An Investigation into Phytochemical Composition of
Caesalpinia bonducella Wild from Chambal Valley, India

Anupam Kr Sachan, Ch V. Rao, Nikhil Kumar Sachan and Vineet Kumar

Abstract: Caesalpinia bonducella (L.) belongs to the family Caesalpiniaecan, is a prickly shrub widely distributed throughout the warm climatic zones of India. The plant is observed being utilized in several curative preparations of the folk medicine practices. It is reported for having wide spectrum of pharmacological properties including antipyretic, antibacterial, anti-anaphylactic, anti-diarrheal, anti-viral, anti-asthamatic and antioxidant properties in extractives from different parts of the plant. The present investigation envisaged conducting phytochemical profiling of C. bonducella, whole plant, reckoning its phytotherapeutic potential. The study involved multiple extractions through successive solvent extraction with varying solvents using Soxhlet apparatus followed by phytochemical tests using standard methods. Fluorescence analysis of different extracts and powdered crude drug were observed under ultra voilet and ordinary light, which signifies as its characteristic feature for certain components. The qualitative chemical tests for different extract depicted presence of alkaloids, carbohydrates and glycosides, flavonoids, tannins, phenolic compounds, fixed oils and fats, protein and amino acids, steroids and terpenoids.

Key words: Caesalpinia bonducella · Successive solvent extraction · Phytochemical

INTRODUCTION

Medicinal plants are the promising source of drugs from ancient time. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to immense potential of medicinal plants used in various traditional systems [1]. An intensive and extensive study of the naturally occurring molecules identified as ‘therapeutically active’ is desired urgently to come out with new therapeutic entities [2]. Caesalpinia bonduc (L.) Roxb (syn. Caesalpinia bonducella (L.) Fleming, Syn. Caesalpinia crista (L.), commonly known as Nata Karanja belonging to the family Feabeae / Caesalpiniaecan, is a prickly shrub widely distributed all over the world, specially in India, Srilanka and Andaman and Nicobar Islands, in India specially found in tropical regions [3, 4]. Generally found up to an altitude of 1,000 m in Himalaya and wild throughout the plains on waste lands or coastal areas of India. It is also found in deltaic region of western, eastern and southern India [5]. Plant has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenes [6, 7], flavonoids, triterpenoids, diterpenoids and steroids [8-10]. All parts of the plant have medicinal properties so it is a very valuable medicinal plant, which is utilized in traditional system of medicine to treat various ailments with respect to heal mankind [11]. It has also been recognized for such multiple therapeutic properties as anti pyretic and antibacterial [12], anti-anaphylactic, anti-diarrheal and anti-viral [13], anti-asthamatic [14] antioxidant [15].

Plants or animals origin is a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Plant derived substances has recently become a great interest owing to their versatile applications. The Pharmacological activities of medicinal plants are due to the presence of various complex chemical substances of different compositions which occur as secondary metabolities. The most important of these bioactive
constituents of Plants are alkaloids, steroids, tannins, flavonoids and phenolic compounds [16]. Hence, it is desirable to know the phytochemical composition of plant material before testing its efficacy for medicinal purpose.

The present study envisaged exploring preliminary phytochemicals of *C. bonducella* which are likely to impart its pharmacological properties.

**MATERIALS AND METHODS**

**Materials:** Chloroform, ethanol, ethyl acetate, n-hexane (Merck, India), double glass distilled purified water and shade dried *C. bonducella* wild plant.

**Collection and Authentication of Plant Material:** The whole plant material was collected from wild sources around the *Udi* area of Chambal valley; district- Etawah, Uttar Pradesh, in month of June and July during 2016. The plant was identified and authenticated at source by Pharmacognosy and Ethnopharmacology Division CSIR-National Botanical Research Institute, Lucknow. A voucher specification (No.: NBRI-SOP-216) has been deposited in Institute repository.

**Preparation of Crude Drug for Extraction:** Plant material was cut into the pieces and shade dried at room temperature; the dried materials were subjected to size reduction to a coarse powder by using a dry grinder (Philips, India) and passed through the sieve before being stored in a closed air tight vessel for further use [17].

**Method of Extraction:** Coarse powder of crude drug was successively extracted with different organic solvents in increasing polarity order such as n-hexane, chloroform, ethyl acetate, 50%v/v ethanol solvents using soxhlet extractor[18, 19].

**n-Hexane Extract:** The shade dried coarse powder of crude drug was extracted with n-Hexane until the extraction was completed. After completion of extraction, solvent was removed by distillation under reduced pressure by using rotary evaporator. Green colour residue was obtained. The residue was then stored in desiccator.

**Chloroform Extract:** The Residue left after n-Hexane extraction was dried and then extracted with chloroform, After 24 hrs the extraction was assumed to be completed, the solvent was removed by distillation. Brownish green colour residue was obtained. The residue was then stored in desiccator.

**Ethyl Acetate Extract:** The marc left after chloroform extraction was dried and then extracted with ethyl acetate, until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Brownish green colour residue was obtained. The residue was then stored in desiccator.

