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PRELIMINARY PHYTOCHEMICAL STUDY OF *NYCTANTHES ARBOR-TRISTIS* L. AND *SANTALUM ALBUM* L. FROM WESTERN GHATS OF KARNATAKA

[1]Ramya and [2*]Chandra M.

Department of Biosciences, Mangalore University, Mangalagangothri -574 199 Dakshina Kannada District, Karnataka, India.

***Corresponding Author: Dr. Chandra M.**
Department of Biosciences, Mangalore University, Mangalagangothri -574 199 Dakshina Kannada District, Karnataka, India.

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ABSTRACT
Plants have an almost endless variety of metabolites which is very useful to human beings. The traditional medicine involves the use of different plant extracts or the bioactive constituents. Primary and secondary metabolites present in plants have been linked with the healing properties of plants. Hence, the present study was carried out to screen the phytochemical constituents in the leaf extracts of two medicinal plants viz., *Nyctanthes arbor-tristis* L. and *Santalum album* L. Different solvents viz., aqueous, ethanol, chloroform and hexane were used to extract the leaf of selected medicinal plants. Phytochemical constituents such as carbohydrates, amino acids, proteins, steroids, alkaloids, flavanoids, glycosides, cardiac glycosides, saponins, tannins, terpenoids, triterpenes, phlobatinnins, fatty acids, anthocyanins, leucoanthocyanins, coumarins, phenols, quinines and emodins were analysed in both the medicinal plants. The study showed that the presence of primary and secondary metabolites in all the four solvent extracts of selected medicinal plants. But *Santalum album* L. showed the highest level of phytochemical constituents compared to *Nyctanthes arbor-tristis* L.The results suggest that the phytochemical properties of these plants may serves for curing various ailments and possess potential antioxidant which leads to the isolation of new and novel compounds.

KEYWORDS: *Nyctanthes arbor-tristis* L., *Santalum album* L., Phytochemicals, Primary and Secondary metabolites.

INTRODUCTION
The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, and antihepatotoxic compounds.[1] According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, efficacy and efficiency.[2] In the present work, qualitative phytochemical analysis was carried out in two plants viz., *Nyctanthes arbor-tristis* L. and *Santalum album* L**.** of Western Ghats of Karnataka.

Nyctanthes arbor-tristis L**.** (Night - flowering Jasmine), commonly known as Parijata, is a species of *Nyctanthes*, native to South Asia. *Nyctanthes arbor-tristis* is a shrub or a small tree growing to 10 m tall, with flaky grey bark. The leaves are opposite, simple, 6–12 cm long and 2–6.5 cm broad, with an entire margin. The flowers are fragrant, with a five- to eight-lobed white corolla with an orange-red centre; they are produced in clusters of two to seven together, with individual flowers opening at dusk and finishing at dawn. The fruit is a flat brown heart-shaped to round capsule 2 cm diameter, with two sections each containing a single seed.[3]

Santalum album L. or Indian sandalwood is a small tropical tree, and is the most commonly known source of sandalwood. It is native to India, Indonesia. This species has historically been cultivated, processed and traded since ancient times. Certain cultures place great significance on its fragrant and medicinal qualities. It is also considered sacred in some religions and is used in different religious traditions. The high value of the species has caused its past exploitation, to the point where the wild population is vulnerable to extinction. Indian sandalwood still commands high prices for its essential oil, but due to lack of sizable trees it is no longer used for fine woodworking as before. The plant is

widely cultivated and long lived, although harvest is viable after 40 years.[4]

MATERIALS AND METHODS

Collection of plant materials

Plants like *Nyctanthes arbor-tristis* and *Santalum album* were collected from Western Ghats of Karnataka and brought to the laboratory. The collected healthy plant leaves were washed with running tap water then rinsed with the distilled water. The plant materials were dried at room temperature and oven dried at 40^0 C and then powdered using a kitchen blender. A sample (50 g) of each powdered plant material was soaked in aqueous, ethanol, chloroform and hexane (100 ml each) for 48 hrs. At the end of the extraction, each extract was filtered using Whatman No.1 filter paper. The filtrate was concentrated in water bath at 30^o C and stored at 4^o C until further use.[5]

Phytochemical Screening

Qualitative Tests for phytochemical was carried out by standard methods described by.[6,7]

Test for carbohydrates

To 2ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of Conc. H_2SO_4 was added on the sides of the test tube. A reddish violet colour ring appeared at the junction of two layers immediately indicates the presence of carbohydrates.

