

QUALITATIVE AND QUANTITATIVE STUDIES OF SANTALAM ALBUM & ROSA BRACTEATAE PLANT EXTRACTS FOR SKIN BENEFICIARIES

Dr. D. Chandra Prabha², Hemalakshmi Mohanarangan^{1*}

¹*Department of Biochemistry, ²Associate Professor,

Sri Ramakrishna College of Arts and Science for Women, Coimbatore, India.

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*Corresponding Author

Hemalakshmi

Mohanarangan

Department of

Biochemistry Sri

Ramakrishna College of

Arts and Science for

Women, Coimbatore,

India.

ABSTRACT

Background: Sandalwood is a parasitic plant, equipped with special structures on its roots that penetrate the roots of host plants and obtain nutrients. Indian sandalwood has the highest oil content (6 to 7%) and a desirable aroma profile. It acts as a facial skin toner and complexion booster. Similarly, roses were originally wild and they come from several parts of the world, North America, Europe, northwest Africa and many parts of Asia and Oceania. There are over 100 different species of roses. Roses possess exotic odor and refreshes the skin. Total phenolics study are highly essential for determining the plant's beneficial characters and provide a way for cosmetic formulations. **Objective:** The main objective of this study is to analyze the secondary metabolites of *Santalum album* and *Rosa bracteatae* using various solvents of extraction. It includes phytochemical studies and

phenolic content studies. **Materials and Method:** Plant extracts of sandalwood and rose petals obtained from three different solvents were subjected to phytochemical analysis and TPC (total phenolic contents) studied using chemical methods and formulations. **Results:** The plants yielded highest beneficial results in ethyl acetate solvent. It also resulted in highest TPC (total phenolic content) with the same solvent thereby showing the appropriateness for choosing a solvent for maximum extraction. **Conclusion:** The study shows the beneficial effects of plant's phyto constituents for skin that was applied for solving various skin problems. These secondary metabolites can replace various cosmetic synthetics and used as natural skin products.

KEYWORDS: Total phenolic content, polyphenolic compounds, sandalwood, rose plants, skin cosmetics.

INTRODUCTION

The outer covering of the body is skin which is the largest organ of the integumentary system. It has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs. Because it interfaces with the environment, skin plays a key immunity role in the protection of body against pathogens and excessive loss of water.

Santalum album or Indian sandalwood is the most well-known and economically important species. New Caledonia's *S. austro caledonicum* and Fiji's *S. Yasi* are also distilled to produce essential oil. *S. spicatum* from Australia has been valued for its wood for many years, and has recently also become a source for essential oil. Many of the other species are used for their wood (for building), firewood and for furniture making.^[1]

Trees are chosen based on age and size, harvested for oil with the higher proportion of heartwood (and thus essential oil) in larger trees. The whole tree is harvested and used including the sawdust and the stump (highest oil content) and the sapwood (contains a small amount of oil). The sapwood (lower grade sandalwood) is used for incense and for chips and powder, while the better logs are used in carving (furniture).^[2]

The rose is a type of flowering shrub. Its name comes from the Latin word Rosa. The flowers of the rose grow in many different colors, from the well-known red rose to yellow roses and sometimes white or purple roses. The wild rose species can be grown in gardens, but most garden roses are cultivars, which have been chosen by people. Roses possess exotic odor and refreshes the skin. It has an anti-inflammatory activity too inhibiting soothing and cooling effects.^[3]

Rosa bracteatae is a plant of the genus roses (*Rosa*) within the family of the rose family (*Rosaceae*). The *Rosa bracteatae* grows as evergreen entwining shrub and reaches stature heights up to 6 meters. It blooms stand on several large, gray-green bracts (bracts). The relatively large, hermaphrodite flowers are at a diameter of 6 to 10 centimeters radial symmetry and double perianth and flow a lemony scent. The five free petals are white. *Rosa bracteatae* in diameter of up to 2.5 centimeters rounded rosehips.^[4]

The present study explores the qualitative and quantitative (total phenolic content) studies of the selected plant extracts through three different solvents to predict the basic pharmaceutical activity of sandalwood and rose petals for skin.

MATERIALS AND METHODS

Preparation of plant extracts

Plant extracts were prepared from the powder of sandalwood and dried rose petals (complete absence of chlorophyll). For a quantity of about 50mg different solvents such as Hexane, Ethyl acetate and Ethyl alcohol were added sequentially to the plant powders in the ratio of 1:3.

They were left undisturbed for dissolving for about 48 hours and then transferred carefully into petri dishes. They were condensed completely in a condenser until the extracts become extremely thick. They were saved in an Eppendorf container for further use.

