

## Quantitative and Qualitative Estimations of Phytoconstituents from Root of *Stereospermum colais* Buch.

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### ABSTRACT

The plant chemistry is based on the study of many therapeutically active chemical constituents associated with many inert substances like cellulose, coloring agents, and lignin etc. The main active constituents are extracted from plants and standardized for its chemical and biological efficacy. The main objective of present study was to find out the active principles from the root of *Stereospermum colais* Buch. Hence for this study air dried powdered root was utilized for extraction purpose. After extraction, different extracts were subjected for preliminary phytochemical screening for the identification of active functional groups. And it showed presence of many active phytoconstituents like phytosterols, proteins, Saponins, Lipids and Flavonoids. Further powdered drug was used for the detection of inorganic elements from the ash of the powdered drug and it showed presence of many inorganic elements like iron, chloride and nitrates. For quantitative estimations total phenolic content, total flavonoid content and total triterpenoids content was determined. Then for the purification of identified active phytoconstituents, TLC was performed by using two different standard samples i.e. beta sitosterol and lupiol. It showed significant result which is given in the figure.

**Keywords:** *Stereospermum colais* root, Qualitative estimation, Quantitative estimation

### INTRODUCTION

Herbal medicines and their preparations are widely used in many developing and developed countries because of natural origin and lesser side effect or dissatisfaction with result of synthetic drugs. However one of most important characteristic of herbal preparation either in single drug or in collection of many drugs is extracted with boiling water during decoction process. This could be the main reason why quality control of herbal drug is more difficult than synthetic drugs. As it is mentioned in "general guidelines for methodologies" on research and evaluation of traditional medicines<sup>1</sup>. The quality and quantity of safety and efficacy information on traditional medicines are not sufficient to meet the criteria to support its use world wide. The reason behind lack of research data are not only due to health policies but also due to lack of methodologies for the evaluation of herbal medicines.<sup>2</sup>

The plants possess many active therapeutically active chemical constituents associated with many inert substances such as cellulose, lignin and coloring agents etc. The active constituents are extracted from plants and purified for their pharmacological utility. So the quality control of herbal drugs is important for their active chemical constituents in modern system of medicine. To meet new thrust of inquisitiveness, standardization of herbal drug is mandatory.<sup>3-7</sup>

### MATERIALS AND METHODS

**Plant material:** The plant specimens for the proposed study were collected from the Pathnur ghat (Taluka Ardhapur, Dist. Nanded, M.S.) and authenticated by Dr. Arvin S. Dhake, Associate professor, Department of Botany, Dr. Babsaheb Ambedkar Marathwada University, Aurangabad, M.S.

About 3kg roots from the ground were uprooted by digging since the selected plant is a large tree. The roots were air dried and powdered by using a big metallic mortar and pestle followed by mechanical grinding. The powdered drug was then stored at room temperature and used for the powder microscopy and extraction purpose.

**Preparation of crude drug for extraction<sup>8-11</sup>:** The entire plant material was dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve No 40 and stored in air tight container for extraction. The collected, cleaned and powdered root of *stereospermum colais* Buch. was used for the extraction purpose. 200gm of powdered material was evenly packed in the Soxhlet apparatus. It was then extracted successively with various solvents of increasing polarity.

**Qualitative Estimations**

**Preliminary Phytochemical screening of extracts<sup>12,13</sup>:** The above extracts obtained from the root were subjected for the various chemical test for the identification of active phytoconstituents groups by following standard procedure. Elemental analysis of Ash for detection of inorganic elements<sup>14</sup>: The powdered drug was incinerated in muffle

Table 1: Showing results of extraction of root of *stereospermum colais*

Sr. No.	Solvents	Estimated wieghts in gm
1	Petroleum ether(60-80°C)	3.38
2	Chloroform	2.64
3	Methanol	18.68

Table 2: Showing results of phytochemical study and detection of inorganic elements.