Fehling's test

2ml of sample was treated with 2 ml of Fehling's solution A or 1 and 2ml of Fehling's solution B or 2. Heated in a boiling water bath results in the formation of brick red precipitate confirms the presence of reducing sugars.

Test for Proteins

To 2ml of the extract, 2ml of Biuret reagent was added. A violet colour ring indicates the presence of peptide linkages of the molecule.

Test for Amino Acids

To the 2ml of extract, 2ml of Ninhydrin reagent was added and kept in hot water bath for 20 minutes. Appearance of purple colour indicates the presence of amino acids in the sample.

Test for Steroids (Salkowski Test)

1ml of extract was added to 10ml of chloroform followed by equal volume of Sulphuric acid, on standing yields red colour.

Test for Alkaloids

To the extract, each of 6 of drops of 1% HCl, Mayer's reagent and Dragendroff's reagent were added. An organic precipitate indicates the presence of alkaloids in the sample.

Test for Flavonoids

5ml of diluted ammonia solution was added to a portion of aqueous extract and 2 ml of Conc. H_2SO_4 was added. A yellow coloration indicates the presence of flavonoids and it disappears on standing.

Test for Glycosides

A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicates the presence of glycosides.

Test for Cardiac Glycosides

5ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayred with 1ml of Conc. H_2SO_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring, whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

Test for Saponins

To the 2 ml extract, 20 ml of distilled water was agitated in a graduated cylinder for 15minutes. The formation of 1cm layer of foam indicates the presence of saponins.

Test for Tannins

5ml of extract was added to few drops of 1% of lead acetate. A yellow precipitate indicates the presence of tannins.

Test for Terpenoids

To 2ml of extract, 2ml of chloroform and 3ml of Conc. H_2SO_4 were added to form a monolayer of reddish brown coloration interface which confirms the presence of terpenoids.

Tests for Triterpenes

To the Chloroform solution of extract, a few drops of acetic acid and 1 ml Conc. H_2SO_4 were added. A deep red color appearance at the junction of 2 layers indicates the presence of triterpenoids.

Test for Phlobatinins

When an aqueous extract was boiled with 1% aqueous HCl, the red precipitate indicates the presence of phlobatinins.

Test for Fatty Acids

0.5 ml of extract was mixed with 5ml of ether. The solution was allowed for evaporation on filter paper and dried the filter paper. The appearance of transparency on filter paper indicates the presence of fatty acids.

Test for Anthocyanins

To 2ml of aqueous extract, 2ml of 2N HCl and 2 ml of ammonia was added. The appearance of pink-red turns blue violet indicates the presence of anthocyanins.

Test for Leucoanthocyanins
5ml of aqueous extract was mixed with 5ml of isoamyl alcohol. Upper layer appears red in colour indicates the presence of leucoanthocyanins.

Test for Coumarins
To 3 ml of 10% NaOH, 2 ml of aqueous extract was added. Formation of yellow colour indicates the presence of coumarins.

Test for Phenols
2ml of extract was mixed with 3ml of ethanol followed by a pinch of FeCl3. The greenish yellow colour indicates the presence of phenols.

Test for Quinones
To 2ml of extract, 3ml of Con. HCl was added to form green colour which indicates the presence of quinines.

Test for Emodins
2 ml of NH_4OH was mixed with 3 ml of benzene and 2 ml of extract was added. Appearance of red colour indicates the presence of emodins.

