RESEARCH ARTICLE
PHYTOCHEMICALS SCREENING AND ELECTROPHORETIC STUDY OF SEED STORAGE PROTEINS OF BAUHINIA ACUMINATA AND CASSIA OCCIDENTALIS

Kumari Nutan Sinha and Tanuja Singh

1Department of Botany, Patna Science College, P.U., Patna, Bihar
2Department of Botany, B.M.D College, Dayalpur, Vaishali, B.R.A. University, Bihar

ABSTRACT
Phytochemical screening of two species viz. Bauhinia acuminata and Cassia occidentalis belonging to family Caesalpiniaceae was performed using generally accepted laboratory technique. Three solvents viz. Chloroform, Benzen and Petroleum ether were used for extraction. The constituents screened were alkaloids, flavonoids, glycosides, saponin, steroid and tannin. The distribution of these constituents in the leaves of selected species were assessed and compared. Preliminary phytochemical screening of Bauhinia acuminata did not reveal alkaloids. Glycoside, steroid and flavonoids were present in both the species. Tannin was present in Cassia occidentalis while absent in Bauhinia acuminata. Saponin was absent in Cassia occidentalis while present in Bauhinia acuminata. Paper chromatography of flavonoids showed presence of Kaempferol, Quercetin, and Apigenin. Kaempferol, Quercetin and Apigenin were present in both the species. Derivatives of Quercetin i.e. Quercetin-3-glucoside was present in B. acuminata while Quercetin-7-glucoside was in C. occidentalis. Beside the common bands among the studied taxa Band 1 (Rf=0.08, mol. wt. 261.143 kDa), band 4 (Rf=0.26, mol. wt. 147.740), Band 7 (Rf=0.42, mol. wt. 102.564), Band 10 (Rf=0.52, mol. wt. 54.854), Band 13 (Rf=0.76, mol. wt. 31.85 kDa) and band 15 (Rf=0.90, mol wt. 7.37 kDa) were exactly alike in both the taxa. The pairing affinity index calculated on the basis of electroforethic patterns of seed protein. The percentage similarity between B. acuminata and C. occidentalis was 40%.

INTRODUCTION
Medicinal plants are nature’s priceless gift to human. Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs. The use of medicinal plants still plays an important role to cover the basic health need in the developing countries. Medicinal plants have been used for centuries as remedies for human diseases because they contain natural components which play a dominant role in the development of novel drug lead for treatment and prevention of diseases (Newman et al., 2003; Rahman and Gilani, 2005). The medicinal value of plants lies in some chemical substances or group of compound that produce a definite physiological action in the human body. These chemical substances are called secondary metabolite. The most important of these bioactive group of plants are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds (Edeoga et al., 2005). These bioactive substances are found to be distributed in plants, yet these compounds were not well established due to lack of knowledge and technique (Hafiza et al., 2003). In recent years secondary metabolites with unknown pharmacological activities have been extensively investigated as a source of medicinal agent (Krisharaju, 2005). Flavonoids and phenols are strong antioxidants and have an important role in the health care system (Dhan Prakash, 2007). Screening of active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer and alzheimer’s disease (Soma et al., 2010). Screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of drug like properties at the onset of drug discovery will pay off later in drug development. The family Caesalpiniaceae is extremely rich in flavonoids. Kaempferol and Quercetin have been reported from flavonol group while Apigenin and Luteolin from flavone group in the members of this family (Harborne, 1967). Flavonoids are known antioxidants, of which Quercetin is a potent antioxidant because of its right structural features and free radical scavenging activity.

