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Original Article

# EXTRACTION, ANTIOXIDANT POTENTIAL AND IDENTIFICATION OF SCONDARY METABOLITES OF WHOLE FRUITS OF *SANTALUM ALBUM* LINNBY GC-MS

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**ABSTRACT**

**Objective:** In order to study the antioxidant potential responsible secondary metabolites the fruit pulp and kernels of Santalum albumwere extracted in different solvents and tried to identify by GC-MS.

**Methods:** The extraction was carried out in various organic solvents by soxhlet by successive manner. The antioxidant capacity of all extracts was determined by FRAP. Identification of various secondary metabolites in the extracts was done by analysing them by GC-MS.

**Results:** Ethyl acetate and methanol extracts of fruit pulp and kernel have shown antioxidant activity. GC-MS identification has shown the presence of many secondary metabolites. Among many compounds cholest-4-en-3-one [11.86 %] obtained as the major constituent in fruit pulp di-chloromethane extract. While kernel methanol extract have showed presence of pyrazinamide [63.70%] and Acetamide-2-cyano [55.40%] in acetonitrile as major compounds.

**Conclusion:** The present study suggests that fruit pulp and kernels of Santalum album can be a new and rich source of medicinally useful secondary metabolites.

**Keywords**: Polyphenolic, Cholest-4en-3-one, Pyrazinamide, Acetamide-2-cyano.

## INTRODUCTION

*S. album* is economically and medicinally important plant of India. Though south western part of India is the major production place of sandalwood, it grows well in other parts also. Because of fragrant wood and oil Powder from the heartwood is used to make incense sticks, burnt as perfumes in houses and temples, or is ground into a paste and used as a cosmetic.

Sandalwood oil has clinical importance against urinary disorders, acts as sedative, also significantly decreases the incidents of papilloma [1].

Among fruits, berries contain high levels of a diversity of phytochemicals known as phenolics including flavonoids (anthocyanins, flavonols and flavanols), proanthocyanidins, ellagitannins and gallotannins, stilbenoids and phenolic acids [2]. *S. Album* produces berries in two seasons (May-June and October-November) in abundance and goes waste because of underutilisation. Fruits are berry like black purple upon ripening; are edible and belongs to drupe category; local tribes and birds enjoy eating these fruits.

Previous reports on santalum album berries have highlighted the general proximate composition, and anthocyanin extraction with its cytotoxic studies [3]. Phenylpropanoids and enzymes in East Indian sandalwood tree undergoing development also have been reported [4]. Through GC-MS analysis Presence of santalbic acid from kernels and alpha santalol acetate and di-n-octylphthalate from stem of *Santalum album*is being reported earlier [5, 6]. There is no evidence on the detailed fruit phytochemical composition.

Present investigation has the objective to identify and quantify the numerous phytoconstituents from fruit pulp and kernels using GC-MS analysis. This study will help in increasing the utilization of plenty and easily available fruits which can open a new door for pharmaceutical industries.

## MATERIALS AND METHODS

### Fruit collection and processing

Plant was identified along with fruit from Botanical Survey of India, Pune Maharashtra. Ripened and disinfected fruits from the home garden tree were collected, washed, pulp was separated carefully. Bony endocarp was removed and white coloured kernel and pulp were dried at 40 °C, powdered and stored in air tight container at 2 °C till further use.

### Successive extraction

Nonpolar to Polar solvents were selected for extraction; Hexane, Ethyl acetate, Acetonitrile, Dichloromethane, Methanol (all spectroscopy grade Merck).

Extraction of both the samples (1:15 w/v) were carried out successively in soxhlet separately at the boiling temperature of each solvent for 16-24 h. Each extract was concentrated under vacuum in the rotary evaporator then subjected to GC-MS analysis.

### Gas chromatography-mass spectrometry (GC-MS) analysis

Phytochemical analysis was done on Jeol AccuTOF GCv. Split was 1:70 with helium as carrier gas at rate of 1 ml/min. The initial oven temperature was 20 °C to 135 °C, and separation was carried out with the hold of 5 min from 135 °C to 220 °C with an increase of 5 °C/min and final holding of 5 min with an increase of 3.5 °C/min up to 270 °C. Nitrogen, hydrogen and air were used as the carrier gas at a linear velocity of 3.5 ml/s.

