

## ASSESSMENT OF INDIAN SANDAL WOOD (*SANTALUM ALBUM L.*, ) SEEDS FOR SEED OIL PRODUCTION AND FATTY ACID METHYL ESTERS

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### ABSTRACT

Sandalwood is one of the most valuable plant species belonging to the family Santalaceae and the genus *Santalum*. It is highly rated for its sweet, fragrant, persistent aroma and the fixative property which is highly in demand by the perfume industry and thus is identified as one of the most sought after aromatic tree in the world. Plant seeds are important source of oil of nutritional, industrial and pharmaceutical importance. Also considered as a valuable secondary income generating product. Indian Sandalwood (*Santalum album.L*) seeds have not been exploited for their therapeutic properties. Thus, the study is aimed to assess to assess the quality of fatty acid extracted from the

seeds of *S. album* by solvent extraction. The fatty acid separation and identification was performed by a Gas-Liquid chromatography. The results have shown have prominent fatty acid content and the presence of Xymemnic acid an unusual acetylenic fatty acid with many reported therapeutic values.

**KEYWORDS:** *Santalum album. L*, Seed oil, Fatty acids.

### 1.0 INTRODUCTION

Sandalwood (*Santalum album L.*) is a prized gift of the plant kingdom woven into the culture and heritage of India (Kumar et al., 2012). It is one of the most valuable plant species in the world belonging to the family Santalaceae and the genus *Santalum*. There are around eighteen sandalwood species belonging to the genus *Santalum* which are; *S. freycinetianum*, *S. haleakalae*, *S. ellipticum*, *S. acumdatum*, *S. involutum*, *S. boninese*, *S. insulare*, *S. austrocaledonicum*, *S. yasi*, *S. macgregorii*, *S. peniculatum*, *S. murrayanum*, *S. obtusifolium*, *S. lanceolatum*, *S. fernandezianum*, *S. salicifolium*, *S. spicatum* and *S. album*. The global

distribution of the sandal family is between 30 degree N and 40 degree S from Indonesia in West to Juan Fernandez Island in the north to New Zealand in the south. These species are mainly found in India, Indonesia, Australia, Timor, Hawaii etc. The main reason for the economic and cultural value of sandalwood is the oil contained in the sandalwood timber, mainly from the heartwood. Heartwood oil content varies, however, widely between species and even within species (Subasinghe, 2013). Among the above mentioned species the East Indian sandalwood tree, *Santalum album L.*, is renowned for its oil, which is highly rated for its sweet, fragrant, persistent aroma and the fixative property which is highly sought after by the perfume industry and thus is identified as one of the most important aromatic tree in the world (Misra and Dey, 2012). *S.album*, is a small evergreen glabrous tree with slender drooping branches. The sapwood is white and odorless. The heartwood is yellowish brown and strongly scented. *Santalum* leaves are of dimension 3.8-6.3 by 1.6 -3.2, and elliptic lanceolate, Subacute glabrous and entire base acute base acute, petioles 1 – 1.3 cm long slender flowers, brownish purple induorous, in terminal and auxiliary paniculate cymes shorter than leaves. Perianth has campanulated limb of four, valvate triangular segments with four stamens, exerted, alternating with four rounded obtuse scales. Fruit is a Drupe, globose, 1.3 cm in diameter ;about the size of a pea; endocarp hard ribbed fruit concealed about size of a pea, spherical, crowned by rim like remains of perianth tube, smooth, rather fleshy, nearly black and seed solitary (Sindhu et al., 2010).

*Santalum album* accumulates mixture of sesquiterpenes and alcohols. These sesquiterpenes, predominantly  $\alpha$ - and  $\beta$ - santalols, constitute sandalwood oil, which is highly valued in the perfume and fragrance industry (Kulheim et al., 2014). The tree *Santalum album* is a native of southern India mainly Coorg, Chennai and Mysore. It generally occurs upto altitudes of 600-900 m. The tree attains the height of 18-20m and is actually an obligate semi parasite plant on various hosts (*Cassia siamea*, *Pongamia glabra* and *Lantana acuminata*). *Santalum album* requires a minimum of 500-650 mm rainfall per year. The finest wood is obtained from driest region particularly on red or stony ground while on rocky ground the tree often remains small but gives the highest yield of oil (Sindhu et al., 2010). Sandalwood trees do not normally begin to yield fragrant heartwood until approximately 10 years of age suggesting that oil accumulation is developmentally controlled and heavily dependent on age. Sandalwood grows relatively slowly and even similarly aged and spaced plantation trees vary widely in the amount of heartwood oil they contain. At harvest, entire trees are uprooted as the lower part of the stems and roots contain the highest amounts of heartwood essential oils.