The seed storage protein analysis helps in identification and characterisation of diversity in crop varieties and also provides information on phylogenetic relationship of the accession (Nisar et al., 2007; Tanksley et al., 1981; Than V.O.C. and Hirata Y. 2002). Electrophoresis of protein is a powerful tool for identification of genetic diversity and the SDS-PAGE is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations (Javid et al., 2004; Iqbal et al., 2005). Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species (Jha, S. S. and Ohri, D. 1996; Seferoglua et al., 2006). The SDS-PAGE is considered to be a practical and reliable method for species identification (Gepts, 1989). Since in mature seeds, type and amount of proteins are more constant than other plant tissues (Magni et al., 2007).
therefore, the SDS-PAGE pattern of seed storage proteins of selected species showed polymorphism on the basis of difference in protein intensity among genotypes. The aims of the present study were to evaluate the chemical constituents of *B. acuminata* and *C. occidentalis* species of family Caesalpiniaceae. These species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative and purgative uses (Hennebelle et al., 2009). *B. acuminata* leaves have antidiabetic action (Ragvan et al., 2006) and *C. occidentalis* is a medicinal herb found to have many disease preventive properties (Nadkarni, 1976; Markham,1982). Hence the present study was carried out to evaluate the phytochemical constituents of *B. acuminata*, and *C. occidentalis* leaf, study of their foliar flavonoids by paper chromatographic method and seed storage protein using SDS PAGE.

**MATERIALS AND METHODS**

**Collection of plant materials:** The leaves of the plants have been collected. The plant samples have been air dried and ground into uniform powder. The extracts have been prepared in three different solutions viz. Benzene, Chloroform and Petroleum ether.

**Test for alkaloids:** *Mayer’s test:* To the 1 ml of extract, add 1 ml of Mayer’s reagent (Potassium mercuric iodide solution). Whitish yellow or Cream coloured precipitate indicates presence of alkaloids.

**Wagner’s test:** To the 1 ml of extract add 2 ml of Wagner’s reagent (Iodine in Potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

**Test for Glycosides:** *Legal’s test:* Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. Appearance of red colour confirmed the presence of glycosides.

**Baljet’s test:** 2 ml of test solution was taken in a test tube followed by the addition of picric acid. Appearance of orange colour revealed the presence of Glycosides.

**Test for Tannins:** *Ferric Chloride Solution test:* Little quantity of extract was taken in a test tube. To this, 2 ml ethanol was added and mixed well followed by the addition of 1 ml of 5% ferric chloride reagent. Deep blue colour was observed which inferred the presence of tannins.

**Test for steroids:** *Salkowski test:* The test extract was treated with few drops of concentrated sulphuric acid. Red colour at lower layer indicated the presence of steroids.

**Test for Flavonoids:** *Shinoda’s test:* (1) The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

(2) The extract is treated with NaOH, formation of yellow colour indicates the presence of flavones.

**Study of Flavonoids:** 5 grams of mature and healthy leaves were collected from each species. After air drying, the phenolic compounds were extracted from these leaves at room temperature in different solvents like 70% Ethanol, Petroleum Ether, Acetone and Methanol using standard procedures. Isolation and purification of the compounds were done by repeated chromatography using Whatman 3mm chromatography paper. Characterisation of compound were carried out following standard technique (Markham,1982). The purified compounds were taken in Ethanol and their UV and visible light spectrum were measured with Spectrophotometer. Further, to know the position of substitution, spectral shifts after the addition of standard diagnostic chemicals were determined.

**Electrophoretic Study of Seed Storage Protein:** Fresh mature seeds of *Bauhinia acuminata* and *Cassia occidentalis* were collected from different localities of Patna.

**Protein Extraction:** Protein was extracted by method given by Jensen and Lixue (1991). Protein was extracted from overnight presoaked seeds in protein solubilization solution (62 m M Tris –HCl, pH 6.8, 10% glycerol, 2% SDS, p-mercaptoethanol and traces of bromophenol blue ) then transferred to Eppendorf tube and centrifuged at 14000 rpm for 30 seconds. The supernatant was transferred to a fresh tube and placed into a boiling water bath for 4 minutes.

