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Optimization of extraction process and phytochemical investigations of Spathodea campanulata flowers

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Since ancient times, plants have been an exemplary source of medicines. Researches conducted in last few decades on the plants mentioned in ancient literature or used as folk medicines have shown potential of phytoconstituents in the treatment of various diseases. Spathodea campanulata is a plant that has been frequently used as a medicine which belongs to the genus Spathodea and family Bignoniaceae. S. campanulata (Bignoniaceae) flowers and bark are used traditionally in the treatment of mental disorders, malaria, hemorrhoids, bacterial infections, HIV, poor blood circulation, gastrointestinal diseases, respiratory ailments and genital-urinary system disorders, etc. Optimization of extraction process of phytoconstituents and phytochemical investigations of the flowers of S. campanulata, and including comparative preliminary phytochemical screening study of the flowers and bark of the plant, S. campanulata have been summarized in the present article.

Key words: Bignoniaceae, Spathodea campanulata, optimization, phytoconstituents, flowers, bark, gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared (FTIR), diffusion ordered spectroscopy-nuclear magnetic resonance (DOSY-NMR).

INTRODUCTION

The variety and sheer number of plants with therapeutic properties is quite astonishing. It is estimated that around 70,000 plant species from lichens to towering trees have been used for treating various ailments. Today, western herbal medicines still makes use of at least a thousand indigenous European plants, as well as many thousand species of other varieties native to America, Africa and Australia. In Ayurveda (Indian system of traditional medicines), about 2000 plants are considered to have medicinal values, while in Chinese pharmacopoeia, 5700 various traditional medicines of plant origin have been reported. About 500 herbs are employed within the conventional medicines, although whole plants are rarely used. From time immemorial, the herbs have played a major role by providing us lead compounds/components for the isolation and synthesis of many conventional drugs.

A general disillusionment with conventional medicines, coupled with the desire for a “natural” life style has resulted in an increasing utilization of alternative or complementary therapies with the natural products in general and phytomedicines in particular. During 1990, sales of over the counter (OTC) phytomedicines in 7 European countries were estimated to be about 2.4 billion US $ at their selling price. Same is the condition in India, where herbal medicine is playing a major role and is being increasingly used by the general public on a self-selection basis to either replace or complement conventional medicines. Medicinal herbs as well as different combinations obtained, is of particular interest to doctors, pharmacists and other healthcare professionals. A pharmacist should be capable of knowing all the reliable information on the quality, safety, efficacy and rational uses of important herbal drugs (Table 1). There are different ways of using herbs, by which their potency is preserved and their active constituents are released to exhibit the desired therapeutic actions.

Traditionally, herbs may be used according to following

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basic methods of preparations:

1. Fresh juice
2. Paste or powder
3. Decoction
4. Hot infusion
5. Cold infusion

This flowchart focuses on the initial need to produce safe and effective galenical products, but it includes the long-term objective of discovering the active principles (Figure 1). These programmes could eventually lead to the development of phyto-pharmaceutical industry in the country.

Currently, natural products pass through a phase of reduced interest in drug discovery, because of the enormous efforts which are necessary to isolate the active principles and to elucidate their structures. However, if one considers the diversity of chemical structures found in nature with the narrow spectrum of structural variations of even the largest combinatorial library, it can be expected that natural products will become even more important. Mainly, actinomycetes, fungi and other higher plants have been proved to biosynthesize the secondary metabolites of obviously unlimited structural diversity that can be further enlarged by structural modification by applying strategies of combinatorial chemistry. Probably, a variety of novel concepts in natural products research is required to draw interest to incorporate natural sources into the high-throughput screening (HTS) process. In various cases, valuable precursors can be made accessible from nature at moderate prices, thus contributing to improved manufacturing processes supplemented by tools of chemical synthesis, biocatalysis or biotransformation, respectively. Precursors are sometimes of considerable advantage, because they also provide access to non-natural analogs or derivatives which may exhibit a superior spectrum of properties referring to the demands for a successful drug development and application. For instance, in order to manufacture oral contraceptives or other steroidal hormones, plant metabolites, such as siosgenin from Dioscorea species and helogenin from Agave sisalana are used as a starting material.