(Kulheim et al., 2014). Medicinally *S. album* is useful in biliousness, fever and thirst and also used in the treatment of skin eruption and related skin diseases. It is commonly used in cosmetic and hair oil and is bitter, cooling, sedative, diuretic, expectorant, stimulant and astringent. Sandal wood oil relieves itching, heat, pruritus, Inflammation of the skin (Patil,2011). The hydrolysed exhausted sandalwood powder demonstrates, anti-inflammatory, anti-mitotic, anti-cancer, antihypertensive, anti-pyretic and sedative properties. possesses anti viral activity against Herplex simplex virus and anti-*Helicobacter pylori* properties, the causative organism for gastric cancer and peptic ulcer (Misra et al., 2012).

Sandalwood seeds are an important source of oils of nutritional, industrial and pharmaceutical importance. The suitability of oil for a particular purpose is however determined by its characteristics and fatty acid composition. Oil from any source any single source has not been found to be suitable for all purposes as oils from different sources generally differs in their fatty acid composition. Fatty acids are utilized in a wide variety of end-use industries that include food, medicine, rubber, plastics, detergents and cosmetics. Fats and oils make up the greatest proportions of raw materials in the chemical industry (Minzangi et al., 2011). *S. album* annually bears a drupe with a hard shelled seed and each tree can produce 1-2 kg of seeds from 3 years of planting. The seed is contained within a hard shelled endocarp, inside a softer pericarp and yields about 50 – 60% of a drying fixed oil. The pale yellow fixed oil is characterized by the presence of a high percentage of an unusual acetylenic fatty acid, the major one being trans-xymenynic acid which occurs only in Santalaceae and Oleaceae families. It has also been reported that xymenynic forms triximenynic glycerides. (Dhanushka et al., 2012, Liu et al., 1997). Santalbic acid (trans 11-octa-decen-9-yonic acid), a major constituent of the seed inhibits gram positive bacteria and several pathogenic fungi. (Misra and Dey, 2013). Studies on unusual acetylenic fatty acids of *Santalum* seed oil genus began in the 1930s and most of them were identified by comparison with those found in seed oils of the *Ximenia* genus (Oleaceae), such as ximenynic acid, E-11- octadecen-9ynoic acid, a long chain acetylenic fatty acid. This rare ximenynic acid previously named santalbic acid, was then identified and reported in various genera of Santalaceae. Proximate and fatty acid composition changes in developed sandalwood (*S. spicatum*) seeds and separation and identification of ximenynic acid isomers in this seed oil as their 4,4-dimethyloxazoline derivatives have also been studied (Butand et al., 2008).

Initial pharmacological studies of seed oil of *S.spicatum* reveal that it does not cause any toxicity or pathological damage to mice, but reduces fat deposition in adipose tissue. However increased aspartate amino transferase enzyme in plasma suggested an increased hepatic activity. Increased n-3 and n-9 fatty acids and decreased arachidonic acid (n-6) were also observed suggested a stimulation of the delta 9- desaturase enzyme. Isolated xymenynic acid from *S. acuminatum* was reported to have anti- inflammatory properties on several rat peritoneal leukocytes. Further studies of Xymenynic acid found it to alter the cytochrome P-450 enzyme in rats, indicating a pharmacological change in the hepatic metabolism (Dhanushka et al., 2012). Studies have also found that rats and mice that consumed sandalwood seed oil deposited less fat on adipose tissue (under the skin) than the control group. Researchers have identified that ximenynic acid produces micro-vascular constriction activity or otherwise increased blood circulation in the skin. The ethyl ester of xymenynic acid has been associated with micro-vascular kinetic properties which could be beneficial in treatment of cellulitis, hair loss and varicose veins. (Dhanushka et al., 2012) Studies have also shown that a highly purified xymenynic acid increases cellular detoxification, anti-oxidation capacity. It leads to a strengthening of the Extra Cellular Matrix (ECM), increases dermal strength and improves skin elasticity.

