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PHYTOCHEMICAL PROFILING AND QUANTITATIVE ESTIMATION OF BIOACTIVE CONSTITUENTS OF *SANTALUM ALBUM* EXTRACT

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ABSTRACT
The present study was undertaken to evaluate the phytochemical profile of *Santalum album* extract and to determine its major phytochemical constituents. Plant material was extracted using a hydroalcoholic solvent system, which yielded 5.26 g of extract with a percentage yield of 10.52% w/w. The obtained extract was subjected to preliminary phytochemical screening using standard chemical tests to detect different classes of secondary metabolites. The results demonstrated the presence of several phytoconstituents, with the distribution varying according to the solvent used for extraction. Alkaloids, flavonoids, phenolic compounds, diterpenes, proteins, carbohydrates, saponins and tannins were detected in selected extracts, whereas glycosides and sterols were not detected under the tested conditions. Quantitative estimation of the hydroalcoholic extract showed a total phenolic content of 0.74 mg/100 mg, total flavonoid content of 0.89 mg/100 mg, and total alkaloid content of 0.42 mg/100 mg of dried extract. Among the estimated phytochemical groups, flavonoids were present in the highest amount, followed by phenolic compounds and alkaloids. These findings indicate that *Santalum album* contains a diverse range of phytochemical constituents and provide a preliminary basis for further pharmacological and phytochemical investigation of the plant extract.

KEYWORDS: *Santalum album*, Hydroalcoholic extract, Phytochemical screening, Flavonoids, Phenolic compounds, Alkaloids, Secondary metabolites, Tannins, Saponins, Medicinal plant.

INTRODUCTION
Medicinal plants have been an important source of therapeutic agents since ancient times and continue to play a significant role in traditional and contemporary healthcare systems [1]. The therapeutic potential of medicinal plants is largely attributed to the presence of biologically active secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides and other phytoconstituents [2]. These compounds may contribute to a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, analgesic, antidiabetic and hepatoprotective effects. Therefore, systematic evaluation of the phytochemical composition of medicinal plants is an important preliminary step in identifying plant materials with potential therapeutic applications [3].
Santalum album L., commonly known as Indian sandalwood, is an important medicinal and aromatic plant belonging to the family Santalaceae. The plant has traditionally been valued for its medicinal and aromatic properties, and different parts of the plant have been used in traditional systems of medicine [4]. The biological activities associated with *S. album* are considered to be related to its diverse chemical constituents, including phenolic compounds, flavonoids, terpenoid constituents and other secondary metabolites. However, the qualitative and quantitative composition of plant extracts can vary considerably depending on the plant part, geographical origin, extraction solvent and extraction conditions [5].
Extraction is a crucial stage in phytochemical investigation because the choice of solvent determines the type and quantity of constituents recovered from plant material. Polar and hydroalcoholic solvents are particularly useful for extracting a broad spectrum of phytochemicals because they can solubilize compounds with different physicochemical properties. Preliminary phytochemical screening using specific chemical reactions provides a useful approach for establishing the qualitative profile of an extract, while quantitative estimation of major groups such as phenolics, flavonoids and alkaloids provides additional information regarding their relative abundance [6].
Phenolic compounds and flavonoids are of particular interest because of their reported antioxidant and anti-inflammatory potential. Alkaloids also represent an important group of plant secondary metabolites with diverse biological activities. Determination of these phytochemical groups can therefore provide valuable preliminary information for subsequent pharmacological and phytochemical investigations [7].
Despite the traditional importance of *Santalum album*, systematic evaluation of its extract with respect to extraction yield, solvent-dependent phytochemical distribution and quantitative estimation of major phytochemical groups remains important. Therefore, the present study was designed to prepare a hydroalcoholic extract of *Santalum album*, determine its percentage yield, perform preliminary phytochemical screening of

different solvent extracts, and quantitatively estimate the total phenolic, flavonoid and alkaloid contents of the hydroalcoholic extract. The findings may provide a preliminary scientific basis for further investigation of *S. album* as a potential source of biologically active phytoconstituents.