**50% V/V Ethanol Extract:** The marc left after ethyl acetate extraction was dried and then extracted with 50% v/v ethanol extract, until the extraction was completed. After completion of extraction, the solvent was evaporated by heating on water bath. Blackish green colour residue was obtained. The residue was then stored in desiccator.

**Identification of Physiochemical Constituents:** The Phytochemical screening was carried out using standard procedures to detect the presence of alkaloids, carbohydrates, glycosides, oils and fats, proteins and amino acids, terpenoids and flavonoids [20-24].

**Detection of Alkaloids:** Small fractions of solvent free extracts were separately stirred with a milliliters of dilute hydrochloric acid and filtered, the filtrate is tested with Mayer’s reagent, Wagner’s reagent, Hanger’s reagent, Dragendorff’s reagent to confirm the presence or absence of alkaloids as indicated by production of cream, reddish brown, yellow or orange-brown colour precipitate respectively with these reagents in presence of alkaloidal substances.

**Detection of Carbohydrates:** Extracts were dissolved separately in distilled water and filtered, then tested with Molisch's reagent, Fehling's reagent, Benedict's reagent and Barfoed’s test for detection of carbohydrates.

**Molisch’s Test:** To the filtrate, added few drops of alcoholic alpha naphthol and 2 ml of concentrated sulfuric acid slowly through the side of test tube; presence of carbohydrate produce a violet colour ring at the junction of two layers.

**Fehling’s Test:** A little fraction of filtrate treated with Fehling’s solution I & II and then heated on a water bath. A brick red precipitate is indicator for reducing sugars.

**Benedict’s Test:** Small quantity of filtrate treated with equal quantities of Benedict’s reagent, heated subsequently on a water bath result to formation of a brown precipitate in presence of reducing sugars.
Barfoed’s Test: The different extracts were treated with Barfoed’s reagent. Monosaccharides, if present, produce a brick red precipitate.

Detection of Glycosides: Glycosides were confirmed by subjecting the acid hydrolysed extract to Legal’s test, Borntrager test and Libermann-Burchard’s test.

Legal’s Test: Hydrolysate was dissolved in pyridine and sodium nitro-prusside solution, added sodium hydroxide; a colour change result in presence of glycosides.

Borntrager’s Test: A few milliliters of hydrolysate treated with chloroform, decanted off chloroform layer, added equal quantity of dilute ammonium solution. A pink colour is produced in ammonical layer in presence of glycosides.

Libermann-Burchard’s Test: Hydrolysate treated with chloroform, to this added Libermann-burchard reagent; a colour change result in presence of glycosides.

Detection of Fixed Oil and Fats
Saponification Test: Few drops of 0.5N potassium hydroxide along with one or two drops of phenolphthalein were added to various extracts, heated on a water bath for 1-2 hours. Saponification or no saponification indicates the presence or absence of oil and fats.

Filter Paper Test: Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil strain on the paper indicated the presence of fixed oils.

Detection of Protein & Amino Acids: The extracts were tested with Million’s test reagent, Biuret test reagent and Ninhydrin test reagent, presence of amino acids and proteins is indicated by production of red, violet and blue colour respectively.

Detection of Phenolic Compounds & Tannins: All the dry extracts were dissolved in minimum amount of water, filtered and subject to Ferric chloride test, Gelatin test. Filtrate on addition of few drops of ferric chloride produce a violet colour precipitate in presence of tannins. A white precipitate is resulted in presence of tannins on addition of 1ml 1% solution of gelatin to the filtrate.

Detection of Phytosterols: Small quantity of the dry extracts dissolved in about 5ml of the chloroform and subjected to Salkowski’s test and Libermann-Burchard’s test.

Salkowski’s Test: One ml of the chloroform solution, prepared as above was added with few drops of concentrated sulfuric acid; green colour is the indicative of phytosterols.

Libermann-burchard’s Test: The chloroform solution, prepared as above was treated with few drops of concentrated sulfuric acid followed by one ml of acetic anhydride. Presence of phytosterols is confirmed by the production of a bluish green colour.

Test for Terpenoids
Chloroform Test: To 5ml of the extract few drops of chloroform and concentrated sulfuric acid was added carefully along the sides of the test tube to form a layer and observed for the presence of reddish brown color.

Detection of Flavonoids
Shinoda’s Test: Small quantity of the extract was dissolved in alcohol, to that pieces of magnesium followed by Concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

Detection of Saponins
Foam Test: The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam shows the presence of saponins.

Fluorescence Analysis: Chemical tests of crude powder drug with different reagents were studies in UV 366nm and ordinary day light. There are many substances in dilute sulfuric acid when suitably illuminated emit light of different wavelength and colour form that which falls on them. Light rich in short wave lengths is very active in producing fluorescence and for this reason strong UV light produces fluorescence in many substances which do not visibly fluorescence in day light [16]. The results are presented in Table 3 and 4.