### Identification of compounds

The spectrum of the unknown components were compared with the spectrum of the known; stored in the Library. The name, molecular weight and structure of the compounds of the unknown were ascertained. The relative % amount of each component was calculated by comparing its average peak area to the total areas.

### Antioxidant activity (FRAP)

The Ferric reducing ability (FRAP) of the extracts were evaluated according to the method described by Benzie & Strain [7]

## RESULTS

GC-MS analysis provides the broad idea about the chemical structure and molecular formula and idea about the functional groups present in the compound. Identification and comparison of the mass spectra is based on the database of the published libraries. The Fatty acid methyl esters and phenolic compounds with their retention time (RT), molecular formula, and concentration % peak area in different solvents extract of *S. album* fruit pulp kernel is presented in table 1 and 2.

**Secondary metabolites**

The secondary metabolites in plants are produces in abundance against self-protection and other functions. Various such compounds were tried to extract in different solvents to identify them. Six bioactive compounds from fruit pulp (table 1.) and seven compounds from kernel (table 2.) could be identified in the present study.

Cholesterol derivative Cholest-4-en-3-one (46.7%) was found to be predominant from dichloromethane extract. While in kernel pyrazinamide (63.70%) from methanol and cyano-acetamide (55.40%) from acetonitrile were found in higher quantity.

**Antioxidant activity (FRAP)**

Various extracts of fruit pulp and kernel were tested for their antioxidant potential (fig. 1). The results suggests that methanol extracts of fruit pulp can provide more antioxidant compounds than ethyl acetate. Kernel ethyl acetate extract have shown higher antioxidant potential than methanol.

**Table 1: Polyphenolics of fruit pulp in different solvents**

| Constituent | Mol. formula | Retention time (min) | % Peak area |
|---|---|---|---|
| **Ethyl acetate** | | | |
| (3-Methyl-oxiran-2-yl)-methanol | ($C_4H_8O_2$) | 3.5 | 6.1 |
| 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- | ($C_6H_8O_4$) | 7.8 | 5.78 |
| **Dichloromethane** | | | |
| Cholest-4-en-3-one | ($C_{27}H_{44}O$) | 46.7 | 11.86 |
| **Acetonitrile** | | | |
| Di-butyl phthalate | ($C_{16}H_{22}O_4$) | 26.6 | 6.97 |
| **Methanol** | | | |
| 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- | ($C_6H_8O_4$) | 4.6 | 0.34 |
| Di-butyl phthalate | ($C_{16}H_{22}O_4$) | 26.6 | 6.97 |

**Table 2: Polyphenolics of kernel in differentsolvents**

| Name of compound | Molecular formula | Retention time (min) | % peak area |
|---|---|---|---|
| **Ethyl acetate** | | | |
| Hen-tri-acontane | ($C_{31}H_{64}$) | 53.2 | 11.77 |
| **Acetonitrile** | | | |
| Phenol | ($C_6H_6O$) | 4.1 | 12.77 |
| Nitrobenzene | ($C_6H_5NO_2$) | 5.8 | 3.95 |
| Acetamide-2-cyano | ($C_3H_4N_2O$) | 3.1 | 55.40 |
| Naphthalene | ($C_{10}H_8$) | 8.2 | 9.20 |
| **Methanol** | | | |
| Pyrazinamide | ($C_5H_5N_3O$) | 4.7 | 63.70 |
| Υ-Sitosterol | ($C_{29}H_{50}O$) | 60.9 | 29.25 |

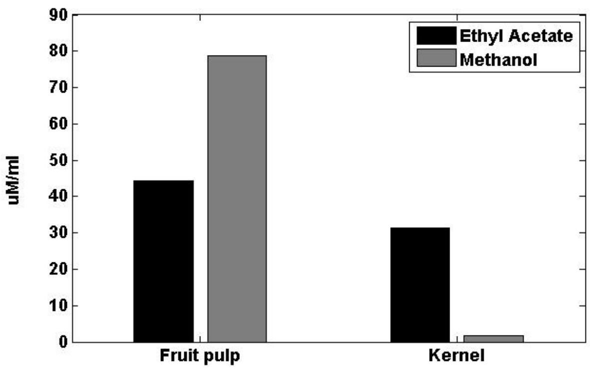


**Fig. 1: Antioxidant potential (FRAP) of fruit pulp and kernel extracts**

Dichloromethane and acetonitrile extracts of both the samples have not shown any antioxidant activity suggesting the less capacity of the solvents to extract antioxidant compounds.