As the global demand for novel cosmetic agents is ever increasing, sandal wood seed oil could enter the market as a cosmetic ingredient that could also act as a vehicle for other oil-soluble agents. Studies continue on oxidative stability and pharmacological effects such as anti-inflammatory and cytotoxicity to extend knowledge of sandal wood seed oil as an acceptable pharmaceutical and cosmetic ingredient (Dhanushka et al., 2012). Acetylenic acids such as xymenynic acid are known to interfere with fatty acid metabolism in a variety of tissues. Seed oil of *S. album* may be a good source of xymenynic acid for cyclo-oxygenase and lipoxygenase enzyme studies (Butand et al., 2008). Hence, an attempt is made to assess the fatty acid methyl esters.

## 2.0 MATERIALS AND METHODS

### 2.1 Seed materials

*Santalum album* seeds were procured from a farmer. They were harvested in October 2014 from a three year old tree and at the time of study they were stored for one month.

## 2.2 Solvent extraction of sandalwood seed oil

The outer husk (Epicarp and Mesocarp) was removed from the seeds manually (Fig. 1). The seeds used in this study consisted of the outer hard shell (Endocarp) and the inner oil-rich kernel. These seeds were crushed manually using a pestle and mortar. The oil was extracted using a Soxhlet apparatus. 10 g of the crushed seed material was mixed with hexane as the extraction solvent. the extraction process lasted for two hours. The solvent was evaporated to dryness using a rotary vacuum evaporator. The oil yield was noted and was refrigerated until further analysis.

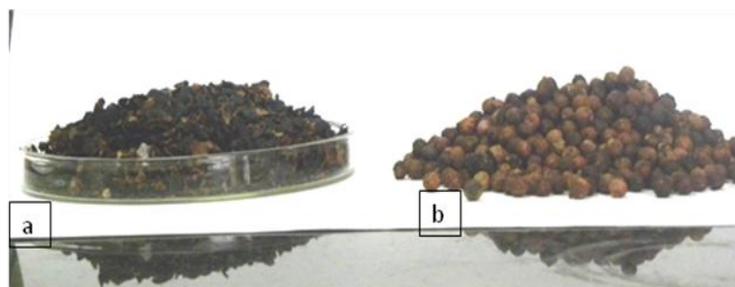


Fig 1: a)Sandal wood Seed coats b) Sandal wood seeds (De shelled)

## 2.3 Physico-chemical analysis

Freshly distilled oil was assessed for its organoleptic (color and odor) and physico-chemical parameters (specific gravity, refractive index, acid value, ester value and saponification value) in order to evaluate the quality of the oil.

### 2.3.1 Physical parameter Analysis

#### 2.3.1.1 Organoleptic properties

The oil was placed in a transparent bottle over a white background and the color and clarity were observed. The characteristic odor was determined by smelling the strip dipped in the oil.

#### 2.3.1.2 Specific gravity

Specific gravity is an important criterion of the quality and the purity of the oil. The actual weight of the empty pycnometer was accurately determined. It was first filled with water and weighed. Then the procedure was repeated for oil. The specific gravity is expressed as the ratio of the weight of the volume of the oil to that of an equal volume of pure water when both are determined at 25°C.

### 2.3.1.3 Refractive index

The digital refractometer was cleaned with lint free tissue paper and calibrated to zero reading with distilled water. Later the distilled water was replaced with oil and refractive index at 25°C was recorded.

## 2.3.2 Chemical parameter analysis

### 2.3.2.1 Acid value

For the determination of the acid value, 1.5g of absolute oil was weighed accurately into a saponification flask to which 10ml of absolute alcohol was added and surface heated for about two minutes. Three drops of phenolphthalein indicator was added and titrated against 0.1N KOH with continuous agitation until the first appearance of the pale pink color. This was considered as the end point and the acid value was calculated using the following formula.