RESULTS AND DISCUSSION

The phytoconstituents were extracted by using different solvents of increasing polarity like n-Hexane, chloroform, ethyl acetate and 50% v/v ethanol. The extractive values are presented in Table 1. The presence and absence of different phyto-constituents are presented in Table 2.
Table 1: Extractive values of *C. bonducella*

<table>
<thead>
<tr>
<th>Parts used</th>
<th>Methods of extraction</th>
<th>n-Hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>50% v/v Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole plant</td>
<td>Successive solvent extraction</td>
<td>0.98%</td>
<td>1.21%</td>
<td>1.32%</td>
<td>2.36%</td>
</tr>
</tbody>
</table>

Table 2: Preliminary phytochemical screening of the various extracts of *C. bonducella*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Constituents</th>
<th>n-Hexane extract</th>
<th>Chloroform extract</th>
<th>Ethyl acetate extract</th>
<th>50% v/v Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Flavanoids</td>
<td>(+)</td>
<td>(+)</td>
<td>(±)</td>
<td>(±)</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrate</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>4</td>
<td>Protein and amino acid</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>5</td>
<td>Phenolic compounds</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(±)</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>9</td>
<td>Phytosterols</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(±)</td>
</tr>
<tr>
<td>10</td>
<td>Fat and oils</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(±)</td>
</tr>
<tr>
<td>11</td>
<td>Terpenoids</td>
<td>(±)</td>
<td>(±)</td>
<td>(±)</td>
<td>(±)</td>
</tr>
</tbody>
</table>

'+' Presence, '-' Absent

Table 3: Fluorescence characteristic of various extract of *C. bonducella*

<table>
<thead>
<tr>
<th>Particulars of the treatment</th>
<th>Under ordinary light</th>
<th>Under UV light (366nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>Green</td>
<td>Light green</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Brownish green</td>
<td>Light green</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Brownish green</td>
<td>Light green</td>
</tr>
<tr>
<td>50% v/v Ethanol</td>
<td>Blackish green</td>
<td>Green</td>
</tr>
</tbody>
</table>

Table 4: Fluorescence characteristic of crude drug of *C. bonducella*

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Particulars of the treatment</th>
<th>Under ordinary light</th>
<th>Under UV light (366nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Brownish green</td>
<td>Dull green</td>
</tr>
<tr>
<td>2</td>
<td>Powder +1N HCl</td>
<td>Dull light brown</td>
<td>Light green</td>
</tr>
<tr>
<td>3</td>
<td>Powder +1N H2SO4</td>
<td>Dull light brown</td>
<td>Light green</td>
</tr>
<tr>
<td>4</td>
<td>Powder +aq. NaOH</td>
<td>Brown</td>
<td>Green</td>
</tr>
<tr>
<td>5</td>
<td>Powder +10% Glacial Acetic Acid</td>
<td>Light brown</td>
<td>Light green</td>
</tr>
<tr>
<td>6</td>
<td>Powder + 5% FeCl3</td>
<td>Orange brown</td>
<td>Blackish green</td>
</tr>
<tr>
<td>7</td>
<td>Powder + Iodine</td>
<td>Greenish brown</td>
<td>Light green</td>
</tr>
<tr>
<td>8</td>
<td>Powder + Picric acid</td>
<td>Yellowish brown</td>
<td>Blackish green</td>
</tr>
<tr>
<td>9</td>
<td>Powder +1% HNO3</td>
<td>Dull light brown</td>
<td>Light green</td>
</tr>
</tbody>
</table>

The phytochemical analysis conducted on *C. bonducella* extracts revealed the presence of phenolic compounds and tannins, steroids, saponins, glycosides, alkaloids in the different fractions of the extract. The various phytoconstituents detected in the fraction obtained through successive extraction using n-hexane, chloroform, ethyl acetate and ethanol are summarized in the Table 2 and the fluorescent characteristic of the extractives in the above fractions is presented in Table 3. Fluorescent characteristics were also observed for the powdered crude drug with different solvents.

The identified phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the various claimed therapeutic activities of this plant; extracts can be separately subjected to the pharmacological screening for further such confirmation of true therapeutic activity of such phytoconstituents revealed in this study [25]. In ethanolic extract alkaloids, carbohydrates and glycosides, flavonoids, tannins, phenolic compounds, amino acids, steroids and terpenoids indicates its possible therapeutic usefulness in the treatment of the inflammatory states of the body and autoimmune disorders like arthritis. The steroids and terpenoids may be possibly actuating the analgesic properties through modulating the nervous system. The presence of hypoglycemic saponins, tannins, triterpenes and flavonoids etc. also indicate the potential antidiabetic property of the extract to be examined. The phytoconstituents like flavonoids may potentially cause the
suppression of inflammatory mediators leading to inhibition of inflammation and better cell repair, maturation and remodeling promoting wound healing activity. The other minor chemical constituents and traces that ordinarily be present in the extracts in very little amount and lead to no noteworthy pharmacological effect.

CONCLUSION

With the growing attention in correlating the phytochemicals of a medicinal plant with its pharmacological activity, the present study on phytochemical profiling of C. bonducella plant with different chemical reagents was conducted to detect the presence of phytoconstituents and could be used for further standardization. Through proper research effort it can be considered as distinctive enough to identify and decide the authenticity of this drug in herbal industry/trade& may help to establish the scientific ground for folklore claims of its medicinal values.

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