Qualitative analysis

The phytochemical analysis of the plants extracts were carried out with the following method of Trease and Evans (1996) and Horborne (1987).

Phytochemical tests

Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for glycosides

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Test for steroids and phytosteroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Test for anthraquinones

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Quantitative analysis of total phenolic content

Total phenolic content (TPC) of extract was assessed according to the Folin–Ciocalteu method (Slinkard & Singleton, 1977) with some modifications.

Briefly, 0.1 ml of extracts (200, 600 and 1000µg/ml), 1.9 ml distilled water and 1 ml of Folin–Ciocalteu's reagent were seeded in a tube, and then 1 ml of 100 g/l Na₂CO₃ was added. The reaction mixture was incubated at 25 °C for 2 hours and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration

curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Amount of TPC = Sample OD/Standard OD * Respective Amount of extract

RESULTS AND DISCUSSION

Sample preparation

The active principles of sandalwood and rose powder were extracted by adding 20ml of hexane, ethyl alcohol and ethyl acetate solution to 1mg of the dry powder. This solution was heated in the boiling water bath at 60 degree centigrade for 60 minutes. The mixture was taken in petridishes and placed over the hot plate for evaporation. It was then scrapped off from the petridishes and weighed to calculate the amount of yield exactly. These yield were collected and used for further analysis.

Table 1: Sample yield from the extract preparation.

Sample and Solvents	Value of yield (gm)
Sandalwood – Hexane	0.127
Rose petals – Hexane	0.544
Sandalwood – Ethyl acetate	0.512
Rose petals – Ethyl acetate	1.004
Sandalwood – Ethyl alcohol	0.26
Rose petals – Ethyl alcohol	0.595

Qualitative test

Potential therapeutic activity of the plants are mostly due to the active compounds residing in them. Following qualitative test are performed to examine the phytochemicals present in each extracts and the results are depicted in table 2 & 3.

Table 2: Phytochemical analysis of sandalwood extracts.

S.No	Phytochemical constituents	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Carbohydrates test	+	+	+
2	Tannins test	-	-	+
3	Saponins test	-	-	-
4	Flavonoids test	+	+	+
5	Alkaloid test	-	-	-
6	Quinones test	+	+	+
7	Glycosides test	-	-	-
8	Cardiac glycosides test	+	+	+
9	Terpenoids test	+	+	+

10	Phenols test	+	+	+
11	Coumarins test	+	+	+
12	Steroids and Phytosteroids test	Steroids	Steroids	Weakly Steroids
13	Phlobtannins test	-	-	-
14	Anthraquinones test	-	-	-

+ Present

- Absent

The extracts of *Santalum album* and *Rosa bracteatae* were analysed qualitatively for their phytochemical contents. Carbohydrates, flavonoids, quinones were found to be present in all the 3 extracts. Phenols are strongly present in *R.bracteatae* compared to that of *S.album*. Steroids are weakly present in the sandalwood extracts. Sandalwood contains sesquiterpenoid more that is reflected in the terpenoids test. It strongly possess terpenoids. Cardiac glycosides are specifically absent in both the extracts in all solvents.

Table 3: Phytochemical analysis of rose petals extracts.

S.No	Phytochemical constituents	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Carbohydrates test	+	+	+
2	Tannins test	-	-	+
3	Saponins test	-	-	-
4	Flavonoids test	+	+	+
5	Alkaloid test	-	-	-
6	Quinones test	+	+	+
7	Glycosides test	+	+	+
8	Cardiac glycosides test	-	-	-
9	Terpenoids test	-	-	-
10	Phenols test	+	+	+
11	Coumarins test	+	+	+
12	Steroids and Phytosteroids test	Steroids	Steroids	Weakly Steroids
13	Phlobtannins test	-	-	-
14	Anthraquinones test	-	-	-

+ Present

- Absent

Coumarin test revealed that the Rose petals possessed large quantity of coumarins compared to that of sandalwood, because the coumarin compound is more important which gives fragrance and colour for the rose flowers. The secondary phytochemicals such as phlobtannins and anthraquinones are found in traces in both the sample extracts. Steroids and phytosteroids are also weakly present in the similar range compared to both the extracts.

Our findings are in accordance with the investigation of^[5] which states that phenols and carbohydrates are the essential components present in the herbal extracts of Hexane, Ethyl acetate and Ethanol.

