-hexane, Chloroform and Ethanol extracts from plant Actiniopteris radiata Linn. Further studies using more specific methods are required to explore the constituents responsible for the activity and the mechanism of this activity which might prove important and improved therapies for the treatment and prevention of tuberculosis.

ACKNOWLEDGEMENT
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BIBLIOGRAPHY

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