Abstract—Dioscorea alata is an important food crop of the genus Dioscorea producing tubers and bulbils rich in medicinal value. Though plant parts are used for the treatment of diverse ailments in Indian and Chinese ethno pharmacological practices, proper documentation with regard to bioactive constituents are still lagging behind. Thus study was undertaken to derive a phytochemical profile of the tuber extracts of the plant. A solvent based extraction method was adopted with solvents chosen in order of increasing polarity. Qualitative analysis for phytochemicals present in tuber extracts confirmed the presence of Alkaloids, Tannins, Phenolics, Glycosides, Saponins, Resins, Terpenoids and Anthraquinones. The report assessed the biomedical application of Dioscorea alata by determining the antimicrobial and cytotoxic properties of tuber extracts. The tests gave positive results regard to antimicrobial activity against both gram negative and gram positive strains. Plant extracts expressed cytotoxicity against Dalton’s lymphoma ascites (DLA) cells which was ascertained by Trypan blue method. Thus study confirms its role as medicinal food apart from its use as a functional food.

Keywords: Dioscorea Alata, Phytochemicals, Antibacterial Activity, Cytotoxic Activity

INTRODUCTION

Dioscorea alata is a vigorous twining herbaceous vine and an important traditional medicine having many bioactive substances, including alkaloids, flavanoids and steroidal saponins. Superiority of D. alata as health food and health medicine is much valued in Chinese system of medicine (Liu et al., 1995). The plant is also known for its high nutritional value, with a crude protein content of 7.4% and vitamin C content ranging from 13.0–24.7 mg per 100 grams (Osagie, 1992). Species have been studied for its antioxidant activity (Das et al., 2012). The studies also report the role of yam extracts in lowering the rate of sugar absorption into the blood stream and thus maintaining blood sugar level (Hou et al., 2001). The effect is mainly attributed to the presence of α-glycosidase inhibitor (Zhang et al., 2011), and some polysaccharides in D. alata capable of lowering sucrase activity to inhibit glucose absorption (Chen et al., 2003). Antibacterial and antifungal activities of D alata have been studied (cordell et.al 2001). Significant hypolipidemic and antioxidative effects are exhibited by D alata extracts (Lin et al., 2006). Dioscorea species also exhibit immune responses which have been attributed by dioscorin content of the plants (Liu et al., 2009). Yams contain high amount of vitamin B6, a good supplement for women under depression as a result of premenstrual syndrome. Traditional system of medicine demands the need of scientific authentication of the herbal extracts for their bioactive repositories. Thus the present study was undertaken to draw out a phytochemical profile of the Dioscorea alata tuber extracts to validate its medicinal application.

MATERIALS AND METHODS

Preparation of Plant Extract

The plant material was separated into its selected part tuber, air dried, ground to moderately fine powder and soxhlet extracted with different solvents in order of...
increasing polarity. Solvents selected were petroleum ether, chloroform, ethyl acetate, and methanol. 5 gm of root powder was introduced into apparatus for each 50 ml of solvent added to it. After 24 hrs of extraction, solvent was recovered by distillation. This process is repeated for all the solvents used. Each extract was evaporated to dryness under reduced pressure using rotary evaporator. Various concentrated extracts were stored in air tight container for further studies.

**PHYTOCHEMICAL SCREENING**

**Test for alkaloids (Mayer’s test)**

1.36 g of mercuric chloride was dissolved in 60ml distilled water with 5g of potassium iodide and diluted to 100ml with distilled water. To 1ml of aqueous solution of sample, few drops of reagent were added. Formation of white pale precipitate showed the presence of alkaloids.

**Test for Tannins (Lead Acetate Test)**

To 5ml of the extract, added 1ml of 1% lead acetate solution. Flocculent white precipitate indicated the presence of tannins.

**Test for Flavanoids (Lead Acetate Test)**

To the alcoholic solution of the extract add few drops of 10% lead acetate solution. Yellow precipitate indicated the presence of flavonoid.

**Test for Phenols (Ferric Chloride Test)**

About 5ml of the extract was dissolved in 1ml of water and 5 drops of 10% ferric chloride solution was added to it. Development of blue or green colour indicates the presence of phenols.