**SDS-PAGE**

SDS-PAGE was done by method suggested by Lamelli, (1970). It was performed on a vertical slab gel. Bromophenol blue was added to the supernant as tracking dye to watch the movement of protein in the gel. Seed protein was analysed through slab type SDS-PAGE using 10% Separating gel and 4% Stacking gel. Molecular weight of different bands were calibrated with a mixture of standard protein markers include Myosin (261.143 kDa), Phosphorylase B (137.190 kDa), BSA (102.564 kDa), Ovalbumin (54.854 kDa), Carbonic Anhydrase (37.670 kDa), Lysozyme (31.854 kDa), Lysozyme (21.769 kDa) and Aprotinin (7.337 kDa). Protein Electode buffer solution was poured into the bottom pool of the apparatus. Gel plate was placed in the apparatus carefully so as to prevent bubbles formation at the bottom of gel plated. Equal quantity of extracted protein from each sample along with Protein molecular weight marker (PAGE mark) was loaded with the micropipette into each wells of the gel. The apparatus was connected with constant electric supply. Electrophoresis was carried out at 20 mA current for 3-4 hours till the tracking dye reaches the bottom of the gel. After electrophoresis, the protein bands were visualized by staining with coomassie brilliant blue G-250 and destained with methanol, acetic acid and water (4:1:5).

**GEL Documentation and Analysis**

Finally gel was photographed. Molecular weight of protein bands were estimated by their relative mobility. Pairing affinity or Similarity index was calculated by the method described by the formula.

\[
P_A = \frac{\text{Bands common to species I and species II}}{\text{Total bands of the species I and Species II}} \times 100
\]

**RESULTS**

The phytochemical characters of the selected medicinal plants viz. *B. acuminata* and *C. occidentalis*, investigated are summarized in Table 1. In the table (+) sign indicates the presence of a particular constituent and (-) indicates absence of a constituent. The data of chromatographic study of flavonoids is tabulated in Table 2. Seed storage protein was analysed through SDS-PAGE using 10% Polyacrylamide gel. The pattern of the total protein *Bauhinia acuminata* and *Cassia occidentalis* showed some variation between them (Plate 1, Fig.1). The Rf value between different species ranged from 0.08 to 0.90 (Table 3).
Table 1 Phytochemical analysis of leaves extract of B. acuminata using various solvents

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Phytochemicals</th>
<th>Benzene</th>
<th>Chloroform</th>
<th>Petroleum ether</th>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>Glycosides</td>
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</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. occidentalis</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glycosides</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

(+)= Present; (-)= Absent

Table 2 Chromatographic and spectral properties of Flavonoid spots of B. acuminata and C. occidentalis

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<tr>
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<th>Visible Light</th>
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<th>UV-NB</th>
<th>BAW</th>
<th>15% AcOH</th>
<th>30% EtOH</th>
<th>Water</th>
<th>Phenol</th>
<th>Forestal</th>
<th>Spectral Max. In EtOH</th>
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<td>--</td>
<td>?</td>
</tr>
<tr>
<td>2</td>
<td>Bl</td>
<td>Bl</td>
<td>Bl</td>
<td>34</td>
<td>36</td>
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<td>-</td>
<td>-</td>
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<td>?</td>
</tr>
<tr>
<td>3</td>
<td>Nc</td>
<td>Bl</td>
<td>Yi</td>
<td>66</td>
<td>41</td>
<td>--</td>
<td>17</td>
<td>27</td>
<td>39</td>
<td>251,373</td>
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<td>56</td>
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<td>72</td>
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<td>BY</td>
<td>84</td>
<td>-</td>
<td>51</td>
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<td>-</td>
<td>-</td>
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</tr>
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<td>86</td>
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<td>66</td>
<td>44</td>
<td>44</td>
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</table>

Abbreviation: (+)= Rf value variable, so not included; (?) = Uncertain chemical identity; BAW = n-butanol acetic acid water; AcOH = aqueous acetic acid; EtOH = Ethanol; TLC = Thin layer chromatography; Nc = Indicates no colour in visible light; Bl = Bright yellow; Yi = Yellow; GY = Greenish yellow; BY = Blackish yellow; B = Brown; RB= Reddish brown.

Fig. 1: Showing diagramatic representation of protein bands of M: Marker; B: Bauhinia acuminata; C: Cassia occidentalis.

Plate 1: Photograph of protein bands of M: Marker; B: Bauhinia acuminata; C: Cassia occidentalis.
DISCUSSION

Phytochemicals are playing vital role for the treatment of different types of diseases and still are use in both traditional and modern system of medication. The present study carried out on the plant samples revealed the presence of medicinally active constituents. Glycoside, tannin, steroids and flavonoids were present in both the species of family Caesalpiniaceae. In Petroleum ether Glycosides was absent in leaf extract of C. occidentalis. Leaf extracts of B. acuminata in chloroform, tannin was absent. Saponin was absent in C. occidentalis while present in B. acuminata. Alkaloid was absent in B. acuminata while present in C. occidentalis.