MATERIAL AND METHODS

MATERIAL

The plant material, *Santalum album* (sandalwood), was used for the preparation and phytochemical evaluation of the extract. Analytical-grade solvents including chloroform, ethyl acetate, ethanol, methanol, distilled water, and hydroalcoholic solvent were used for extraction and phytochemical screening. Reagents required for qualitative phytochemical tests, including Ferric chloride, Hager's reagent, Wagner's reagent, concentrated sulphuric acid, lead acetate, sodium hydroxide, zinc hydrochloride, ninhydrin, Biuret reagent, Fehling's reagent, Benedict's reagent, and gelatin, were of analytical grade. All chemicals and reagents were procured from Merck Life Science Pvt. Ltd., Mumbai, India, unless otherwise specified.

Methods

Collection of plant materials

Leaves of *Santalum album* were collected from Vindhya Herbals, Bhopal (M.P.). The fresh leaves parts of this species were washed under running tap water, shade dried at room temperature and powdered following which they were ground to a fine powder, sieved through a 500-µm sieve, and stored until the extraction.

Extraction by maceration process

Fifty grams (50 g) of dried, coarsely powdered *Santalum album* was subjected to extraction using the maceration method [8]. The powdered material was mixed with a hydroalcoholic solvent consisting of ethanol and water (80:20 v/v) and allowed to macerate for 48 hours with occasional shaking. After maceration, the extract was filtered through Whatman No. 40 filter paper to remove the plant residue. The resulting filtrate was concentrated on a water bath maintained at 80–90°C until most of the solvent was evaporated. The concentrated extract was further dried to obtain a thick, sticky mass, which was collected and stored in a suitable airtight container until further use.

Storage of extract

The concentrated extract was collected in 25 ml McCartney bottles using a clean spatula. The bottles were then tightly stoppered to prevent contamination and moisture absorption and stored in a refrigerator at 4°C until further use.

Biochemical Assays

Preliminary screening of biochemical tests of all three extracts were done for testing various phytochemicals found in plants [9]. The crude extracts were tested for the presence or absence of secondary metabolites such as alkaloids, phenolic compound, flavonoids, saponins, tannins and glycosides. The following biochemical tests have been performed to confirm the presence or absence of the secondary metabolites in the plant extract.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

b) Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2. Detection of Glycoside

a) Conc. H_2SO_4 Test: Extract dissolved in distilled water and treated with few drops of conc. Sulphuric acid. Formation of red color indicates the presence of glycoside.

b) Liebermann's Burchard's Test: To 2ml of extract, 2ml of acetic acid and conc. H_2SO_4 carefully added and cool appearance of brownish green colour indicates presence of glycoside.

c) Raymond's Test: To 2ml of extract, add 0.1ml of 1% m- Dinitrobenzene in ethanol and add 2-3 drop of 20% NaOH, appearance of violet colour indicates the presence of glycoside.

3. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

4. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicate the presence of diterpenes.

5. Detection of phenols

a) Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

b) Zinc hydrochloride test: To the test solution add a pinch of zinc dust and few drops of concentrated hydrochloric acid, after few minutes a yellow or orange color indicates the presence of phenols.

c) Shinoda test: Add few fragments of magnesium ribbon into the test solution and also add concentrated hydrochloric acid in a drop wise set aside for few minutes, yellow or orange color indicates the presence of

phenols.

6. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin test: To the extract solution add 0.2% ninhydrin (indane 1, 2, 3-trione hydrate) solution shows purple color indicates the presence of proteins.

c) Biuret test: To the extract solution add 1% copper sulphate solution forms pink or purple color indicates the presence of proteins.

7. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

8. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes indicates the presence of saponins.

9. Detection of tannins

a) Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

b) Ferric chloride test: To the extract solution add 1 ml of 1% neutral ferric chloride solution shows green or violet color indicates the presence of tannins.

10. Detection of Sterols

a) Salkowski Test: 3-4 drops of Conc. Sulphuric acid were added to the extract in chloroform. Formation of red color appears at lower layer indicates presence of sterols.

b) Libermann Buchard test: To the extract solution add few drops of acetic anhydride boil and cool. Add concentrated Sulfuric acid from the sides of the test tube, a brown ring appears at the junction of two layers green color in the upper layer indicates the presence of sterols.

Quantitative studies of phytoconstituents

Estimation of total phenol content

The total phenolic content of the extract was determined using the modified Folin–Ciocalteu method [10]. For the preparation of the standard solution, 10 mg of gallic acid was accurately weighed and dissolved in 10 ml of methanol to obtain a stock solution. Different concentrations of gallic acid ranging from 10–50 µg/ml were prepared by suitable dilution with methanol.