Test for organic elements			Test for inorganic elemnts	
Sr.no	Test	Inference	Test	Inference
1	Carbohydrate	Negative	Calcium	Negative
2	Protein	Positive	Iron	Positive
3	Glycoside	Negative	Magnesium	Negative
4	Saponin	Positive	Potassium	Negative
5	Coumarin	Negative	Sulphate	Negative
6	Flavanoid	Positive	Phosphate	Negative
7	Anthraquinone glycoside	Negative	Choride	Positive
8	Phytosterol	Positive	Carbonate	Negative
9	Phenol	Negative	Nitrate	Positive
10	Alkaloids	Negative	----	----
11	Lipid	Positive	----	----

Table 3: showing result of Total flavonoid content

Sr. No.	Standard (Gallic acid ppm)	Absorbance			Mean
		I	II	III	
1	20	0.053	0.055	0.054	0.054
2	40	0.133	0.132	0.133	0.132
3	60	0.223	0.226	0.224	0.224
4	80	0.309	0.311	0.308	0.309
5	100	0.377	0.374	0.379	0.376
Sample	77.5	0.282	0.282	0.281	0.281

furnace to obtain ash. The ash was treated with 50% hydrochloric acid for 30 minutes and filtered. The filtrate was used for the detection of elements by specific test.

#### Test for Calcium

- Test solution when treated with ammonium carbonate solution gives white precipitate which after boiling and cooling is insoluble in solution of ammonium sulphide.
- Test solution treated with potassium chromate gives yellow crystalline precipitate.

#### Test for Iron

- Test solution when mixed with dilute hydrochloric acid and potassium permanganate shows faint pink coloration.
- Test solution when mixed with dilute hydrochloric acid and solution of ammonium thiocyanate gives blood red coloration.

#### Test for Magnesium

- Test solution shows white precipitation after boiling with ammonium carbonate.
- Test solution, dilute ammonia and sodium phosphate solution produces white crystalline precipitate.

Test for Potassium: Test solution with perchloric acid gives white precipitation.

#### Test for sulphate:

- Test solution and few drops of 5% BaCl<sub>2</sub> solution gives white crystalline BaSO<sub>4</sub> precipitate.

- White lead acetate reagent produces white precipitate with test solution.

Test for phosphate: Test solution prepared in HNO<sub>3</sub> when heated with few drops of ammonium molybdate solution has not shown yellow crystalline precipitate.

#### Test for chloride

- Test solution prepared in HNO<sub>3</sub> when mixed with few drops of 10% AgNO<sub>3</sub> Produces white precipitate.
- Test solution with lead acetate solution gives white precipitate soluble in hot water.

#### Test for carbonate

- Test solution with dilute acid does not liberated carbon dioxide.
- Test solution with mercuric chloride solution does not produce brownish red precipitate.
- Test solution with magnesium sulphate, white precipitate was not formed.

Test for nitrate: Test solution when warmed with sulphuric acid and copper, no red fumes were liberated.

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 -Sitosterol. Improvements were made to the sample preparation and the mobile phase used in the method.

Sample preparation: 0.2g of *Stereospermum colias* root methanolic extract was diluted with 10ml methanol. Steroids are non-polar in nature so the methanol extract of

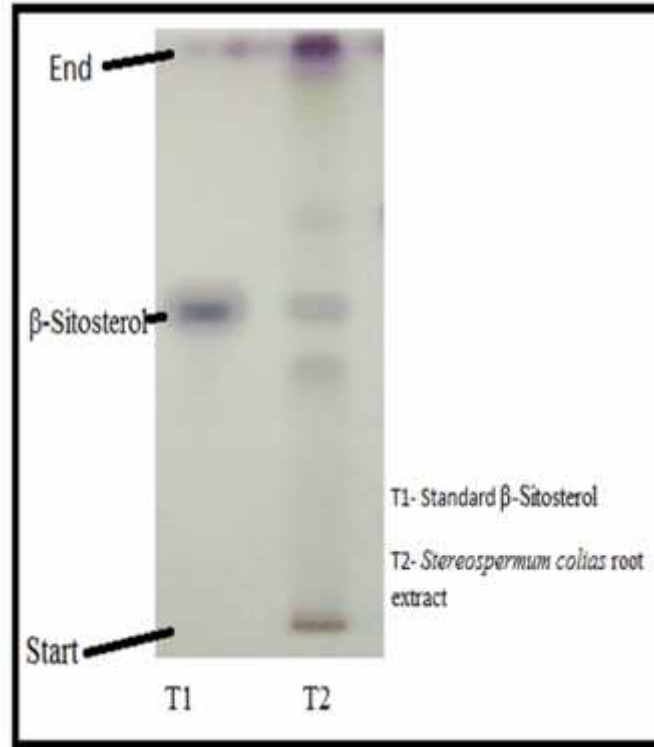


Fig. 1: TLC of powdered root of *Stereospermum colias* Buch compared to  $\beta$ -Sitosterol