_Spathodea_ is a monotypic genus in the flowering plant family Bignoniacaeae. The single species is _Spathodea campanulata_, known as the Fountain Tree, African Tulip Tree, Flame-of-the-Forest or Nandi Flame. It is a tree that grows between 7 to 25 m (23 to 82 ft) tall, native to tropical Africa and Southern Asia. This tree is planted extensively as an ornamental tree throughout the tropics and is much appreciated for its very showy reddish-orange or crimson (rarely yellow) campionul flower. However, it has the potential to become an invasive species. The flower bud is amule-shaped and contains water. These buds are often used by children who play with its ability to squirt the water. The sap sometimes stains yellow on fingers and clothes. The open flowers are cup-shaped and hold rain and dew, making them attractive to many species of birds. In neotropical gardens and parks, their nectar is popular with many hummingbirds, such as the Black-throated Mango (Anthracothorax nigricollis), the Black Jacobin (Florisuga fusca) or the Gilded Hummingbird (Hylocharis chrysura).

### Table 1. Drugs derived from plants and their ethno-medical co-relation and sources.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Drug</th>
<th>Action or clinical use</th>
<th>Plant source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ajmalicine</td>
<td>Circulatory disorder</td>
<td>Rauwolfia serpentina L.</td>
</tr>
<tr>
<td>2</td>
<td>Arecoline</td>
<td>Anthelmingtic</td>
<td>Benth ex. Kurz, Areca catechu L.</td>
</tr>
<tr>
<td>3</td>
<td>Atropine</td>
<td>Anticholinergic</td>
<td>Atropa belladonna L.</td>
</tr>
<tr>
<td>4</td>
<td>Bromelain</td>
<td>Anti-inflammatory, Proteolytic agent</td>
<td>Ananas comosus L. Merrill.</td>
</tr>
<tr>
<td>5</td>
<td>Caffeine</td>
<td>CNS stimulant</td>
<td>Camellia sinensis L. Kuntze</td>
</tr>
<tr>
<td>6</td>
<td>Cocaine</td>
<td>Local anaesthetic</td>
<td>Erythroxylum cocoa Lam K.</td>
</tr>
<tr>
<td>7</td>
<td>Colchicine</td>
<td>Anti tumor agent, Anti gout</td>
<td>Colchicum autumnale L.</td>
</tr>
<tr>
<td>8</td>
<td>Curcumin</td>
<td>Choleretic</td>
<td>Curcuma longa L.</td>
</tr>
<tr>
<td>9</td>
<td>Digitoxin</td>
<td>Cardiotoxic</td>
<td>Digitalis purpurea L.</td>
</tr>
<tr>
<td>10</td>
<td>Emetine</td>
<td>Amoebicide; emetic</td>
<td>Cephalis ipecacuanha (Brotero) a. Richard.</td>
</tr>
<tr>
<td>11</td>
<td>Ephedrine</td>
<td>Sympathomimetic</td>
<td>Ephedra sinica stap.</td>
</tr>
<tr>
<td>12</td>
<td>Glycyrrhizin</td>
<td>Sweetner</td>
<td>Glycyrrhiza glabra L.</td>
</tr>
<tr>
<td>13</td>
<td>Hyoscamine</td>
<td>Anticholinergic</td>
<td>Hyoscamus niger L.</td>
</tr>
<tr>
<td>14</td>
<td>Silymarin</td>
<td>Antiepatoxic</td>
<td>Silybum marianum L. Gaerth.</td>
</tr>
<tr>
<td>15</td>
<td>Stevioside</td>
<td>Sweetner</td>
<td>Stevia rebaudiana Bertonici.</td>
</tr>
<tr>
<td>16</td>
<td>Theobromine</td>
<td>Diuretic; bronchodilator</td>
<td>Theobroma cocoa L.</td>
</tr>
<tr>
<td>17</td>
<td>Tubocurarine</td>
<td>Skeletal muscle relaxant</td>
<td>Chondrodendron tomentosum R. &amp; P.</td>
</tr>
<tr>
<td>18</td>
<td>Yohimbine</td>
<td>Aphrodisiac</td>
<td>Pausinystalia yohimbe (K. Schum.) Pierre.</td>
</tr>
</tbody>
</table>
The wood of the tree is soft and is used for nesting by many hole-building birds, such as Barbets. The generic name comes from the Ancient Greek word *Spathe*, in reference to the spadix-like calyx. *S. campanulata* (Bignoniaceae) flowers and bark are used traditionally in the treatment of mental disorders, malaria, hemorrhoids, bacterial infections, HIV, poor blood circulation, gastrointestinal diseases, respiratory ailments, genital-urinary system disorders, etc. (Vinayaka et al., 2009; Mbosso et al., 2008; Trigo and Santos, 2000; Niyonzima et al., 1999; Makinde et al., 1988; Kovoor, 1953; Wealth of India, Raw Material, 2005).