According to the work of^[6], in the phytochemical analysis of the four extracts, saponins, tannins, anthraquinones, terpenoids, and flavonoids were found in all the extracts. Alkaloids were detected in extracts of *Vitex doniana* only, while cardiac glycosides were also present in extracts of *Mucuna pruriens* only. The medicinal values of the plant leaves may be related to their constituent phytochemicals.

Phytochemical screening of petroleum ether, chloroform, ethanol, aqueous and hydro-alcoholic extracts revealed the presence flavonoids, tannins triterpenoids, saponins, sterols, alkaloids and carbohydrates by positive reaction with the respective test reagent.^[7] Phytochemical screening showed that maximum presence of phytoconstituents in ethanolic and hydro-alcoholic extracts.

The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value.^[8] For example, saponins are glycosides of both triterpene and steroids having hypotensive and cardio depressant properties, while anthraquinones possess astringent, purgative, anti-inflammatory, moderate antitumor, and bactericidal effects. Cardiac glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia.

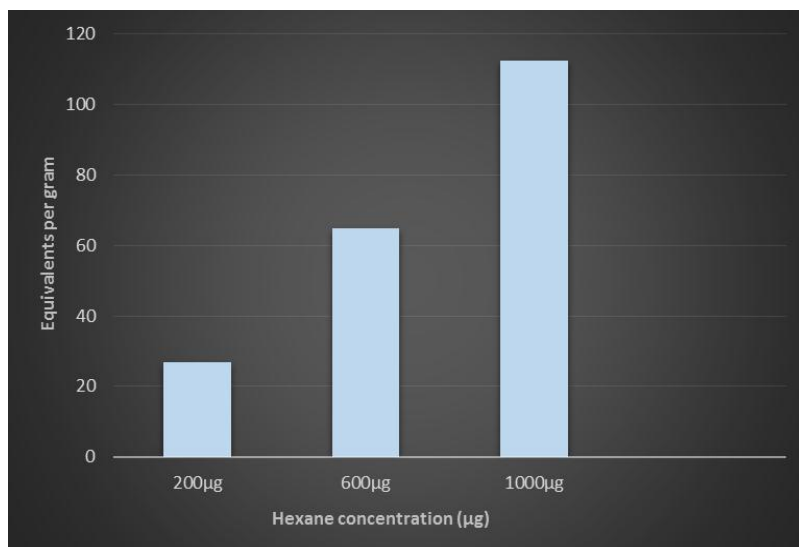
Quantitative test

Total phenolic content

The phenolics are a class of antioxidant compounds which serve as free radical terminators by scavenging or chelating process. The free radical induced lipid peroxidation is inhibited by the proton donating ability of hydroxyl groups in phenolic compounds. The quantitative test are performed to examine the amount of phenolics present in each extracts in various solvents used. The catechol solution is taken as the working standard for the samples.

Table 4: Total phenolic content of Sandalwood (Hexane extract).

Concentration (μg)	Amount of Phenol (μg)
200	26.87
600	64.89
1000	112.39

**Fig 1: Graph showing total phenolic content of sandalwood.****Table 5: Total Phenolic Content of Rose Petals (hexane extract).**

Concentration (μg)	Amount of Phenol (μg)
200	14.27
600	46.33
1000	187.59

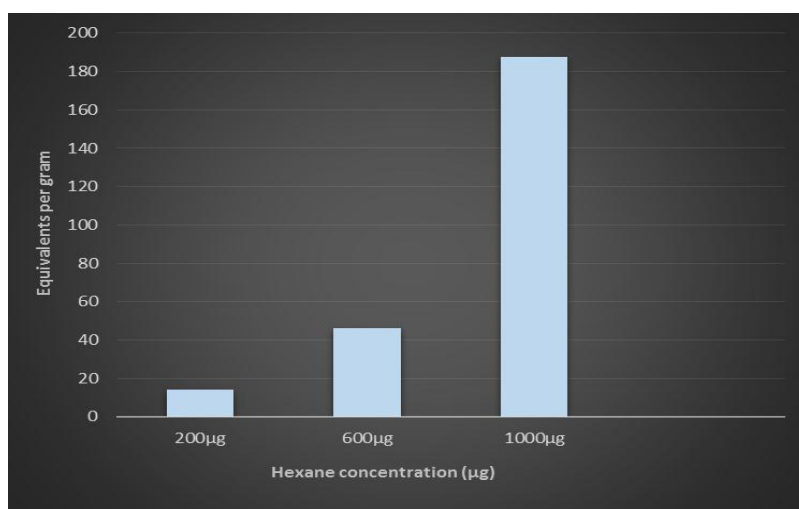
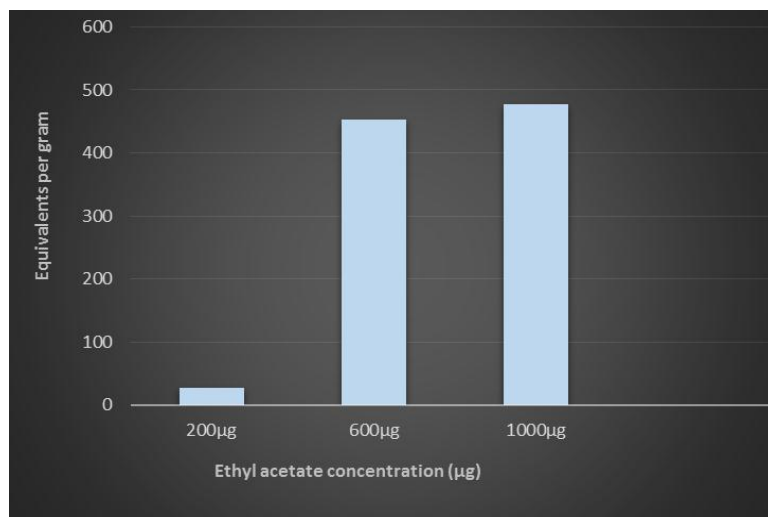
**Fig 2: Graph showing total phenolic concentration of Rose Petals.**