Estimation of total chlorophyll content
100 mg leaf tissues were soaked in 10 ml of DMSO: Acetone mixture (1:1) for overnight incubation (in the dark) and absorbance read at 663 and 645 nm and total chlorophyll content was calculated using the following equations.[8]
Chlorophyll $_a$ (C_a) = (12.25 × OD at 663) - (2.79× OD at 645) ×10/ (1000×wt)
Chlorophyll $_b$ (C_b) = (21.50 × OD at 645) - (5.10× OD at 663) ×10/ (1000×wt)
Total Chlorophyll (C) = (7.15 × OD at 663) + (18.71× OD at 645) ×10/ (1000×wt)

RESULTS AND DISCUSSION
The phytochemical screening demonstrated the presence of different types of phytocompounds like alkaloids, saponins, flavonids, steroids, tannins etc which could be responsible for the various pharmacological properties. Preliminary phytochemical investigation of *Santalum album* revealed the presence of Carbohydrates, Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Triterpenes, Fatty acids, Glycosides, Cardiac glycosides, Steroids, Phenols, Quinones, Coumarins, Emodins, Chlorophyll a, Chlorophyll b and total Chlorophyll which are represented in the table 1, 2 and 3. The results of proximate analysis are depicted in the table 4.

Carbohydrates, fatty acids were present in all the solvent extracts of leaves of *N. arbor-tristis* and *S. album.* Amino acids, proteins, anthocyanins and leucoanthocyanins were absent in all the solvent extracts of leaves of both the plants. Out of four solvent extracts, ethanol and hexane showed the presence of steroids in leaf of *S. album* but interestingly, steroids were not detected in any of the solvent extract of leaf of *N. arbor-tristis*. Carbohydrates are macromolecules, and it's composed of the elements of water and carbon. All carbohydrates are polar it can be readily converted into glucose which is used as an ultimate source of energy.[9] The presence of flavonoids and polyphenols in plants due to the presence of tannins[10,11] and also act as ecofriendly waste because of its numerous uses such as reducing agent in making silver nanoparticles.[12,13]

Alkaloids were detected in ethanol, chloroform and hexane extracts of leaves of selected plants but absent in aqueous extracts of both the plants. Ethanol and hexane extracts of leaf of *S. album* and aqueous and hexane extracts of leaf of *N. arbor-tristis* showed the presence of flavonoids. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall.[14] The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumours.[15] Quinones were detected in all the three solvent extracts of leaf of *S. album* but absent in aqueous extract. Leaf of *N. arbor-tristis* showed the presence of quinones in chloroform and hexane extracts. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defence mechanism against predation by many microorganism, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids etc.[16,17] Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity (alkaloids, phenolics, essential oils and terpenes, sterols, flavonoids, lignins, tannins, etc.).[18] These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides, and herbicides.[19,20-21] Generally, the presence of different phytochemicals in crude plant extracts has been linked to the detrimental effects of leachates, root exudates or decomposing residues of such plants on the other vegetation or succeeding crops.[22,23] Glycosides and cardiac glycosides were detected in all the solvent extracts of leaf of *S. album*. Glycosides were present in chloroform and hexane extracts of leaf of *N. arbour-tristis* and absent in aqueous and ethanol extracts. Cardiac glycosides were present in all the three extracts of leaf of *N. arbour-tristis* except hexane. Glycosides are known to lower the blood pressure according to many reports.[24,25,26] Cardiac glycosides (CGs) are natural compounds that are potent inhibitors of the plasma membrane Na+/ K+-ATPase leading indirectly to the intracellular accumulation of Ca2+ ions which can lead to myocardial contractility. CGs tends to exert potent antineoplastic effects by increasing the immunogenicity

