Phytochemical Screening of the Pollen of some selected plants with antidiabetic properties.

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Abstract: Diabetes has been known since ages in Ayurveda by Shushruta. It is a chronic metabolic disorder which results in an increased cane of sugar in blood. In the present investigation phytochemical screening of pollens of some selected plants with known antidiabetic properties was carried out like Catheranthus roseus, Momordica charentia, Butea monosperma and Syzygium cuminii. As pollen is very important entity carrying paternal genome to next generation, It is quite likely that different important constituents present in the plant parts may be present in the pollen also. Owing to the economic value and medicinal importance some plants like Cathareanthus roseus, Momordica charantia, Butea monosperma and Syzygium cuminii were selected and pollens of these plants were screened for their phytochemical constituents. The results showed the presence of antidiabetic constituents in the pollen parts of all the above selected plants. They showed the presence of alkaloids, steroids, flavinoids, tannins, saponins and carbohydrates in them. Thus the important antidiabetic constituents could be detected in the pollens of all the above selected medicinal plants in preliminary phytochemical screening.

Key words: Phytochemical, Pollen, Antidiabetic.

INTRODUCTION

About 80% of the world’s population relies on traditional medicines, which are prominently based on plants (Subramanium, 2001, WHO 1993). A good number of herbal remedies have stood the test of time particularly for the treatment of allergic, metabolic and various degenerative diseases. (Gupta, 1994). The plant based formulations have proved biologically more compatible with human system and are comparatively less toxic and have fewer side effects even on long usages than the synthetics (Chaudhari, R. R. 1970). The Pioneering research on indigenous medicinal plants was initiated by Shri Ramnath Chopra which is well documented in his comprehensive treaties (Chopra et al 1958). The scientific data on a good number of medicinal plants investigated has been well documented (Satyavati, et al, 1987). Development of traditional system of medicine for alleviating human diseases is now growing up very fast. Indian council of Medical Research, New Delhi, in its revived research on traditional medicine has adopted diabetes as one among six thrust area for multidisciplinary study. Under this programme, screening of active constituents from various plants have shown marked protection against various disease like Bhoomyamalaki (Phyllanthus niruri Fam Euphorbiaceae) has showed marked protection against jaundice due to viral hepatitis.

Mamardicine (Momordica charantia Fam Cucurbitaceae), Palsoninin (Butea frendosa Fam Fabaceae) gained importance for their hypoglycemic activity. Owing to their medicinal importance, in the present investigation plants known for their antidiabetic activity were selected for their phytochemical studies. As pollen is very important entity carrying paternal genome to next generation, it is quite likely that different important constituents may be present in them. Therefore, preliminary phytochemical screening of pollen grains was carried out in all the plants under investigation.

MATERIALS AND METHODS

For phytochemical screening sufficient pollen grains material was collected from the above selected plants. It was dried, weighed and used for Extraction of constituents.

Extraction of Constituents:

The weighed quantity of pollen material was successfully extracted with different organic solvents. The solvents used for successive extraction were petroleum ether (60 to 80°C) and alcohol. The solvent was removed by Evaporation from the extract and the residue left was weighed from this, the percentage yield to solvent was calculated. Then it was subjected to various chemical tests as follows.
I - Test for Sterols:
(a) Salkowski reaction: Few mg of residue of each extract was taken in 2ml of chloroform and 2ml of sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red color in the chloroform layers indicated the presence of sterols.
(b) Liebermann-Burchard Reaction: Few mg of residue was dissolved in chloroform and few drops of acetic unhydride were added followed by conc. sulphuric acid from the side of the test tube. A transient colour development from red to blue and finally green indicated the presence of sterols.

II - Test of Alkaloids:
Few mg of residue of each extract was taken separately in 5ml of 1.5% v/v hydrochloric acid and filtered. These filtrates were then used for testing alkaloids.

   a) Mayer’s Reagent test: To a little of the test filtrate taken in a watch glass a few drops of Mayer’s reagent was added. Formation of cream coloured ppt. showed the presence of alkaloids.
   b) Wagner’s Reagent: Few ml of test filtrate was taken in test tube and to this wagner’s reagent was added, a brown flocculent precipitate was formed indicating the presence of alkaloids.