Acid value= [Titer value x 56.11 x 0.1/weight of the oil (g)].

### 2.3.2.2 Ester value (Saponification value)

About 1.5g of absolute oil was weighed accurately into a saponification flask to which 10ml of neutral 95% alcohol was added and surface heated for about two minutes. Three drops of phenolphthalein indicator was added and titrated against 0.1N KOH until a pale pink color appeared. After adding 25mL of 0.5N alcoholic KOH, a reflux condenser was connected and was then heated on a boiling water bath for two hours. At the completion of the time period it was cooled and then excess alkali was titrated with 0.5N hydrochloric acid. A blank was performed and the total ester was calculated using the following formula.

Ester value= (Blank value – Titre value) x 56.11 x 0.5/(weight of the oil (g)).

## 2.4 Fatty acid analysis

The fatty acids were analysed as their respective fatty acid methyl esters (FAMES) by acid catalyzed trans-esterification. In a conical flask 0.5g of oil was taken. 100 ml of methanol and 7-8 drops of concentrated hydrochloric acid was added to the mixture. The flask was attached to a condenser and refluxed for 2 hrs at 100°C. The above mixture was then transferred to a separating funnel. The conical flask was washed with distilled water and this step was repeated again. The washings were collected into a separating funnel. Diethyl ether was added to the separating funnel and was shaken well and allowed to settle. The diethyl ether layer was collected into a beaker. The aqueous layer was again washed with ether and all the

diethyl ether layer was pooled together and evaporated to dryness to get the fatty acid methyl esters.

### 2.5 Gas Chromatographic analysis

FAME analysis was carried out in a Gas-Liquid chromatography (Perkin-Elmer) equipped with Flame Ionization Detector, dimethyl polysiloxane column. Sample volume of 0.1  $\mu$ L with the split less mode of injection was used. Nitrogen was used as a carrier gas with the flow rate of 1mL/min. Initial Oven temperature was programmed at 180°C, held for 0 minutes, then raised to 200°C at the rate of 2°C/min and held for 2 minutes, finally raised to 225°C at the rate of 4°C/min and was held for 5 minutes. Temperature of the Injector and detector was maintained at 275 and 300°C respectively. The components were identified by comparing the retention time with those reported in the literatures. Percentage of individual component was calculated based on the GC peak areas.

### 3.0 RESULTS AND DISCUSSION

Sandalwood seed oil is used in medicinal (cure for arthritis, pain reliever) and cosmetic (heals sores, skin lesions, burns, protects skin) field. In the current study, the physico-chemical parameters and fatty acid composition for the seeds of *Santalum album* Linn. belonging to the family Santalaceae have been evaluated. The solvent extraction of seeds using hexane as the extraction solvent yielded 27.8% of pale yellow colored, highly viscous oil which was then subjected to physico-chemical parameter analysis. The purity and quality of oil was assessed in terms of specific gravity, refractive index, acid value, ester value and saponification value that serves to detect adulteration as well as for identification of valued oils (Vijayalakshmi *et al.*, 2010). Also physical-chemical properties of triglyceride and its applications depends upon fatty acid constituents in molecule and are very important in the determination of the components of seed oil (Botinestean *et al.*, 2012). Refractive Index of an oil increases with the increase in unsaturation and chain length of fatty acids and it is correlated to the molecular weight (Gunkel and Fraser, 2010). Refractive Index of sandalwood seed oil was found to be 1.4898 and specific gravity was found to be 0.9406. Sandalwood seed oil has a high level of freshness as it has very low acid value i.e., 0.748. The saponification value gives an indication of the nature of fatty acids in the essential oil since longer the carbon chains the less acid is liberated per gram of fat hydrolyzed and the value was found to be 157.856. All the results (Table 1) were on par with the literature data (Ananthapadmanabha, 2011). It is known that individual fatty acids can be identified by Gas chromatography because of their