Table 6: Total Phenolic Content of Sandalwood (Ethyl acetate extract).

Concentration (μg)	Amount of Phenol (μg)
200	27.37
600	452.59
1000	476.59

**Fig 3: Graph showing Total Phenolic Content of Sandalwood.****Table 7: Total Phenolic Content of Rose Petals (Ethyl acetate extract).**

Concentration (μg)	Amount of Phenol (μg)
200	117.58
600	484.12
1000	524.60

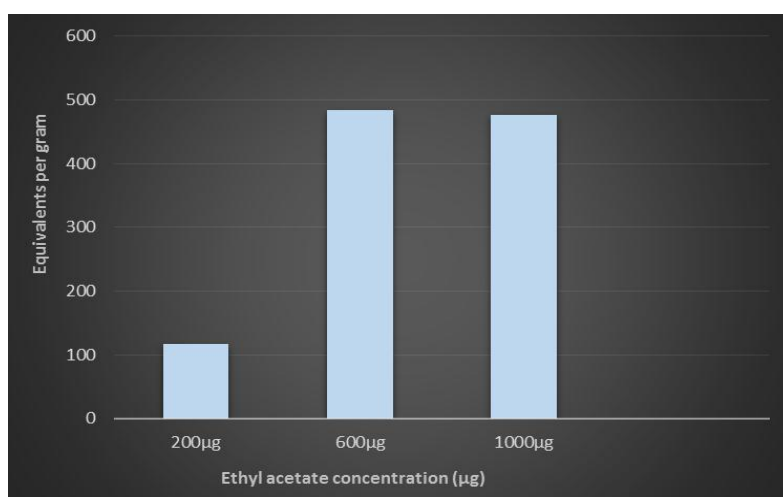
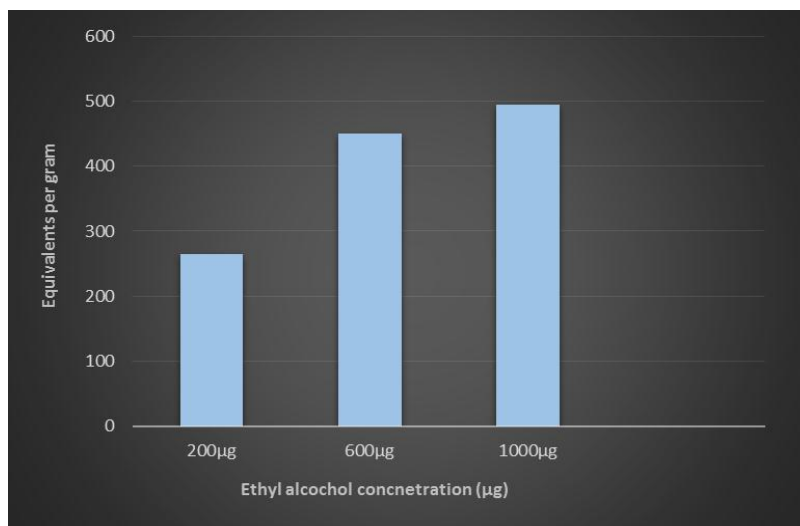
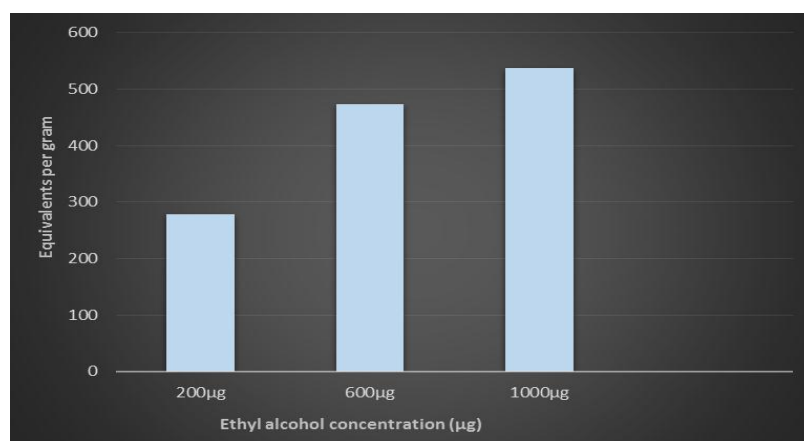
**Fig.4: Graph showing Total Phenolic Content of Rose petals.**