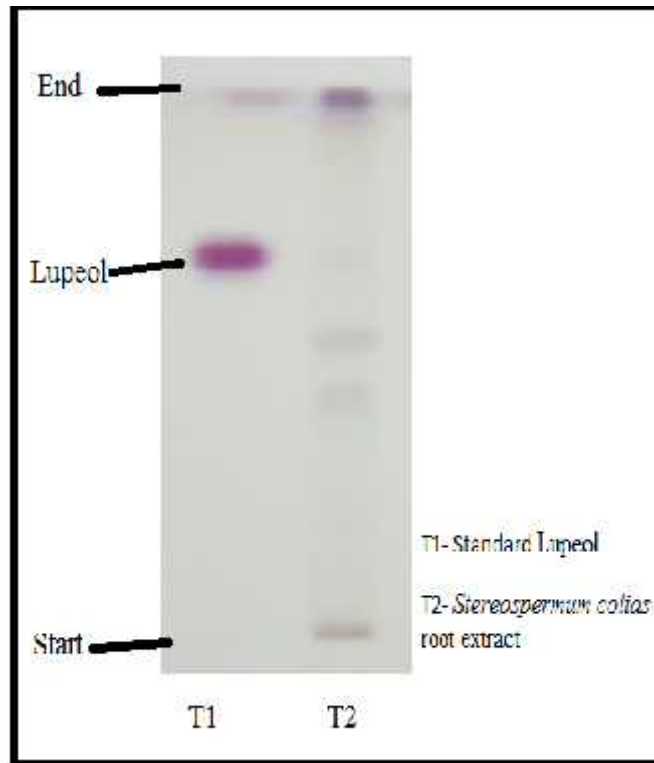


Fig 2: TLC of powdered root of *Stereospermum colias* Buch compared to Lupeol

*Stereospermum colias* root 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.

Standard preparation

1. Dissolve 10 mg of  $\beta$ -Sitosterol (available from Total Herb solution) in 10 mL of methanol.

2. Dissolve 10 mg of lupeol (available from Total Herb solution) in 10 mL of methanol.

Reagent preparation: Prepare anisaldehyde-sulfuric acid reagent by slowly adding 9 mL of 98% sulphuric acid to

Table 4: showing result of Total Phenolic content

Sr. No.	Standard (Gallic acid mg/ml)	Absorbance			Mean
		I	II	III	
1	20	0.275	0.273	0.278	0.275
2	40	0.56	0.56	0.57	0.563
3	60	0.887	0.888	0.884	0.886
4	80	1.188	1.190	1.185	1.187
5	100	1.458	1.460	1.457	1.458
Sample	71.07	1.042	1.042	1.043	1.042