**Test for Glycosides (Keller-kiliani Test)**

1ml of glacial acetic acid containing traces of ferric chloride and 1ml of conc.sulphuric acid was added to 1ml of alcoholic extract. Observed for the formation of reddish brown colour at the junction of two layers and for upper layer to turn greenish blue.

**Test for Saponins (Froth Test)**

The extract was diluted with distilled water to 20ml in the test tube. The solution was shaken vigorously for 15 minutes and observed for stable persistent froth. If there is formation of 1cm layer of foam indicates presence of saponins.

**Test for Anthraquinones (Borntrager’s Test)**

Boil the test extract with 1ml of sulphuric acid in a test tube for five minutes. Filter white hot. Cool the filtrate and shake with equal volume of chloroform. Separate the lower layer of chloroform and shake it with half of its volume of dil. Ammonia. A rose pink to red colour is produced in the ammonial layer which indicates the presence of anthraquinones.

**Test for Resins**

Extract treated with conc sulphuric acid forms red or reddish brown colour, when treated with 50% HNO3 gives green colour.
Test for Carbohydrates (Molisch’s Test)

To the test solution add few drops of alcoholic α naphthol, then add few drops of conc. Sulphuric acid through sides of test tube purple to violet colour ring appears at the junction of two layers.

Test for Terpenoids (Salkowski Test)

To 1ml of the extract 2ml of chloroform was added, 3ml conc. Sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Anti Microbial Screening

Anti microbial screening was done using disc diffusion method. The prepared discs saturated with plant extracts along with the positive (streptomycin) and negative (pure solvent) controls were placed on the inoculated nutrient agar plates previously inoculated with respective bacterial strains. Petriplates incubated at 37°C for 24 hours and observed for zone of inhibition.

Measuring Invitro Cytotoxicity (Trypan Blue Method)

DLA (Dalton’s lymphoma ascities) cells were aspirated from the peritoneal cavity of tumour bearing mice. The experiments were set up by incubating different concentrations of plant extract with 1×10^6 cells. The final volume of the assay mixture was made up to 1ml using PBS and was incubated at 37°C for about 3 hours. 100ml of trypan blue was added after incubation and the number of dead cell was counted using haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately.

\[
\text{Percentage of cytotoxicity} = \left( \frac{\text{No of dead cells} \times 100}{\text{No of live cells} + \text{No of dead cells}} \right)
\]

RESULTS AND CONCLUSION

Phyto Chemical Analysis of Dioscorea Alata

Phyto chemical analysis of root extracts of Dioscorea alata revealed the presence of few type of phytochemicals. Results are illucidated in Table 1

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Test</th>
<th>PE</th>
<th>CHCl₃</th>
<th>EA</th>
<th>MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer's test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Lead acetate test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric chloride test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller killani test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Borntrager's test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>General test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch's test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Presence of various phyto chemicals confirms the use of Dioscorea alata as a potent source of modern drug.
Anti Bacterial Screening

Inhibition zone diameter was observed for all the tested extracts Table 2. Results imply the possibility of using *Dioscorea alata* root extract in antibacterial drug designing.

<table>
<thead>
<tr>
<th>Test extract</th>
<th>Diameter of Inhibition Zone (mm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>E Schericia Coli</strong></td>
<td><strong>Staphylococcus Aureus</strong></td>
<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>14</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>16</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Cytotoxicity of Dioscorea alata to Dalton’s Lymphoma Ascites (DLA) Tumour Cells

It was found that 200µg/ml of *dioscorea alata* produce 32% cytotoxicity on Dalton’s Lymphoma Ascites (DLA) tumour cells. Cytotoxicity was found to be concentration dependent.

**CONCLUSION**

Traditional Indian system of medicine attains much attention in being a safe alternative to the chemical drugs. *Dioscorea alata*, have been used in a number of herbal formulations but still many of the phytochemical parameters have not been standardized. This study will help in authentication of the plant and ensures reproducible quality of herbal products.
REFERENCES