Table 8: Total Phenolic Content of Sandalwood (Ethyl alcohol extract).

Concentration (μg)	Amount of Phenol (μg)
200	264.38
600	449.60
1000	493.78

**Fig 5: Graph showing Total phenolic content of Sandalwood.****Table 9: Total Phenolic Content of Rose Petals (Ethyl alcohol extract).**

Concentration (μg)	Amount of Phenol (μg)
200	279.17
600	473.79
1000	536.70

**Fig 6: Graph showing Total Phenolic Content of Rose petals.**

The concentration the extracts are taken in accordance with the work of.^[5] In the current study, *Santalum album* and *Rosa bracteatae*, rose petals in ethyl acetate extract solvent

yielded highest amount of phenol content than the other extracts. Whereas the sandalwood in hexane extract found to possess least amount of phenol. Plant phenolics constitute a major group of phytochemicals that acts as primary antioxidants. They have high redox potentials which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

Among the ethanolic extract of five medicinal plants, *Holarrhena antidysentrica*, *Pachygone ovata*, *Asparagus racemosus*, *Lippia nodiflora* and *Curcuma zedoaria* studied for their phenolic content, large amount was detected in *Pachygone ovata*. This was followed by *Holarrhena antidysentrica*, *Asparagus racemosus* and *Lippia nodiflora*. The least amount of phenolics was observed in *Curcuma zedoaria*. These studies were revealed from the work of.^[9] *Holarrhena antidysentrica* had the highest level of phenolics among the five medicinal plants tested in petroleum ether solvent.

Petroleum ether extract of was found to be the rich in the total phenol content among the rest of the medicinal plants screened. The amount of phenolics in petroleum ether extract of medicinal plants clearly demonstrated that the extractability of phenolic compounds is too low in this particular solvent.

Among the various solvent extracts of medicinal plants examined for their total phenolic content, it was noticed that all the five herbs exhibited good phenolic content in aqueous extract. This clearly indicates that the extractability of phenolic compounds is good in aqueous medium. Eventhough the extractability of total phenols in the aqueous medium is comparatively better, the ethanolic extract of *Pachygone ovata* has shown to have the maximum content of phenolics expressed in terms of catechol equivalents. These *invitro* studies were revealed in accordance from study of.^[10]

4. CONCLUSION

The findings of the study entitled “qualitative and quantitative studies of *Santalum album* and *Rosa bracteatae* plant extracts” is summarized. The extracts of *Santalum album* and *Rosa bracteatae* were found to be rich in the carbohydrates, flavonoids and quinones. Coumarin present in high levels are highly responsible for their fragrance and colouration in *R.bracteatae* extracts. These phytochemicals accounts for the potent medicinal benefits. Ethyl acetate extracts of *S.album* and *R.bracteatae* possess a high phenolic content which is an indicative measure of high redox potentials acting as reducing agents. Maximum phenol contents of the plants are extracted by the ethyl acetate.

From the above findings, it can be concluded that the phytoconstituents – the secondary metabolites present in the plants are highly responsible for their fragrance effective pharmaceutical characters for skin that are made use in the manufacturing of cosmetic and herbal products. Further investigations are in line to extend the study for potential activities of secondary metabolites which play a major role in exerting the oxidant and tyrosinase inhibition.

Future recommendations

Further investigations are in line to extend the study for anti-oxidant and anti-tyrosinase studies that are essential for skin melanin production incorporated for skin whitening assays.

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