PHYTOCHEMICAL INVESTIGATION OF DOLICHOS BIFLORUS (HORSEGRAM) SEED EXTRACT

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ABSTRACT
Dolichos biflorus Linn, commonly known as ‘horse gram’ is a common twining plant grown all over India. In traditional medicine, the decoction of the grain is indicated in Scrofula (glandular swelling) and Corpulence (obesity). Hence this study was planned to identify first the nature of the compounds present in aqueous extract before identification and isolation of the active principle associated with anti obesity potential of horse gram. The aqueous soluble fraction of horse gram seed was extracted using Soxhlet Assembly. Then it was subjected to qualitative tests for identification of its constituents. Identified constituents were separated using Thin Layer Chromatography (TLC). The photochemical analysis of the aqueous extract was tabulated (Table 1): Carbohydrates, proteins, steroids, tannins and lactones were present in the extract. Gums, mucilage, alkaloids, glycosides, fats and volatile oils were absent in the extract.

Dolichos biflorus seed, commonly known as ‘horse gram’ is a routine ingredient in Indian diet as a pulse. In traditional medicine, the decoction of the grain is indicated in Scrofula (glandular swelling) and Corpulence (obesity)1. Obesity is a global problem and intense research is going on in different directions to identify anti obesity potential in drugs already in use as well as screening newer agents for this aspect. While chemical agents as drugs are inherently associated with adverse effects, a welcome alternative will are edible natural food products with time proven safety potential along with anti obesity effect.

Key Words: Dolichos biflorus seed extract, horse gram, phytochemical analysis

INTRODUCTION
A literature survey on horse gram resulted in identifying only the food & caloric value2. The constituents reported to be present in the pulse were starch, oil, fiber, ash, phosphoric acid and the enzyme urease3. A methanolic extract of the whole plant of Dolichos biflorus was reported to have lipid lowering activity4 and antioxidant activity5. The methanolic extract was reported to contain phyto constituents such as alkaloids, steroids, flavonoids and isoflavones6. There was no reference available as to the presence or absence of bio constituents of medicinal interest in aqueous extract of horse gram seed. Therefore, a preliminary phytochemical screening involving qualitative tests for identification of various plant constituents like alkaloids, glycosides, phytosterols, phenolic compounds, saponins etc became mandatory.

AIM AND OBJECTIVES:
The aim of this study was to identify the phyto chemical constituents of medicinal interest in the aqueous extract of Dolichos biflorus seed.

The objectives are:
- To extract the aqueous soluble fraction of horse gram seed using Soxhlet Assembly.
- To subject the extract to qualitative tests for identification of its constituents.
- To separate the identified constituents using Thin Layer Chromatography (TLC).

MATERIALS AND METHODS:
Institutional Human Ethics Committee approval was obtained.

Preparation of aqueous extract of horse gram seed:
Coarse powder of horse gram seed was packed in Soxhlet
assembly and subjected to hot extraction with water for 24 hours. The collected extract was air dried and made into a solution of 100mg/ml.

**Qualitative chemical examination:**

The above solution was examined by standard Detection Tests for the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, proteins and amino acids.

**Characterization of identified plant constituents using Thin Layer Chromatography:**

To run a TLC using commercially available pre coated plates, by selecting the mobile phase based on the identified constituents and then to resolve the components of original mixture and eluting them out from chromogram by treating the cut-out spots with a suitable solvent and to quantify the same using suitable instrumental method of analysis.

**RESULTS:**

The photochemical analysis of the aqueous extract was tabulated (Table 1): Carbohydrates, proteins, steroids, tannins and lactones were present in the extract. Gums, mucilage, alkaloids, glycosides, fats and volatile oils were absent in the extract.

**Table 1: List of constituents present and absent in Dolichos biflorus seed extract**

<table>
<thead>
<tr>
<th>Constituents present</th>
<th>Constituents absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Gums</td>
</tr>
<tr>
<td>Proteins</td>
<td>Mucilage</td>
</tr>
<tr>
<td>Steroids</td>
<td>Alkaloids</td>
</tr>
<tr>
<td>Tannins</td>
<td>Glycosides</td>
</tr>
<tr>
<td>Lactones</td>
<td>Fats</td>
</tr>
<tr>
<td></td>
<td>Volatile oils</td>
</tr>
</tbody>
</table>

The following were the tests done to find out the ingredients:

**Molish’s test for carbohydrate:**

A violet ring was formed at the junction of the two liquids indicating the presence of carbohydrates.

**Biuret Test for proteins:**

Violet colour was appeared indicating the presence of proteins.

**Test for steroids:**

1. Salkowski reaction:
   The chloroform layer appeared red and the acid layer showed greenish yellow fluorescence
2. Liebermann - burchard reaction:
   Initially a red colour appeared and this turned to blue next and finally a green colour was appeared.
3. Lieberman’s reaction:
   Blue colour appeared indicating the presence of steroids(bufadienolides)

Thus the above tests results were indicative of the presence of steroids (phytosterols)

**Test for tannins:**

Black colour was appeared with lead acetate indicating the presence of tannins.

**Test for lactones:**

Appearance of red colour with acetic anhydride and concentrated sulphuric acid showed the presence of lactones.

**Test for glycosides & cardiac glycoside:**

Borntrager’s test for anthraquinone glycosides, Baljet’s test, Legal’s test & Keller-Kiliani test for cardiac glycosides and Foam test for saponins glycosides were all negative indicating the absence of cardiac glycosides in the aqueous extract of horse gram seed.

**Test Alkaloids:**

With Mayer’s test a light cream precipitate was appeared. With Hager’s test, a dim yellow precipitate was appeared, suggesting weakly positive reaction for alkaloids.

But the Dragendorff’s test and the Wagner’s test for alkaloids were negative suggesting the absence of alkaloids in aqueous extract.

Therefore a TLC was done to confirm whether any alkaloid was present or not in the aqueous extract. The spots did not turn orange brown on the addition of Dragendorff’s reagent, confirming the absence of any alkaloid in the aqueous extract.

The positive findings confirming the presence of carbohydrates, proteins, steroids, tannins and lactones were shown in Figure 1.

**Figure 1: Lab tests of constituents present in Horse gram**

**DISCUSSION:**
The phytochemical analysis confirmed the presence of carbohydrates, proteins, phytosterols, tannins, and lactones. This also confirmed the absence of gums & mucilages, various glycosides, fixed & volatile oils as well as fats. Though there was a doubtful indication for the presence of alkaloids (with Mayer’s test and Hager’s test), the Thin Layer Chromatography confirmed the absence of alkaloids in the aqueous extract. The slightly positive indication for alkaloids in Mayer’s test and Hager’s test could probably due to the presence of impurities in the extract.

Phytosterols have medicinal value. Hence this fraction can be isolated from the aqueous extract and tested for anti-obesity potential in rats and human volunteers. The same can be tested for appetite suppressant activity in rats. This if proves positive can form the basis for developing a molecule of interest for anti-obesity activity.

REFERENCES:


