Original Article

Qualitative and quantitative characterization of phytoconstituents from stem bark of *Stereospermum colais* Buch

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ABSTRACT

Upon the qualitative analysis work on *Stereospermum colais* stem bark belongs to family Bignoniaceae, the phytochemical constituents such as steroids, carbohydrates, triterpenoids, phenolic compounds, coumarins, flavonoids, saponins, anthraquinones, proteins, and lipids were identified in stem bark of *S. colais*. Quantitative analysis of phenols, flavonoids, and triterpenoids was further performed. The active phytoconstituent was analyzed using thin-layer chromatography (TLC) and preliminary phytochemical screening of stem bark; from the methanolic extracts of stem bark showed various phytoconstituents such as flavonoids, phytosterols, saponins, and coumarins. Some important inorganic elements such as iron, chloride, potassium, phosphate, nitrate, and sulfate were identified by total ash analysis additionally. The quantitative determination of total phenolic content (72.5 mg/g), total flavonoid content (70.53 mg/g), and total triterpenoid content (12.68 mg/g) was performed using the same extract. In addition, for further purification of identified chemical constituents, TLC was performed and it showed remarkable results. The methanolic extract of *S. colais* stem bark contains beta-sitosterol and lupeol which are very useful therapeutically mostly to treat diabetes and heart diseases.

Keywords: Phytochemical, phytoconstituents, qualitative and quantitative screening, stem bark of *Stereospermum colais*

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Introduction

The plant have various phytochemical constituents such as phenolic acids, terpenoids, lignins, stilbenes, tannins, vitamins, amines, betalains, alkaloids, flavonoids, quinones and coumarins, and other metabolites, which are rich in antioxidant activity.[1,2] Studies have manifested that a lot of these antioxidant compounds have anticarcinogenic, antimutagenic, anti-viral activity, anti-inflammatory, antiatherosclerotic, antitumor, and antibacterial activities.[3,4] Treatment with natural antioxidants has beneficial effects such as reduced risks of cancer, cardiovascular disease, diabetes, and other diseases. The natural phytochemicals presentare present in stem barks, fruits, oilseeds, beans, berry crops, tea, herbs, and vegetables.[5,6] In a going years, phytochemicals (secondary metabolites) having unknown pharmacological activities. These have been generally studied as a source of therapeutic agents.[7]

There is an obstruction in the use of traditional medicines worldwide due to imperfection of quality and quantity and safety and efficacy information on traditional medicines. The imperfection of research data is not only due to lack of methodologies for the evaluation of herbal medicines but also due to health policies.[8] The plant contains a number of active chemical and therapeutically active constituents. Therefore, in modern systems of medicine, it is very important to study the quality control of herbal medicines for their active chemical constituents to satisfy new belief of curious, compulsion of standardization of herbal medicine.[9,10] *Stereospermum colais* has a wide range of medicinally active compounds in it, earlier studies which deal with qualitative and quantitative analysis have been done on the root, fruit, and leaf parts of the *S. colais* plant.

Since stem bark part is also medicinally active part, but not a single study has been performed on stem bark part of the *S. colais* plant,

Access this article online

Website: http://www.jpbs-online.com
DOI: 10.31555/jpbs/2017/5/2/20-22
E-ISSN: 2321-0125

How to cite this article: Sonawane Y, Imran M, Akhtar R, Ahmed S. Qualitative and quantitative characterization of phytoconstituents from stem bark of *Stereospermum colais* Buch. J Pharm BioSci 2017;5(2):20-22.

Source of Support: Nil, **Conflict of Interest:** None declared.

therefore the present study deals with the qualitative and quantitative analysis of the stem bark part of the *S. colais* plant.

Materials and Methods

Plant material

S. colais plant was identified by Dr. Arvind S Dhabe, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (Maharashtra), India. *S. colais* plant was collected from Patnurghat, Nanded district (Maharashtra), India, in July.

Preparation method of crude extract

For the preparation of extract, stem bark was air dried for 1 week and powdered using a big metallic mortar and pestle followed by domestic mechanical grinder, and then the powder was passed through sieve No. 40 and stored in an air-tight container for extraction.[10] A composition of 200 g of powdered material was packed in the Soxhlet apparatus and then extracted successively with various solvents such as petroleum ether, chloroform, and methanol.[11,12]

Qualitative method for phytochemical analysis

For the qualitative phytochemical analysis of methanol extracts of stem bark, they were prepared using standard procedures,[13] and also elemental analysis of ash of stem bark for the detection of inorganic elements such as magnesium, calcium, potassium, iron, sulfate, phosphate, chloride, carbonate, and nitrate was performed using specific tests.[14]

Quantitative methods for phytochemical analysis

For the quantitative analysis of phytochemicals present in the methanol extract of stem bark of *S. colais,* they were determined and quantified by standard procedures.[6-12,14-17]

Determination method for total phenolic content

For the determination of total phenolic content from the methanolic extract of stem bark of *S. 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 stem bark *S. colais* was prepared simultaneously. Each sample was mixed with 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml of sodium carbonate solution. The mixtures were allowed to react for 40 min at room temperature. After the above reaction, mixture produces blue color which was measured at 725 nm on ultraviolet-visible spectrophotometer of LABINDIA 3000+ and calculated the amount of total phenolic content from calibration curve of standard gallic acid.[9-12,14,15]