The presence of flavonoids, glycoside in leaf of B. purpurea and root and leaf of B. tomentosa are earlier reported (Sanjoshi et al., 2007; Gupta et al., 2011 and Madhavan et al., 2012). The data of paper chromatographic study of flavonoids showed the presence of Kaemferol, Quercetin, Apigenin, and derivatives of Quercetin viz. Quercetin-7-glucoside, Quercetin-3-glucoside. Kaemferol, Quercetin and Apigenin were common in both the taxa. The derivatives of Quercetin, viz. Quercetin-3-glucoside (Isoquercitrin) was present in B. acuminata, while Quercetin-7-glucoside was present in C. occidentalis. Flavonoids are a group of naturally occurring polyphenolic compounds primarily from fruits and vegetables.

They are one of the most numerous and wide spread groups of phenolic compound seen in higher plant (Carini et al., 2001). It has been reported that the presence of bioactive substances in plants play a role in preventing colorectal carcinoma, hypercholesterolemia and renal calculi (Marchounch et al., 2001). It is documented that the presence of saponins can control human cardiovascular disease and reduce cholesterol, also tannin may provide protection against microbiological degradation of dietary protein in the semen (Aletor et al., 1993). Seed storage protein was analysed through SDS-PAGE using 10% Polyacrylamide gel. The pattern of the total protein Bauhinia acuminata and Cassia occidentalis showed some variation between them. The Rf value between different species ranged from 0.08 to 0.90 (Table 3). The value depict the mobility of the protein on gel surface. Polymorphism was observed in three variable regions i.e high, medium and low molecular weight. Molecular weight of proteins ranged from 7.37 kDa to 261.143 kDa. Band 1 (Rf=0.08, mol. wt. 261.143 kDa), Band 2 (Rf=0.12, mol. wt. 198.09 kDa), Band 3 (Rf=0.20, mol. wt. 178.34 kDa), Band 4 (Rf=0.26, mol. wt. 147.740 kDa), Band 5 (Rf=0.28, mol. wt. 137.190 kDa), Band 6 (Rf=0.32, mol. wt. 120.04 kDa), Band 7 (Rf=0.42, mol. wt. 102.564 kDa), Band 8 (Rf=0.44, mol. wt. 97.90 kDa), Band 9 (Rf=0.48, mol. wt. 91.68 kDa), Band 10 (Rf=0.52, mol. wt. 54.854 kDa), Band 11 (Rf=0.58, mol. wt. 41.56 kDa), Band 12 (Rf=0.64, mol. wt. 37.67 kDa), Band 13 (Rf=0.76, mol. wt. 31.85 kDa), Band 14 (Rf=0.82, mol. wt. 21.50 kDa) and Band 15 (Rf=0.90, mol. wt. 7.37 kDa) were exactly alike in both the taxa.

The pairing affinity index calculated on the basis of electrophoretic patterns of seed protein. The percentage similarities between B. acuminata and C. occidentalis was 40%. According to the result of above findings, Bauhinia acuminata and C. occidentalis showed some diversity. The diversity in seed storage protein has also been reported by Khan et al., (2002).

CONCLUSION

In conclusion, this finding justifies the traditional use of the plants, for prophylactic and therapeutic purposes. Leaf of B. acuminata and C. occidentalis, has an opportunity to explore the species both phytochemically and pharmacologically. There for, ethnopharmacology can bridge between the folklore use and actual pharmacological efficacy of the medicinal plants. Studied plants can be seen as the potential source of useful drugs. There is need for further studies on the plant parts for structure and quantity of these bioactive compounds.

Reference


<table>
<thead>
<tr>
<th>Band no.</th>
<th>Rf Value</th>
<th>Mol. Wt. In KDa</th>
<th>Marker</th>
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<th>C. occidentalis</th>
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