QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF PHYTOCONSTITUENTS FROM FRUITS OF STEREOSPERMUM COLAIS (BUCH.-HAM. EX DILLWYN) MABB

MD. Imran 1*, Mohib Khan 2
1 Department of Pharmaceutical Sciences, JJT University, (Rajasthan) India.
2 Oriental college of Pharmacy, Sanpada, New Mumbai, (MS) India.

ABSTRACT
In qualitative analysis, the phytochemical compounds such as steroids, carbohydrates, triterpenoids, coumarines, phenolic compounds, flavonoids, saponins, anthroquinones, Proteins and lipids were screened in fruit of S. colais. Quantitative analysis of phenols, flavonoids and Triterpenoids was further performed. Thin layer chromatography was used to analyze the active Phytoconstituents. Preliminary phytochemical screening of fruit methanolic extracts identified 11 major functional groups, amongst them flavonoid, phytosterols, saponins and coumarins. Additionally total ash analysis of identified three inorganic elements, iron, chloride and sulphate. The same extract was used for quantitative determination of total phenolic content (72.5 mg/g), total flavonoid content (70.53 mg/g) and total triterpenoids content (12.68 mg/g). For further purification of identified chemical constituents, TLC was performed and it showed remarkable results. The methanolic extract of S. colais fruit contains beta sitesterol and lupeol which are very useful therapeutically.

Keywords
Phytochemical, Fruit of Stereospermum Colais, Qualitative and Quantitative Screening.

Corresponding author
MD. Imran
Department of Pharmaceutical Sciences, JJT University, (Rajasthan) India.
Mobile: 09970929176;
imransa99@rediffmail.com

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INTRODUCTION
Plants are furnished with various phytochemical molecules such as terpenoids, phenolic acids, vitamins, lignins, stilbenes, tannins, amines, betalains, flavonoids, quinones, coumarins, alkaloids, and other metabolites, which are rich in antioxidant activity. Studies have revealed that most of these antioxidant compounds have anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities. The treatment with natural antioxidants has been connected with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases related to age. In current years, there has been a worldwide movement towards the exercise of the natural phytochemicals present in oilseeds, beans, fruits berry crops, teas, herbs, and vegetables. In current years, phytochemicals (secondary metabolites) with unknown pharmacological activities have been comprehensively studied as a source of therapeutic agents.

Since there is a hurdle in use of traditional medicines worldwide due to lack of quality and quantity safety and efficacy information on traditional medicines. The lack of research data are not only due to lack of methodologies for the evaluation of herbal drugs but also due to health policies. The plant contains lots of active chemical and therapeutically constituents. Hence in modern systems of medicine it important to study quality control of herbal medicines for their active chemical constituents. To satisfy new thrust of inquisitiveness, standardization of herbal medicine is compulsory. Stereospermum colais has many medicinally active compounds in it, earlier studies which deals with qualitative and quantitative analysis has been done on the root and leaf part of the plant, since fruit part is also medicinally active part but not a single study has been performed on fruit part of the plant, hence present study deals with the qualitative and quantitative analysis of the fruit part of the plant.

MATERIALS AND METHODS
Plant material
Stereospermum colais plant was collected from the Patnur ghat, Nanded district (MS), India in the month of July. The plant was identified by Dr. Arvind S Dhabe, Department of botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS), India.

Preparation of crude extract
The fruits were air dried for one week and powdered by using a big metallic mortar and pestle followed by domestic mechanical grinder. The powder was passed through sieve No 40 and stored in air tight container for extraction. 200gm of powdered material was evenly packed in the Soxhlet apparatus and then extracted successively with various solvents like petroleum ether, chloroform and methanol.

Qualitative phytochemical analysis
Qualitative phytochemical analysis of methanol extracts of fruits was conducted following standard procedures. Elemental analysis of Ash for detection of inorganic elements (Calcium, Iron, Magnesium, Potassium, Sulphate, phosphate, chloride, carbonate and Nitrate) was performed by specific tests.

Quantitative phytochemical analysis
The phytochemicals are present in the methanol extract of fruits of S. colais were determined and quantified by standard procedures.

Determination of total phenolic content
To determine total phenolic content from the methanolic extract of fruit of Stereospermum colais, calibration curve of standard Gallic acid of 20, 40, 60, 80 and 100 mg/ml was prepared in water and 1 mg/ml of methanolic extract of fruit Stereospermum colais was prepared simultaneously. Each sample was mixed with 0.25 ml of Folin ciocalteu reagent and 1.25 ml sodium carbonate solution. The mixtures were allowed to react for 40 minutes at room temperature. After the reaction period the blue color was measured at 725 nm on UV-visible spectrophotometer of LABINDIA 3000+ and calculated the amount of total phenolic content from calibration curve as Gallic acid.

Determination of total flavonoid content
An aliquot (1 ml) of standard solution of quercetin (20, 40, 60, 80 and 100 μg/ml) was added to 10 ml volumetric flask containing 4 ml of 5% NaNO2 into it. After 5 minute 0.3 ml of 10% AlCl3 was added. Then 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Same dilutions were also prepared for the test solution. Blank determination was done by using methanol in place of test or standard solutions. Mixed well and taken the absorbance at 358 nm against blank. From the obtained standard curve of quercetin the total flavonoids content of methanolic extract of fruit of Stereospermum colais was determined.