For the sample preparation, 10 mg of the dried extract was accurately weighed, dissolved in 10 ml of methanol, and filtered. From this solution, 2 ml (1 mg/ml) was taken for the estimation of total phenolic content.

For the assay, 2 ml of the extract or standard solution was mixed with 1 ml of Folin–Ciocalteu reagent, previously diluted with distilled water in a 1:10 (v/v) ratio, followed by the addition of 1 ml of sodium carbonate solution (7.5 g/L). The mixture was vortexed for 15 seconds and allowed to stand for 15 minutes at room temperature for colour development. The absorbance of the resulting blue-coloured solution was measured at 765 nm using a UV–Visible spectrophotometer. The total phenolic content was calculated from the calibration curve of gallic acid and expressed as mg gallic acid equivalent (GAE) per 100 mg of dried extract.

Estimation of total flavonoids content

The total flavonoid content of the extract was determined using the aluminium chloride ($AlCl_3$) colorimetric method [11]. For preparation of the standard solution, 10 mg of quercetin was accurately weighed and dissolved in 10 ml of methanol to obtain a stock solution. Different concentrations of quercetin ranging from 5–25 µg/ml were prepared by suitable dilution with methanol.

For sample preparation, 10 mg of the dried extract was accurately weighed, dissolved in 10 ml of methanol, and filtered. From this solution, 3 ml (1 mg/ml) was used for the estimation of total flavonoid content.

For the assay, 3 ml of the extract or standard solution was mixed with 1 ml of 2% aluminium chloride solution and allowed to stand for 15 minutes at room temperature for colour development. The absorbance of the resulting solution was measured at 420 nm using a UV–Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve prepared using quercetin and expressed as mg quercetin equivalent (QE) per 100 mg of dried extract.

Estimation of total alkaloids content

A part of extract residue was dissolved in 2N HCl and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform [11].

Preparation of standard curve

Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform. The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer against the blank prepared as above but without Atropine.

RESULTS AND DISCUSSION

The extraction of *Santalum album* was carried out using a hydroalcoholic solvent system to obtain a broad range of polar and moderately polar phytoconstituents. As presented in Table 1, the hydroalcoholic extract was obtained as a dark black-coloured extract, with a recovered extract weight of 5.26 g and an extraction yield of 10.52% w/w. The obtained yield indicates that the extraction procedure was effective in recovering extractable constituents from the plant material. The hydroalcoholic solvent system is capable of extracting diverse classes of phytochemicals, including phenolic compounds, flavonoids, alkaloids, glycosides and other secondary metabolites. The dark colour of the extract may be attributed to the presence of concentrated plant constituents and naturally occurring coloured compounds.

The preliminary phytochemical screening results of the different extracts of *Santalum album* are presented in Table 2. The screening demonstrated variation in the distribution of phytoconstituents among chloroform, ethyl acetate, ethanol and aqueous extracts, indicating that solvent polarity influenced the extraction of different chemical constituents. Alkaloids were detected in the ethanol and aqueous extracts in selected tests, whereas the chloroform and ethyl acetate extracts showed predominantly negative reactions. This suggests that relatively polar solvents were more suitable for extraction of the alkaloidal constituents detected by the applied tests.

The screening of glycosides using concentrated sulphuric acid, Liebermann–Burchard and Raymond's tests produced negative results in all the tested extracts, indicating that glycosides were not detected under the experimental conditions employed. Similarly, sterols were not detected by the Salkowski and Liebermann–Burchard tests in any of the extracts. These findings suggest that these particular classes of constituents were either absent, present below the detection level of the preliminary tests, or not sufficiently extracted under the conditions used.

Flavonoids showed a more prominent distribution among the extracts. The ethyl acetate and ethanol extracts gave positive reactions with both the lead acetate and alkaline reagent tests, while the aqueous extract showed a positive reaction with the lead acetate test but a negative reaction with the alkaline reagent test. The chloroform extract showed negative reactions for both tests. The comparatively positive response of the ethyl acetate and ethanol extracts indicates that these solvents were more effective in extracting the flavonoid constituents detected by the screening procedures.