The application of leaves and stem barks in most herbal preparations can be attributed to the fact that, human body organs are known to accumulate in high concentrations, active components of most of the herbal preparations. These components which have been shown to relieve diseased conditions in patients include alkaloids, tannins and inulin. The leaves have also been reported to be the most commonly used plant part in other parts of Africa.

**MATERIALS AND METHODS**

**Plant and chemicals**

The plant material (flowers and bark) was collected in the month of November to December, 2009 from Aurangabad. The botanical identity of the plant was confirmed at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad by preparing Herbarium of the plant. A voucher specimen has been deposited at the Museum of the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. All the reagents and chemicals used in the study were procured from Dipal Laboratory, Aurangabad and were of analytical grade.

**Extraction, optimization of extraction process and isolation of phytoconstituents**

Extraction of phytoconstituents was done using two techniques (Monica et al., 2004; Khandelwal, 2000; Fernando et al., 1996):

1. Soxhlet (continuous) extraction method, and
2. Maceration (at cold and at room temperature).

Collected plant material (flowers and bark) was kept in cool place for drying and to avoid direct loss of phytoconstituents from sunlight. Dried plant material (flowers and bark) was ground into powder by using mixer. This powder (190 g each) was ready for preliminary tests and extraction process. Powder (flowers) of the plant material was then extracted with solvents petroleum ether, chloroform and methanol using both techniques, namely, soxhlet extraction method and maceration. The obtained extracts (three) were processed separately. Petroleum ether extract: solvent was evaporated to give dark green material, chloroform extract: solvent was evaporated to give dark green material and methanolic extract: solvent was evaporated to give a dark green semisolid material.
Various parameters for the effective extraction of phytoconstituents from flowers using the same two techniques (soxhlet and maceration) of the plant were studied for solvents, namely, petroleum ether and methanol, and was optimized by repeating the extraction process twice (a total of three times).

Optimized parameters for soxhlet extraction procedure

**Petroleum ether extraction**

Volume of soxhlet extractor: 1000 ml.
Solvent used: petroleum ether (60 to 80°C).
Quantity of material loaded: 250 g per batch.
Quantity of solvent loaded: 950 ml per batch.
Number of cycles required: Approximately 50 to 55 cycles.
Time required for completion: Approximately 72 to 75 h.
Temperature maintained: 60 to 80°C.

**Methanolic extraction**

Volume of soxhlet extractor: 1000 ml.
Solvent used: chloroform.
Quantity of material loaded: 250 g per batch.
Quantity of solvent loaded: 900 ml per batch.
Number of cycles required: Approximately 50 to 55 cycles.
Time required for completion: Approximately 72 to 75 h.
Temperature maintained: 60 to 80°C.

Optimized parameters for maceration procedure

Maceration of both the flowers and stem bark of *S. campanulata* was (optimized) carried out using petroleum ether and methanol as solvents and at two temperature conditions of room temperature and at cold for 70 to 72 h.

For optimization of extraction processes, that is, for optimization of soxhlet extraction process and maceration process, the aforementioned extraction processes, respectively, were repeated twice (total number of 3 times) in both the cases.

Preliminary phytochemical screening

Preliminary phytochemical screening of flowers and bark extracts was carried out by using standard tests laid down in official books (Tables 2 and 3).