of dying cancer cells.[27] *S. album* leaf showed the presence of saponins in all the solvent extracts, where as *N. arbor-tristis* leaf showed the presence of saponins in aqueous and hexane solvents extracts. Saponins have natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, which help the body to fight infections and microbial invasion.[28] Plant steroids are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics.[29] Saponins are used as mild detergents and in intracellular histochemistal staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholestrolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. it is also known to have antifungal properties.[30] Tannins were detected in all the solvent extracts of leaf of *N. arbor-tristis* except ethanol extract but in *S. album* tannins were absent only in chloroform. Tannins are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzyme and affect the absorption of vitamins and minerals. However, some kinds of tannins can reduce the mutagenicity of a number of mutagens and display anticarcinogenic, antimicrobial and antioxidant activities.[31] Terpenoides were observed in all the solvent extracts of *S. album* except hexane where as *N. arbor-tristis* showed the presence of terpenoids in aqueous and ethanol leaf extracts. *S. album* leaf extracts detected the triterpenoids in ethanol and hexane extracts and *N. arbor-tristis* leaf extracts detected the terpenoids in ethanol and hexane. Coumarins were detected in all the solvent extracts of *S. album* except aqueous extract and the content were present in aqueous, chloroform and hexane leaf extracts of *N. arbour-tristis*. Coumarin is a natural substance that has shown to have in vivo anti-tumor activity. A recent study has shown that 7-hydroxycoumarin inhibited the release of cyclin D1, which is over expressed in many cancer types.[32] Many compounds derived from coumarin have numerous therapeutic applications, which include photo chemotherapy, antitumor and anti-HIV therapy.[33] Phenols were present in all the solvent extracts of leaf of *S. album* where as in ethanol leaf extracts of *N. arbor-tristis* phenolic contents were absent. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bacterisides are compared.[34] Emodins were absent in all the four solvent extract of *S. album* but *N. arbor-tristis* showed the presence of emodins in all the three solvent extract of leaf except aqueous extract. Phytochemical constituents such as tannins, alkaloids, flavonoids and several other aromatic compounds or secondary metabolites of plants serve as defence mechanism against predation by many microorganism, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, terpenoids and quinone etc.[35,36] Photosynthetic pigments like total chlorophyll, a, and b ratio were found to be higher in *N. arbor-tristis* but lower in *S. album.* Chlorophyll is the primary compounds that as the only substances that captures sunlight and makes it available to plant system for its cultivation on photosynthesis.[37,38] According to some authors, the quantity and the composition of bioactive compounds present in plants are influenced by the genotype, extraction procedure, geographic and climatic conditions, and the growth phase of the plants.[39,40]

Table 1: Phytochemical analysis of *Santalum album* L.

Phytochemicals	Aqueous	Ethanol	Chloroform	Hexane
Carbohydrates	+	+	+	+
Proteins	-	-	-	-
Amino acids	-	-	-	-
Steroids	-	+	-	+
Alkaloids	-	+	+	+
Flavonoids	-	+	-	+
Glycosides	+	+	+	+
Cardiac glycosides	+	+	+	+
Saponins	+	-	-	+
Tannins	+	+	-	+
Terpenoids	+	+	+	-
Triterpenes	+	-	-	+
Phlobatinnins	-	-	-	+
Fatty acids	+	+	+	+
Anthocyanins	-	-	-	-
Leucoanthocyanins	-	-	-	-
Coumarins	-	+	+	+
Phenols	+	+	+	+
Quinones	-	+	+	+
Emodins	-	+	+	+

Table 2: **Phytochemical analysis of *Nyctanthes arbor-tristis* L.**

Phytochemicals	Aqueous	Ethanol	Chloroform	Hexane
Carbohydrates	+	+	+	+
Proteins	-	-	-	-
Amino acids	-	-	-	-
Steroids	-	-	-	-
Alkaloids	-	+	+	+
Flavonoids	+	-	-	+
Glycosides	-	-	+	+
Cardiac glycosides	+	+	+	-
Saponins	+	+	+	+
Tannins	+	-	+	+
Terpenoids	+	+	-	-
Triterpenes	-	+	-	+
Phlobatinnins	-	-	-	-
Fatty acids	+	+	+	+
Anthocyanins	-	-	-	-
Leucoanthocyanins	-	-	-	-
Coumarins	+	-	+	+
Phenols	+	-	+	+
Quinones	-	-	+	+
Emodins	-	-	-	-

Table 3: Estimation of chlorophyll pigments.

Pigments	*Santalum album*	*Nyctanthes arbor-tristis*
Chl a (mg/g tissue)	0.538	0.718
Chl b (mg/g tissue)	0.590	0.918
Total Chl a + b (mg/g tissue)	1.127	1.636

Table 4: Results of the proximate analysis.

Parameter	*Santalum album* L.	*Nyctanthes arbor-tristis* L.
Moisture (%)	16.2	12.0
Total Ash value (%)	8.5	29.5
Water insoluble ash (%w/w)	5.5	4.0
Acid insoluble ash (%w/w)	1.8	0.5
Alcohol soluble (%w/w)	42.8	31.2
Water soluble (%w/w)	6.0	4.8

CONCLUSION

The results of the present study indicate that the phytochemical compounds found in the selected two medicinal plants possess potential sources of antioxidant and antimicrobial effect which leads to isolation of novel compounds.

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