**DISCUSSION**

**Secondary metabolites**

**Fruit pulp**

Ethyl acetate extract have shown many compounds (more than 50), out of which two (table 2) were identified; 4H-Pyran-4-one2, 3-dihydro-3, 5-dihydroxy-6-methyl (5.78%), and (3-Methyl-oxiran-2-yl)-methanol (6.1 %).

Pyran compounds and their flavonoid derivative are known for their anti-mycobacterial, antibacterial and anti-inflammatory activity, also are responsible for aroma [8]. Recent research has reported the strong antioxidant activity and inhibition of lipid peroxidation [9, 10].

(3-Methyl-oxiran-2-yl)-methanol another compound found in ethyl acetate extract of fruit pulp; is reported to be the major constituent of volatile oil and is reported to be present in oil of, *Oldham gypsophila*, and Wisteria pod [11, 12].

In dichloromethane extracts out of 32 compounds only sterol derivative Cholest-4-en-3-one (11.86 %) was identified. In general phytosterols are of pharmaceutical importance. Glycosylated derivative of cholest-4-en-3-one are reported as the novel cytotoxic compound from the marine red alga Peyssonnelia sp. [13].

Acetonitrile extract have shown 23 peaks from which only one compound di butyl phthalate (DBP) has been identified (26.97%).

It is a bio plasticizer; such compounds are reported to be useful in making soft and flexible plastic and pesticides. It is widely used in fragrance bases for household, personal care and cosmetic products [14]. Among all types of phthalates DBP is considered to be safe and hence widely used.

**Kernel**

Defatted kernel powder yielded various polyphenolic compounds in different solvents extracts. The list of identified compounds is summarised in table 2.

In successive extractions from ethyl acetate extract of kernel only one compound was identified from more than 50 compounds. Hen tri-acontane which is a high molecular weight saturated hydrocarbon found in most of the plant in the form of wax. Hen tri-acontane have many biological functions in plants and thus reported to be useful for humans and animals.

The major functions reported earlier are antitumor [15], anti-inflammatory [16], UV protection and antioxidant [17] activity. These properties suggests the use of the kernel as anti-sunburn product and traditional use similar to sandalwood and wood oil. Its presence in other plants like *Scabiosa comosa* and spinach leaves has been reported earlier. It is the first report of its presence in presently studied kernel ethyl acetate extract. This suggests the use of kernels in skin ointments.

Kernel dichloromethane extract was not analysed for GC-MS

Acetonitrile extract have shown the presence of 22 peaks out of which four compounds could be identified. Phenol (12.77%) a backbone component of all poly phenolics have been found in free form which is a primary compound in secondary metabolite synthesis in plants. Nitrobenzene (3.95%) is the basic backbone compound required in synthesis of many secondary metabolites in plant. Synthetically it is useful pharmaceutical product and pesticides.

2-cyano Acetamide (55.40%) found in acetonitrile extract. Synthetically it is reported as highly reactive molecule and potential chemotherapeutic agent [18]. This suggests the important role of kernel in chemotherapy.

Naphthalene (9.20%) have found in less quantity which is volatile insecticide its presence in kernel shows the self-protecting mechanism of plants and also support the traditional method of eating kernels (Roasting and shade drying). In endophytic fungus Muscodor vitigenus have been reported the production of volatile insect repellent naphthalene [19].

Methanol extract showed 24 peaks of which two were identified. Pyrazinamide (63.70%) have found in highest quantity. Pyrazinamide nucleus drugs are famous and safe anticancer drugs, novel for tuberculosis and good bactericidal [20]. This again suggests the usefulness of the kernels in the treatment of tuberculosis as a neutraceutical and an antibiotic.

### Antioxidant activity (FRAP)

Antioxidant activity of different solvent extracts suggests that non polar solvent ethyl acetate as well as polar one methanol can extract many antioxidant compounds from fruits of *Santalum album*. With proper separation technique, these compounds can be made useful in pharmaceutics.

## CONCLUSION

Over all studies suggests that the fruit pulp and kernels can be an additional source of useful phytochemicals along with the heart wood of *Santalum album* tree. With proper toxicity studies large amount of underutilized and easily available fruits and seeds can be make useful and will avoid wastage. Further studies are necessary to ascertain the bioactivity of individual compound, with other pharmaceutically important properties.

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## CONFLICT OF INTERESTS

Declared None

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