Determination of total Triterpenoids
5 g of powder extracted with 50 ml distilled water by heating on water bath for 30 min. then the extract was allow to cool and then filter. 75 ml chloroform and diethyl ether was added in 1:2 concentrations by continuous stirring for 30 min. after 5 gm of sodium carboxyl methyl cellulose was added to forms lumps and sticky mass and then separated. Further marc subjected to extraction with 75 ml chloroform: diethyl ether (1:2) for four times. The obtained residue was dissolved in 50 ml of neutral absolute alcohol. Then the
mixture were titrated with 0.1 N NaOH using phenolphthalein as an indicator. Similarly blank readings were taken without addition of sample. Percentage of Triterpenoids content was calculated as per the given factor. Factor for the calculation: each ml of 0.1N NaOH = 48.8 mg of Triterpenoids.

Thin layer chromatography
For thin layer chromatography analysis, the method used was taken from Quality standards of Indian medicinal plants ICMR (volume 4, 2006) for β-Sitosterol. Improvements were made to the sample preparation and the mobile phase used in the method.

Sample preparation
0.2g of fruit methanolic extracts were diluted with 10ml methanol. The methanol extract was then partitioned with petroleum ether (PE) so that the steroids separate in the petroleum ether layer and all the other polar components remain in the methanol layer. The petroleum ether layer was further used for TLC.

Preparation of Standards
1. 10 mg of β-Sitosterol was dissolved in 10 mL of methanol.
2. 10 mg of lupeol was dissolved in 10 mL of methanol.

Preparation of reagent
Anisaldehyde-sulfuric acid reagent was prepared by slowly adding 9 mL of 98% H₂SO₄ to an ice cooled mixture of 85 mL of methanol and 10 mL of glacial acetic acid. To this solution 0.5 mL of anisaldehyde was added and mixed well.

Chromatographic conditions
Stationary phase: Silica gel 60F₂₅₄ pre-coated TLC plate (Merck)
Mobile phase: Toluene: Ethyl Acetate (80: 20)
Spraying reagent: Anisaldehyde-sulfuric acid reagent.

RESULTS AND DISCUSSION
Phytochemical investigation was carried out previously on leaves of S colais. which showed the presence of bioactive compounds like Cardiac glycosides, Flavonoid, Quinones, Terpenoids, Alkaloids and Steroids and inorganic elements like magnesium, iron, sulphate, phosphate, chloride and fluoride were reported in leaves part. While fruit part shows the presence of carbohydrate, protein, saponin, coumarin and flavonoid and inorganic elements like iron, sulphate and chloride. Hence fruit part can also be used as source of medicine for treatment of many diseases.

Qualitative phytochemical analysis
Extraction of powdered fruits of S colais was carried out by using various solvents of increasing polarity and then the methanolic extract was subjected to preliminary phytochemical screening for the identification of active major functional gro

Table 01: Showing results of phytochemical study and detection of inorganic elements.

<table>
<thead>
<tr>
<th>Phytochemical analysis</th>
<th>Test for inorganic elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr. No.</td>
<td>Test</td>
</tr>
<tr>
<td>1</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>2</td>
<td>Protein</td>
</tr>
<tr>
<td>3</td>
<td>Glycoside</td>
</tr>
<tr>
<td>4</td>
<td>Saponin</td>
</tr>
<tr>
<td>5</td>
<td>Coumarin</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>7</td>
<td>Anthraquinone glycoside</td>
</tr>
<tr>
<td>8</td>
<td>Phytosterol</td>
</tr>
<tr>
<td>9</td>
<td>Phenol</td>
</tr>
<tr>
<td>10</td>
<td>Alkaloids</td>
</tr>
<tr>
<td>11</td>
<td>Lipid</td>
</tr>
</tbody>
</table>

Quantitative phytochemical analysis
The results of quantitative analysis are presented in Table 02.
Table 02: Quantitative analysis of phytochemicals (mg/g).

<table>
<thead>
<tr>
<th></th>
<th>Total flavonoids</th>
<th>Total phenols</th>
<th>Total Triterpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Stereospermum colias Fruit</em></td>
<td>72.5*</td>
<td>70.53*</td>
<td>12.68*</td>
</tr>
</tbody>
</table>

* Mean of six determinations

**Thin layer chromatography**

For the purification of identified active phytoconstituents, TLC was performed by using two different standard samples i.e. beta sitosterol and lupeol which showed presence of the same.

Following application of the anisaldehyde-sulfuric acid reagent, this band appears as violet color in visible light.
CONCLUSIONS

The fruit of *Stereospermum colais* screened for phytochemical constituents seemed to have the potential to act as a source of useful medicines and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Hence, *Stereospermum colais* fruits can be utilized in herbal drug formulation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS’ CONTRIBUTIONS

This work was carried out in collaboration between all authors. MD. Imran designed & performed the study, wrote the protocol, and wrote the first draft of the Manuscript. Mohib Khan managed the analyses of the study. MD. Imran managed the literature searches. All authors read and approved the final manuscript.

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