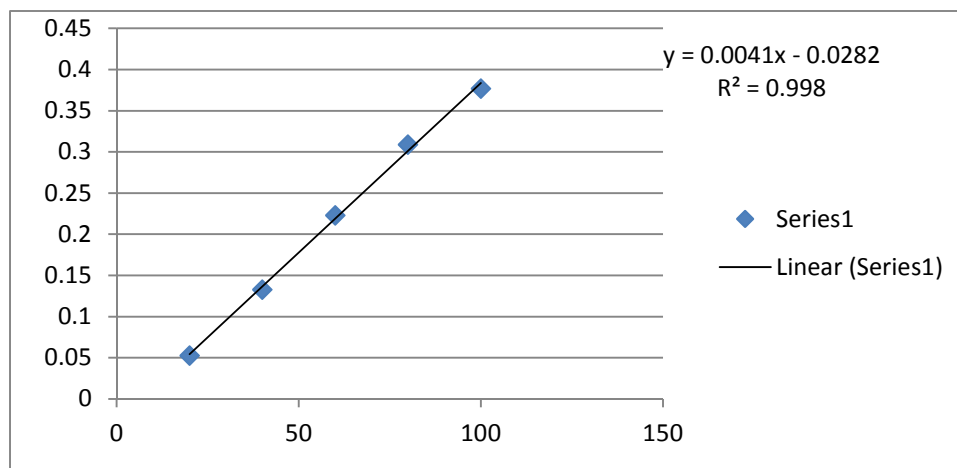


Fig. 3: Calibration curve of standard quercetin

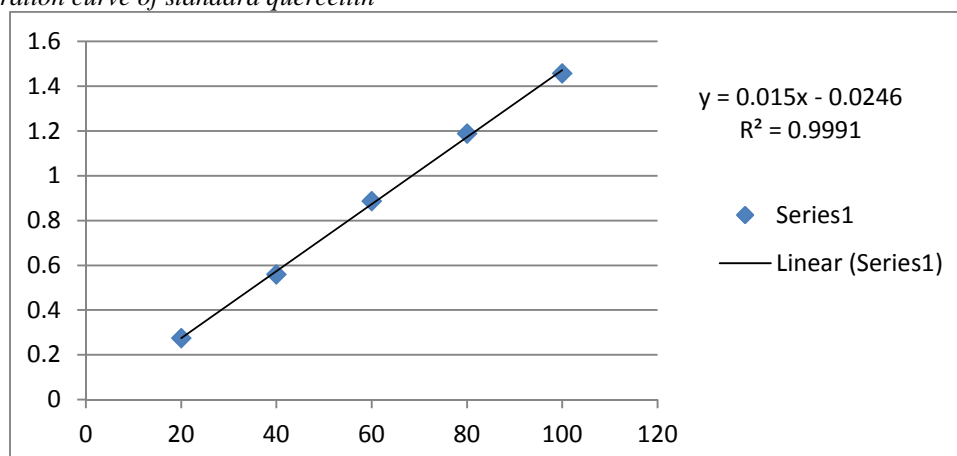


Fig. 4: Calibration curve of standard gallic acid

an ice cooled mixture of 85 mL of methanol and 10 mL of glacial acetic acid. To this solution add 0.5 mL of anisaldehyde and mix well. The anisaldehyde-sulfuric acid reagent is colorless and should be stored in a refrigerator. If a color develops, the reagent must be discarded

#### Chromatographic conditions

Silica gel 60F<sub>254</sub> pre-coated TLC plate (Merck)

Toluene: Ethyl Acetate (80: 20)

Spray plate with the anisaldehyde-sulfuric acid reagent, heat to 105°C for 15 minutes. Inspect plate in visible light.

#### Quantitative Estimations<sup>15,16</sup>

Determination of total phenolic content: To determine total phenolic content from the methanolic extract of root 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 root *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, 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of 5% NaNO<sub>2</sub> into it. After 5 minutes 0.3 ml of 10% AlCl<sub>3</sub> 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

Table 5: showing result of total triterpenoids

Sr. No.	Sample	Burette reading (ml)			Mean B.R.
		I	II	III	
1	Methanolic extract of root of <i>stereospermum colais</i>	0.3	0.3	0.2	0.26

total flavonoids content of methanolic extract of root of *Stereospermum colais* was determined.

Determination of total terpenoids: 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

#### RESULTS AND DISCUSSION

Extraction of plant material: The powdered root of *stereospermum colais* Buch. Was extracted in Soxhlet extracto by using different solvents of increasing polarity. 200gm of drug was used for extraction purpose and the quantity of extracts obtained are given in table number 01. Qualitative estimation: Extraction of powdered root of *stereospermum 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 active major functional groups. And ash of powdered drug was utilized for the detection of inorganic elements. Results for both the studies are given table no 2.

After identification of major active functional groups the drug was subjected for the comparative TLC for further purification purpose by using two different standard compounds namely Sitosterol and Lupeol. It has been observed that the bands in TLC obtained of the test drug have same Rf values as standard. The results for TLC is given in figure number 1 and 2.

**Quantitative estimations:** For quantitative estimations parameters like total flavonoid content, total phenol content and total triterpenoid content have been determined from the root of *stereospermum colais* Buch. It was found to be 7.75%, 7.107% and respectively. Results for quantitative estimation is given in table number 03, 04 and 05 respectively.

#### CALCULATION

Factor for the calculation:  
 each ml of 0.1N NaOH = 48.8 mg of triterpenoids  
 0.26 ml of 0.1N NaOH = X mg of triterpenoid  
 Therefore  $X = 48.8 \times 0.26 \times 0.1 / 0.1$

X=12.68 mg of triperpenoids

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