different retention time, the sandal wood seed oil were esterified using acid catalyzed transesterification and transforming the fatty acid into fatty acid methyl esters (FAME). The gas chromatographic studies of FAME revealed two major peaks at 18.084 and 21.802 minutes which were identified as oleic acid and xymenynic acid respectively. (Table 2) Seed oil of *Santalum album* contained 10.07% oleic acid (C<sub>18:1</sub>; Mono unsaturated fatty acid) and 83.95% of xymenynic acid (C<sub>18:2</sub>; Poly unsaturated fatty acid) which represented about 94% of the relative percentage of the oil. (Fig. 2) This is similar to the results reported by (Butand et al., 2008) where, *S. spicatum* seed oil has only 33.4 – 40.34% of xymenynic acid where as *S.album* contains very high percentage of xymenynic acid 75 – 82.8%. xymenynic acid is a rarest fatty acid found in the plant kingdom (Dhanushka, 2012). xymenynic acid is an effective anti-inflammatory agent, which attributes to the indigenous use of the sandalwood seed kernel. This unique activity resulted reducing cellulitis, oiliness (sebum secretion), and hair loss, thus this novel finding has opened the doors of the cosmetic world for xymenynic acid extractable from *Santalum album*. There are a number of patented formulas where xymenynic acid is used in cosmetic formulations for hair-loss, anti-aging and cellulitis, based on control clinical studies. Several cosmetic preparations claim to have plant extracts and powders containing xymenynic acid. Till now oleic acid has been the major fatty acid in most of the commonly used cosmetics oils such as olive and almond. (Dhanushka, 2012).

**Table 1: Physico-Chemical parameter analysis of *Santalum album* seed oil.**

Sl.No	Parameter	Reference Value (The wealth of India, 1992; Ananthapadmanabha, 2011)	Sample
Physical parameters			
1.	Color	Yellow	Yellow
2.	Texture	Viscous	Viscous
3.	Specific gravity@ 25°C	0.9356	0.94061
4.	Refractive Index @ 23.3°C	1.4891	1.4898
Chemical parameters			
1.	Acid value	NA	0.748
2.	Ester value	NA	157.108
3.	Saponification value	176	157.856

(NA: NOT AVAILABLE).

**Table 2: Gas chromatographic report.**

Si. No	Components identified	Retention time (min)	Area %
1.	Oleic acid	18.084	10.07
2.	Xymenynic acid	21.802	83.95

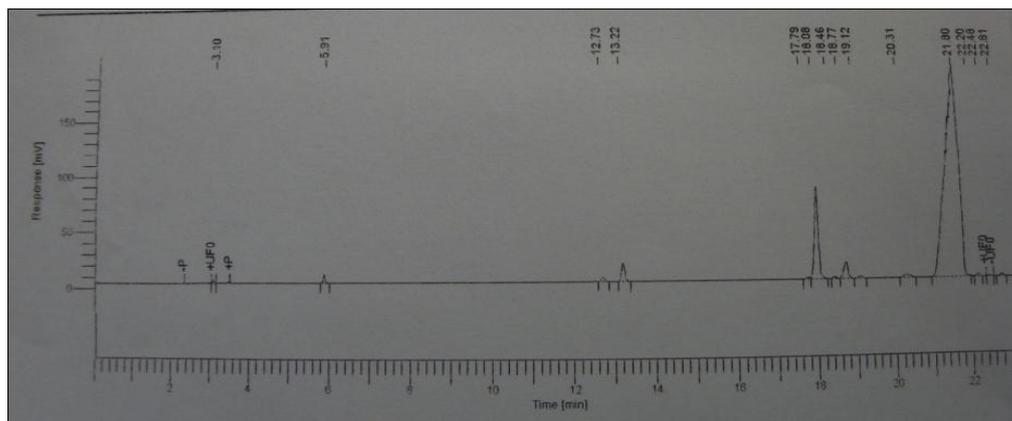


Figure 2: Gas chromatograph of *Santalum album*. L seed oil.

#### 4.0 CONCLUSION

From the large number of papers published so far it is clear that not only *Santalum album* seed oil possesses interesting pharmacological potential for practical purposes but also has great potential for various applications in cosmetics and others such as surface coating and low pour point bio diesel. From the current study it can be concluded that the Indian Sandalwood *S.album* seed oil is an excellent source of xymenynic acid and oleic acid, an essential fatty acid and is hitherto unexploited. Present study highlights the seed oil of *Santalum album* L. to be a good source of xymenynic acid.