### Table 2. Preliminary phytochemical screening of flowers of *S. campanulata.*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Steroid</th>
<th>Carbohydrate</th>
<th>Gum</th>
<th>Protein</th>
<th>Tannins</th>
<th>Glycoside</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 3. Preliminary phytochemical screening of bark of *S. campanulata.*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Steroid</th>
<th>Carbohydrates</th>
<th>Gum</th>
<th>Protein</th>
<th>Tannins</th>
<th>Glycoside</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Chromatographic technique used for separation and isolation of phytoconstituents (Thin Layer Chromatography)

**Steps involved in performing TLC of extracts**

Silica gel 60 F254 TLC precoated plates supported with aluminium were used.

**Activation of TLC plate**

TLC plate was activated by heating in oven for 30 min at 105°C.

**Sample application**

Dipping the capillary into the solution to be mixed and applied the sample by capillary touched to the thin layer plate at a point about 2 cm from the bottom. Spot was air dried.

**Chamber saturation**

The glass chamber for TLC was saturated for 30 min with mobile phase. Mobile phase was poured into the chamber and capped with lid.

**Chromatogram development**

After the saturation of chamber and samples loaded on plate and kept in chamber. The solvent level in the bottom of the chamber must not be above the spot, as the spotted material will dissolve in the pool of the solvent instead of migration on chromatographic plate. Chromatographic plate was allowed to develop to about 12 to 15 cm.

**Visualization**

Plates were removed and examined using visualizing agent (Vanillin + H2SO4, Anisaldehyde + H2SO4 solution) after which *R*<sub>f</sub> value was calculated using following formula:

\[
R_f \text{ Value} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}
\]
Column chromatography

The obtained extracts were subjected to analysis by column chromatography. The phytoconstituents were thus separated by subsequent elution using the optimized parameters of mobile phase and other factors from the data obtained through optimization of mobile phase in TLC study. The columns were prepared from borosilicate glass using standard parameters. The obtained extracts from large columns were further purified using columns of small dimensions, that is, 10 cm in length and 1 cm in diameter.

Experimental

- Height of column: 45 cm.
- Height of silica gel in column: 30 cm.
- Stationary phase: Silica gel for column chromatography (# 230-240, Merck).
- Elution type: isocratic.
- Fraction quantity: 20 ml.
- Sample preparation: Ratio of sample to Silica gel (1:8).
- Number of fractions collected: 30.
- Detection: Under UV (365 nm) light and by anisaldehyde-H\textsubscript{2}SO\textsubscript{4} solution.

RESULTS AND DISCUSSION

When literature survey of the plant was examined, it revealed that many scientists worked on isolation and separation of the phytoconstituents present in leaves, bark and flowers of plant some of which includes: Vinayak et al. (2009) and Gouda (2009) conducted the research on flowers of the S. campanulata plant in terms of phytochemical investigations, where they reported Spathoside as one of the important phytoconstituent present in the flowers of the plant in their research results. They also further studied the plant flowers for their anti-solar potential in the same year and reported the pharmacological/medicinal use, that is, anti-solar potential of phytoconstituent (Spathoside) obtained from flowers of the plant.

Researchers at Assiut University, Egypt conducted a research on leaves of the S. campanulata plant in terms of phytochemical investigations in year 2009 where they reported iridoids (that is, 6-O-trans-caffeoyl-decinnamoyl globularimin, 6-O-trans-caffeoyl-asystasioside E and 6-O-trans-caffeoyl-5,7-bisdexoxy cynanchoside) as important phytoconstituents of the plant their research results. They also added the method of extraction of iridoids (that is, 6-O-trans-caffeoyl-decinnamoyl globularimin, 6-O-trans-caffeoyl-asystasioside E and 6-O-trans-caffeoyl-5,7-bisdexoxy cynanchoside) from flowers of the plant.

Mbosso et al. (2008) conducted the research on flowers of the plant S. campanulata in terms of phytochemical investigations, where they reported spathadol as one of the important phytoconstituent present in the stem of the plant in their research results.

They also further studied the stem of the plant for its anti-bacterial potential in the same year and reported the pharmacological/medicinal use, that is, the anti-bacterial potential of phytoconstituents obtained from the stem of the plant; but the literature review also showed that very few work has been done on the flowers of this plant and no work has been reported on the optimization process of the phytoconstituents from the flower of the plant. Hence, this work could contribute to a better valorization of this medicinal plant.