III Test of Proteins:
   a) Bureit test: A few ml of test residue was taken in water and 1 ml of 4% sodium hydroxide solution was added followed by a 1% solution of copper sulphate. Violet Pink colour development indicates the presence of protein.
   b) Xanthoprotein test: Few 2 ml of extract was taken to this was added 5ml of Nitric acid, white precipitate indicates the presence of protein.

IV Test for Saponins:
   a) Foam Test: A few mg of test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. If stable, characteristic honey comb like froth is obtained, saponins are present.

V Test for Caumarins:
A small amount of test residue moistened with water was taken in test tube. The mouth of test tube was covered with paper mounted with dilute sodium hydroxide solution. The covered test tube was placed in boiling water bath for several minutes. The paper was removed and exposed to ultraviolet light. If yellowish green fluorescence is obtained, the caumarins are present.

VI Test for Tanins:
The test residue of each extract was taken separately in water, warmed and filtered.
Tests are carried out with the filtrate using following reagents.
   a) Ferric chloride solution: Few drops of ferric chloride were added to a little of the above test filtrate. If greenish or bluish black colour is obtained, Tanins are present.
   b) Lead acetate Test: Few drops of lead acetate solution were added to the test filtrate. If precipitate is obtained, tannins are present.

VII Test for Flavinoids:
   a) Shinoda Test: A small quantity of test residue was dissolved in 5ml of ethanol (95%) and added small piece of magnesium ribbon followed by few drops of concentrated hydrochloric acid, colours ranging from orange to red (flavones), red to crimson (flavorals) crimson to magenta (flavonones) and occasionally to green or blue are developed within 3 minutes if flavinoids are present.
VIII Test for sugars:

Fehlings Solution Test: - The test residue solution was heated and drop by drop fehling solution was added to it and warmed. If red precipitate of cuprous oxide is obtained, reducing sugars including all monosaccharides and many disaccharides are present.

Results obtained are shown in the tables below:-

Table 1: Extractive values and Physical characteristics of selected plants pollens.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Material</th>
<th>Solvent</th>
<th>% of extractive</th>
<th>Colour and appearance of extractive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pollen of <em>C. roseus</em></td>
<td>Petroleum Ether, Ethanol</td>
<td>26.58, 32.91</td>
<td>Yellowish brown, oily mass, Dark brown mass</td>
</tr>
<tr>
<td>2</td>
<td>Pollens of <em>M. charantia</em></td>
<td>Ethanol, P.Ether</td>
<td>1.96, 18.5</td>
<td>Yellowish brown, oily mass, Brown dry mass</td>
</tr>
<tr>
<td>3</td>
<td>Pollen of <em>B. monosperma</em></td>
<td>Ethanol, P Ether</td>
<td>1.42, 21.42</td>
<td>Yellowish, oily mass, Brown mass</td>
</tr>
<tr>
<td>4</td>
<td>Pollen and <em>S. cuminii</em></td>
<td>Ethanol, P Ether</td>
<td>5.50, 22</td>
<td>Light yellowish oily mass, Dark brown and dry mass</td>
</tr>
</tbody>
</table>

Table 2: Summarizes the qualitative determination of phytochemical constituents for *Catheranthes roseus* –

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sterols</td>
<td>Petroleum Ether (P.E) Solvent extract alcohol after P.E</td>
</tr>
<tr>
<td></td>
<td>a. Salkowski reaction</td>
<td>+ +</td>
</tr>
<tr>
<td></td>
<td>b. Liebermann-Burchard reaction</td>
<td>+ +</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Mayer’s reagent</td>
<td>+ -</td>
</tr>
<tr>
<td></td>
<td>b. Wagner’s reagent</td>
<td>+ -</td>
</tr>
<tr>
<td>3</td>
<td>Proteins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Bureit test</td>
<td>- -</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Foam test</td>
<td>+ -</td>
</tr>
<tr>
<td>5</td>
<td>Caumarins</td>
<td>- -</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Ferric Chloride reagent</td>
<td>- -</td>
</tr>
<tr>
<td></td>
<td>b. Lead acetate reagent</td>
<td>- -</td>
</tr>
<tr>
<td>7</td>
<td>Flavinoids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Shinoda Test</td>
<td>+ +</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Fehling’s Test</td>
<td>+ +</td>
</tr>
</tbody>
</table>

Qualitative determination of phytochemical constituents for *Momordica charantia* –

1. Sterols + -
2. Alkaloids + -
3. Proteins + -
4. Saponins + -
5. Caumarins + -
6. Tannins + -
7. Flavinoids + -
8. Carbohydrates + -