Diterpenes were detected only in the ethanol extract by the copper acetate test, while the chloroform, ethyl acetate and aqueous extracts showed negative results. Phenolic constituents were detected in different extracts through the ferric chloride, zinc hydrochloride and Shinoda tests. The ethanol extract showed positive reactions in the zinc hydrochloride and Shinoda tests, while the aqueous extract showed a positive ferric chloride reaction. The presence of phenolic and flavonoid constituents is particularly relevant because these classes of phytochemicals are commonly associated with antioxidant and other pharmacological activities.

The protein screening results also demonstrated solvent-dependent variation. The ethyl acetate extract showed a positive ninhydrin reaction, the ethanol extract showed a positive xanthoproteic reaction, and the aqueous extract showed a positive Biuret reaction. The remaining reactions were negative. Carbohydrates were detected in the ethyl acetate, ethanol and aqueous extracts by selected Fehling's and Benedict's tests, whereas the chloroform extract showed negative reactions. Saponins were detected in the ethanol and aqueous extracts, particularly through the foam test, while the chloroform and ethyl acetate extracts were negative. Tannins were detected through the gelatin test in the ethanol and aqueous extracts, whereas the other extracts showed negative reactions. Overall, the results in Table 2 demonstrate that the phytochemical profile varied considerably according to the solvent used for extraction.

Quantitative estimation of major phytochemical groups in the hydroalcoholic extract is presented in Table 3. The extract contained 0.74 mg/100 mg of total phenolic content, 0.89 mg/100 mg of total flavonoid content, and 0.42 mg/100 mg of total alkaloid content of dried extract. Among the three estimated groups, flavonoids showed the highest measured content, followed by phenolic compounds and alkaloids. The relatively higher flavonoid content is consistent with the positive flavonoid screening reactions observed particularly in the ethyl acetate and ethanol extracts in Table 2.

The presence of phenolic and flavonoid constituents in measurable amounts is important for further pharmacological investigation because these phytochemical groups may contribute to the biological properties of *Santalum album* extract. However, the quantitative values obtained represent the estimated content under the specific analytical procedures used and should not be interpreted as identification of individual compounds.

Table 1: % Yield of extract of *Santalum album*

S. No.	Extract	Colour	Weight of extract	% Yield (w/w)
1.	Hydroalcoholic	Dark black	5.26	10.52

Table 2: Result of phytochemical screening of extract of *Santalum album*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	**Alkaloids**				
	Hager's Test:	-ve	-ve	+ve	-ve
	Wagner's Test:	-ve	-ve	-ve	+ve
2.	**Glycosides**				
	Conc. H_2SO_4 Test:	-ve	-ve	-ve	-ve
	Liebermann's	-ve	-ve	-ve	-ve
	Burchard's Test:	-ve	-ve	-ve	-ve
	Raymond's Test:				
3.	**Flavonoids**				
	Lead acetate Test:	-ve	-ve	+ve	+ve
	Alkaline reagent test:	-ve	+ve	+ve	-ve
4.	**Diterpenes**				
	Copper acetate Test:	-ve	-ve	+ve	-ve
5.	**Phenol**				
	Ferric chloride Test:	-ve	-ve	+ve	-ve
	Zinc hydrochloride test:	-ve	+ve	+ve	+ve
	Shinoda test:	-ve	-ve	-ve	+ve
6.	**Proteins**				
	Xanthoproteic Test:	-ve	-ve	+ve	-ve
	Ninhydrin test:	-ve	+ve	-ve	-ve
	Biuret test:	-ve	-ve	-ve	+ve
7.	**Carbohydrate**				
	Fehling's Test:	-ve	+ve	+ve	+ve
	Benedict's Test	-ve	-ve	+ve	+ve
8.	**Saponins**				
	Froth Test:	-ve	-ve	-ve	+ve
	Foam Test:	-ve	-ve	+ve	+ve
9.	**Tannins**				
	Gelatin test:	-ve	-ve	+ve	+ve
	Ferric chloride test:	-ve	-ve	-ve	-ve
10.	**Sterols**				
	Salkowski Test:	-ve	-ve	-ve	-ve
	Libermann Buchard test:	-ve	-ve	-ve	-ve

Table 3: Estimation of total phenol and flavonoids content of *Santalum album* extract

S. No.	Extract	Total phenol content	Total flavonoids content	Total alkaloid content
		(mg/100mg of dried extract)		
1.	Hydroalcoholic	0.74	0.89	0.42

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