In the present study, to explore the field of optimization of parameters for better extraction and isolation methodology, shade dried flowers of the plant S. campanulata P. Beauv belonging to family Bignoniaceae, having bioactive phytoconstituents were studied with special emphasis on the optimization process for the extraction of phytoconstituents from these flowers by various methods and techniques along with the phytochemical investigations of the plant. The dried flowers and bark powder were subjected to preliminary phytochemical analysis using various tests and methods to indicate presence/absence of bioactive phytoconstituents. The preliminary phytochemical investigation revealed the presence of tannins, alkaloids and glycosides in the flower part of the plant and tannins, alkaloids, glycosides and flavonoids in the bark part of the plant. Hence, to extract these phytoconstituents present in the plant, soxhlet extraction and maceration techniques were utilized. Soxhlet extraction (continuous hot extraction) was carried out for flower part of the plant using solvents of increasing polarity, namely, petroleum ether, chloroform and methanol. Various parameters were taken into account and studied during soxhlet extraction procedure of the phytoconstituents from the flower of the plant. The soxhlet extraction procedure for the extraction of phytoconstituents was repeated twice using selected parameters (a total of three times) for the optimization of the procedure for extraction of phytoconstituents using two solvents, such as, petroleum ether and methanol. Maceration technique was also used and the maceration was carried out at two temperature conditions as maceration at room temperature and maceration at cold. Various parameters for maceration technique were also taken into account and studied for the optimization of the extraction procedure of phytoconstituents using the same two solvents, such as, petroleum ether and methanol. Thus, the technique of soxhlet extraction and maceration and its various parameters used for the extraction of phytoconstituents from the flower of the plant S. campanulata using solvents petroleum ether and methanol were optimized. All the obtained extracts, such as petroleum ether extracts (soxhlet and maceration), methanolic extracts (soxhlet and maceration), etc., were subjected to further phytochemical investigations.

Petroleum ether extracts were further subjected to purification by column chromatography. The isolated compound was subjected to qualitative GC-MS/DOSY-NMR analysis (Table 4) and from its spectrum, peaks
components were identified as hexadecanoic acid, methyl ester (13.42%), tricosane (9.64%), n-Heneicosane (13.57%), bis (2-ethylhexyl) phthalate (22.50%), oleic acid (19.58%), 1, 2-Benzenedicarboxylic acid, diisooctyl ester (14.71%), levodopa (18.49%). Methanolic extracts were also subjected to column chromatography for isolation of compounds followed by qualitative analysis by GC-MS/DOSY-NMR (Table 4) and from its spectrum peaks components were identified as 4,8-Methanoazulen-9-ol, decahydro-2,24,8-tetramethyl-, stereoisomer (77.31%) and thujopsene (22.69%).

In a nutshell, in the present study, procedure for the extraction (soxhlet and maceration) of phytoconstituents from flowers of the plant S. campanulata using two solvents such as petroleum ether and methanol was optimized along with the phytochemical investigations of the plant involving isolation and characterization of phytoconstituents from flowers of the plant.

REFERENCES


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<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytoconstituent</th>
<th>Identification</th>
<th>Pharmacological activity/use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether extract</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
<tr>
<td>2</td>
<td>Petroleum ether extract</td>
<td>Tricosane</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether extract</td>
<td>n- Heneicosane</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
<tr>
<td>4</td>
<td>Petroleum ether extract</td>
<td>Bis (2-ethylhexyl) phthalate</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
<tr>
<td>5</td>
<td>Petroleum ether extract</td>
<td>Oleic acid</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
<tr>
<td>6</td>
<td>Petroleum ether extract</td>
<td>1,2-Benzenedicarboxylic acid, disoocyt ester</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
<tr>
<td>7</td>
<td>Petroleum ether extract</td>
<td>Levodopa</td>
<td>GC-MS/DOSY-NMR</td>
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<tr>
<td>1</td>
<td>Methanolic extract</td>
<td>4,8-Methanoazulen-9-ol, decahydro-2,24,8-tetramethyl-, stereoisomer</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
<tr>
<td>2</td>
<td>Methanolic extract</td>
<td>Thujopsene</td>
<td>GC-MS/DOSY-NMR</td>
</tr>
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