Determination method for total flavonoid content

For the determination of total flavonoid, a liquid (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% $NaNO_2$ into it. Then, after 5 min, 0.3 ml of 10% $AlCl_3$ and 2 ml of 1 M NaOH were added and the total volume was made up to 10 ml with distilled water. The same dilutions were also prepared for the test solution. Blank determination was done using methanol in the place of test or standard solutions. The mixture was mixed well and the absorbance was taken at 358 nm against blank. From the obtained standard curve of quercetin, the total flavonoid content of methanolic extract of fruit of *S. colais* was determined.[9-12,14,15]

Determination method for total triterpenoids

For the determination of total triterpenoids, 5 g of powder were extracted with 50 ml of distilled water by heating on a water bath for 30 min. Then, the extract was allowed to cool at room temperature and then it was filtered. Exactly 75 ml of chloroform and diethyl ether were added as 1:2 concentrations by continuous stirring for 30 min. Then, 5 g of sodium carboxyl methyl cellulose was added to form lumps and sticky mass and then it was separated. Further, it is subjected to extraction with 75 ml chloroform:diethyl ether (1:2) for 4 times. The resultant residue was dissolved in 50 ml of neutral absolute alcohol. Then, the mixture was titrated with 0.1 N NaOH using phenolphthalein as an indicator. Similarly, blank readings were taken without addition of sample. Percentage of triterpenoid content was calculated as per the given factor. Factor for the calculation was as follows: Each ml of 0.1 N NaOH = 48.8 mg of triterpenoids.[9-12,14,15]

Thin-layer chromatography (TLC)

For performing TLC analysis for β-sitosterol, the method used for TLC profiles was taken from quality standards of Indian medicinal plants (volume 4, 2006).[16] Improvements were made to the sample preparation and the mobile phase used in the method.

Sample preparation

Accurately weighed 0.2 g of stem bark methanolic extracts was diluted with 10 ml methanol. This methanol extract was partitioned with petroleum ether that leads to steroids' separation in the petroleum ether layer and all the other polar components remain in the methanol layer. Then, petroleum ether layer was further used for TLC.

Preparation of standards

Accurately weighed 10 mg of β-sitosterol was dissolved in 10 ml of methanol. And, 10 mg of lupeol was dissolved in 10 ml of methanol.

Preparation of reagent

The required reagent such as anisaldehyde-sulfuric acid reagent was prepared by slowly adding 9 ml of 98% H_2SO_4 to an ice-cooled mixture of 85 ml of methanol and 10 ml of glacial acetic acid. To the above solution, 0.5 ml of anisaldehyde was added and mixed well.

Chromatographic conditions

- Stationary phase: Silica gel $60F_{254}$ pre-coated TLC plate (Merck)
- Mobile phase: Toluene: ethyl acetate (80:20)
- Spraying reagent: Anisaldehyde-sulfuric acid reagent.

Results and Discussion

The qualitative and quantitative phytochemical investigation was carried out previously on stem bark of *S. colais*[17] which showed the presence of various bioactive compounds such as cardioglycosides, flavonoids, quinones, terpenoids, alkaloids, and steroids, and inorganic elements such as magnesium, iron, sulfate, phosphate, chloride, and fluoride were reported in leaf and fruit parts. While stem bark part shows the presence of carbohydrate, protein, saponin, coumarin, and flavonoid, and inorganic elements such as iron, sulfate, and chloride. Hence, stem bark part can also be used as the source of medicine for the treatment of many diseases.

Qualitative phytochemical analysis

The various solvents of increasing polarity were used for the extraction of powdered stem bark of *S. colais*. Then, methanolic extract was used for preliminary phytochemical investigation for the identification of active major functional group, and ash of powdered stem bark was used for the identification of inorganic element; this shows the medicinal important of stem bark part. In addition, the ash of powdered stem bark was utilized for the detection of inorganic elements, which shows the medicinal importance of stem bark part of the plant. The results are shown in Table 1.

Quantitative phytochemical analysis

The results of quantitative analysis are shown in Table 2.

TLC

TLC was performed for purification and identification of active constitutes using two different standard samples, i.e., β-sitosterol and lupeol which showed the presence of active constituents to be separated. Following application of the anisaldehyde-sulfuric acid reagent, this band appears as violet in visible light.

Table 1: Results of phytochemical study and detection of inorganic elements

Phytochemical analysis		Test for inorganic elements	
Test	**Inference**	**Test**	**Inference**
Carbohydrate	+	Calcium	−
Protein	+	Iron	+
Glycoside	−	Magnesium	−
Saponin	+	Potassium	−
Coumarin	+	Sulfate	+
Flavonoid	+	Phosphate	−
Anthraquinone glycoside	−	Chloride	+
Phytosterol	−	Carbonate	−
Phenol	−	Nitrate	−
Alkaloids	−	-	-
Lipid	+	-	-

Table 2: Quantitative analysis of phytochemicals (mg/g)

Plant material	Total flavonoids	Total phenols	Total triterpenoids
S. colais stem bark	72.5*	70.53*	12.68*

*Mean of six determinations. *S. colais*: *Stereospermum colais*

Conclusion

The stem bark of *S. colais* investigated for phytochemical constituents seems to have the potential to act as a source of useful medicines and also to enhance the health status of the consumers due to the presence of various compounds they are plays vital role for good health. Hence, *S. colais* stem bark can be used in herbal drug formulation.

Acknowledgments

The authors thank KBHSST'S Institute of Pharmacy Malegaon for providing facilities to conduct this interested research work.

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