#### 5.0 REFERENCES

1. Arun KAN, Geeta J and Mohan Ram HT, Sandalwood: history, uses, present status and the future. *Current science*, 2012; 103(12): 1408 – 1416.
2. Ananthapadmanabha HS, Sandal wood, *The fragrance of the World*, Navbharath Press, 2011; 24.
3. Biswapriya BM, Satyahari D, Phytochemical analysis and evaluation of antioxidant efficacy of in vitro callus extract of East Indian sandalwood tree. *Journal of pharmacognosy and phytochemistry*, 2012; 1(3): 7 – 16.
4. Butand JF, Raharivelomanana P, Bianchini JP, Gaydou EM, *Santalum insulare* Acetylenic fatty acid seed oils: Comparison within the genus, *J Am Oil Chem Soc.*, 2008; 85: 353 – 356.
5. Biswapriya BM, Satyahari D, 2013. *Biological Activities of East Indian Sandalwood Tree, Santalum album*, Peer J Pre Prints.
6. Cristina B, Nicoleta GH, Daniel IH, Ionel J, Fatty acid composition by Gas Chromatographic Mass Spectrometry (GC-MS) and most important physical-chemicals

- parameters of Tomato seed oil. *Journal of Agroalimentary processes and technologies*, 2012; 18(1): 89 – 94.
7. Carsten K, Christopher GJ, Julie AP , Emilio LG , Liz B , Jorg B, Foliar application of methyl jasmonate does not increase terpenoid accumulation, but weakly elicits terpenoid pathway genes in sandalwood (*Santalum album* L.) seedlings, *Plant Biotechnology*, 2014; 31: 585–591.
  8. Dhanushka S, Hettiarachchi, Yandi L, Sonia J, Michael R B, John EDF and Bruce S, Assessment of western Australian sandalwood seeds for seed oil production. *Australian Forestry*, 2012; 75(4): 246 – 250.
  9. Gunkel W, Fraser LC, *Hand Book of Essential Oils, Science & Technology and Applications, Volume 1*, CRC press publications, New Delhi, 2010; 156 – 162: 330 – 332.
  10. Misra B.B and Dey S, Comparative phytochemical analysis and antibacterial efficacy of in vitro and in vivo extracts from East Indian sandalwood tree (*Santalum album* L.).*Letters in Applied Microbiology*, 2012; 55: 476 – 486.
  11. Minzangi K, Kaaya A.N, Kansime F, Tabuti J.R.S, Samvura. B and Grahi Nialsen. O, Fatty acid composition of seed oils from selected wild plants of Kahuzi- Biega national park and surrounding, democratic republic of Congo, *African Journal of Food science*, 2011; 5(4): 219 – 226.
  12. Rakesh K. Sindhu, Upma, Ashok Kumar, Sahil Arora, *Santalum album* Linn: A review on morphology, phytochemistry and pharmacological aspects. *International journal of pharm tech research*, 2010; 2(1): 917 – 919.
  13. Subasinghe S.M.C.U.P., *Santalum Research: A global perspective*. *Journal of tropical forestry and environment*, 2013; 3(1): 1 – 8.
  14. Vaishali Patil, *Pharmacognostical study on the seed of Santalum album Linn*. *International Journal of Pharm Tech Research*, 2011; 3(3): 1600 – 1602.
  15. Vijayalakshmi. A, Rakesh Tripathi and Ravichandiran. V, Characterization and evaluation of anti dermatophytic activity of the essential oil from *Artimisia nilagirica* Leaves growing wild in Nilgiris. *Int. J. Pharm. Pharm Sci*, 2010; 2(4): 93 – 97.
  16. Yandi D. Liu, Robert B. Longmore, Michael R. Boddy and John E. D. Fox, Separation and identification of triximenynin from *Santalum spicatum* R. Br. *JAOCS*, 1997; 74(10): 1269 – 1272.
  17. *The wealth of India, A dictionary of Indian raw materials & Industrial products*, Publication & information directorate, CSIR, New Delhi, 1992; 9: 223 – 224.