Qualitative determination of phytochemical constituents for *Butea monosperma* –

1. Sterols + -
2. Alkaloids + -
3. Proteins + -
4. Saponins + -
5. Caumarins + -
6. Tannins + -
7. Flavinoids + -
8. Carbohydrates + -
RESULT AND DISCUSSION

Phytochemical screening for different constituents present in the pollen part of the above selected plants showed following results. Pollens of *Catheranthus roseus* showed the presence of steroids and Alkaloids which are reported to have antidiabetic properties derived from other parts of the plant. The earliest chemical investigation of *Catheranthus roseus* was carried out by M. Creshoff (1890) who indicated the presence of alkaloidal constituents in them. More than 100 alkaloids have been isolated from the plant and their biological uses have been widely explored. (Trelease and Evans, 1978, Fransworth 1973). These alkaloids possess antifibrillic, hypotensive, sedative and tranquilizing properties.

In addition to alkaloids leaves also contain an alcohol called lochnrol, several monoterpen glycosides including adenosine and resoside. Leaf decoction and aqueous extract of fruits and seeds showed hypoglycemic activity. The main alkaloid is vindoline, dehydraouidolinine and coromaridine which are used for the treatment of diabetes. The pollens of *C. roseus* showed strong presence of alkaloids in them.

*Momordica charantia* commonly known as ‘Karela’ has been used in traditional medicine as an appetite stimulant, a treatment for gastro intestinal infection and to lower blood sugar level in diabetes. The fruit extract of *Momordica charantia* has been reported to cause hypoglycaemic effect (Principal constituent Charantin) similar to insulin in juvenile diabetes (Goel *et al.* (2002).

The steroidal saponins known as charantin present in the fruit extracts of *Momordica charantia* are being reported to have antidiabetic properties and are being considered as principal constituents having hypoglycemic activity. The pollens of *Momordica charactia* showed the presence of saponins along with flavinoids, carbohydrates and tannins in them.

Similarly *Butca monosperma* commonly known as ‘palas’ belonging to family Fabaceae is a medicinally important plant. Almost all parts like flower stem, gum leaves, seeds, root and bark are believe to have medicinal properties. The main constituents in flower are butrin (1.5%) besides butein (0.37%) and butin (0.04%) also contains flavinoids and steroids (Murti and Rao, 1971). The terpinoid namely (-). Palasonin was found to be effective inhibiting glucose uptake and depleted the glycogen content in Ascaridia galli (Amrit Pal Singh, 2005).

The presence of flavinoids and sterols in *Butea monosperma* is reported to have antidiabetic properties. The pollens of *Butea monosperma* showed the presence of carbohydrates, flavinoids and sterols in the them.

*Syzygium cumini* (L) a members of family Myrtaceae is of wider interest for its medicinal application. Literature reveals that the seed extract is found to be effective against diabetes. They also possess anti inflammatory, antiarthritic and antipyretic properties. Seeds contain an alkaloid Jambosine and a glycoside. Jamoblin or antimellin, which halts the diastatic conversion of starch into sugar. Seeds also contain ellagic acid. Other 34 polyphenols in the seeds and bark have been isolated and identified by Bhatia and Bajaj. The other constituents of the seeds are protein (6-8%) 0.41%phosphorus 0.17%, fatty acids, starch 41% dextrin 6.1% and 6 to 19% tannins. The leaves, steeped in alcohol, are prescribed in diabetes. They yield 12 to 13% tannin (by dry weight).

Amongst the above mentioned constituents presence of alkaloids and tannins in the pollen parts of *Syzygium cumintii* is reported to have antidiabetic properties.

**Conclusion:**

Now a day’s few drugs are available in market for the treatment of diabetes. Diets, quality of food and life habits are good remedy. In this situation use of herbal can be a safe and efficient method of treatment. The point base formulations have proven biologically more compatible to human system and are comparatively less toxic...
and have less side effects. In the present investigation phytochemical screening for different constituents present in the pollen part was carried out. The important antidiabetic constituents (like alkaloids, steroids, flavonoids glycosides, tannins, saponins) present in other parts of plants were detected in the pollen part of all the selected plants.

All these plants can be further studied for isolation of active compound and the activity of these compounds can be screened for hypoglycemic activity.

REFERENCES

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