Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(1): 2587-2592
Received: 19-11-2018
Accepted: 22-12-2018

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Pharmaceutical importance, physico-chemical analysis and utilisation of Indian sandalwood (*Santalum album* Linn.) seed oil

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Abstract
The *Santalum album* L. of genus *Santalum* and oil obtained from has got more commercial value than other species in the world. Traditionally, sandalwood oil has unique stability, and provides perfume with its best fixative property. Sandalwood oil has antipyretic, antiseptic and diuretic properties. The oil has an important place in the indigenous system of medicine. Indian sandalwood tree is becoming endangered, and measures to sustain this valued bio resources, took prominence and flaws in policy which endangered the species were amended. Government of Karnataka promulgated an amendment to Karnataka Forest Act in 2001 to encourage private domestication of sandalwood as a means to conserve and enhance the status of this resource. With all these positive measures the private growers have taken up more farming of sandalwood and in continuation to this positive measures sandalwood seeds are also available in plenty and could be collected in huge quantities and presently these seed and oil obtained from it has limited use. On an average, the 3-5 year old plant could produce around 2-3 Kgs of sandalwood seeds and the matured trees of 8-10yrs of aged tree can produce around 4-6kgs of sandalwood seeds per tree. The seed index analysis experimented shows that on an average there are around 4000 -4500 number of seeds in one Kg and each seed weigh around 0.23gms. The average yield of oil is around 35-40%. The seed oil possesses diuretic, anti-tremorogenic, antiviral and hypotensive activities, lymphatic, treatment of skin conditions such as eczema, psoriasis, rash or other inflammation. Sandalwood Seed Oil is a rich source of a natural and highly stable, acetylenic fatty acid called Ximenynic Acid, with known pharmacological results with its anti-aging property. The physicochemical investigations and its pharmaceutical importance of seed oil extracted from 6 year old plant are attempted in this research work.

Keywords: Sandalwood seed oil, anti-aging, seed index, ximenynic acid, *Santalum album*

Introduction
Sandalwood (*Santalum album* Linn.) known as Chandana in India, also known as the East Indian sandalwood is commercially an important tree species in peninsular India because of its high economic value and is the best endemic tree in the world. The average yield of oil ranges from 2-6% w/w of heartwood content. The volatile oil distilled from Indian Sandalwood derived from the heartwood is colourless to yellowish viscous liquid with heavy sweet odour (*Anon*, 1972) [2]. The top note (the first aroma you smell) is very soft with a sweet-woody, balsamic body note and a long-lasting bottom note which makes this oil as a good perfume. The sweet powerful and lasting odour makes Sandalwood oil useful in perfume industry. The fragrant parts of sandalwood oil constitutes of α- and β-santalol. For more than five decades, India has been the traditional leader of sandalwood oil production for perfumery and pharmaceuticals.

Traditionally, sandalwood oil has unique stability, and provides perfume with its best fixative property. The heartwood, sandalwood's fragrant timber, has long been used traditionally in important religious ceremonies not only in India but in many parts of the world (Srinivasa Rao *et al*., 2016) [17]. Sandalwood oil has antipyretic, antiseptic, anti-scabietic, and diuretic properties. It is also effective in the treatment of bronchitis, cystitis, dysuria and diseases of the urinary tract. Markets worldwide are currently facing a serious shortage of this increasingly expensive, high quality Indian sandalwood (*Santalum album*) – largely as a result of unsustainable harvesting of the species over a long period of time in its native environments of India (Venkatesha Gowda, 2011) [21].

Indian sandalwood tree is becoming endangered, and in an attempt to curb its possible extinction increased interest by both forest departments and private growers towards expansion of plantations has added to the resource building of this valuable tree (Soundararajan *et al*.,

2015) [18]. The use of Sandalwood oil in treatment of skin problems is legendary. It is an excellent moisturizer and nourishes all type of skins. The astringent, anti-inflammatory, antiseptic, and pain relieving properties of sandalwood have been put to good used in healing wounds, scars, and acne (Soundararajan *et al*., 2017) [19].

Sandalwood plants are widely distributed in Southern parts of peninsular India mainly in the States like, Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. East Indian sandalwood, *Santalum album* L., is the queen among the species yielding supreme quality essential oil much required by domestic and international perfume and allied industries. India realised the value of sandalwood trade and took positive steps to protect the natural population of sandalwood. Measures to sustain this valued bio resources, took prominence and flaws in policy which endangered the species were amended (Venkatesha Gowda, 2011; Srinivasa Rao *et al*., 2016) [21, 17].

It has been reported that Sandal seed oil could be reacted with zinc chloride, yielding a dark plastic solid which when dissolved in benzene forms an ideal base for insulation tapes. It reacts with sulphur at 220° C yielding a dark sticky rubber like product, suggesting it to be vulcanisable oil. Resins like colophony and copal could be dissolved in oil at 200 °C producing an orange coloured varnish, which may be used in the manufacture of pigmented enamels. By partial hydrogenation a semidrying oil of utility in soap industry could be obtained. A number of surface active products having excellent foaming capacity and foam suitability products which are commercially valuable as foam boosters, germicides plasticizers emulsifying, thickening and wetting agents and for use in liquid detergents have been reported (Desai *et al.,* 1990) [5].

Sandalwood seed oil contains a fat rich in santalbic acid (Ximenynic Acid), which is an acetylenic acid. Partial hydrogenation using Lindlar's catalyst has yielded an oil containing 80% of C18:1 and C18:2 fatty acids, 13% of C18:3, 6% C20 fatty acids and traces of Lauric acid(C12), Palmitic acid (C16) Stearic acid (C18) fatty acids making it more semi drying oil in the process (Shankaranarayana, 1979) [14].

Sandalwood Seed Oil in its triglyceride form needs to be hydrolysed to release the active free Ximenynic Acid. Santalbic acid (Ximenynic Acid) in its free form shows anti-inflammatory activity and a low level of toxicity. Upon hydrolysis, the majority of the free fatty acids are of oleic acid, a common ingredient in many cosmetic preparations. Hydrolysed Sandalwood Seed Oil exerts the effects of Ximenynic Acid while balancing the emollient and soothing effects of oleic acid Sandalwood Seed Oil is a reliable source of Ximenynic Acid which is an established anti-inflammatory lipid. This rare active oil can be formulated and delivered in a similar manner to normal carrier oils, having excellent stability and favourable physicochemical characteristics (Hopkins, *et al.,* 1969; Shankaranarayana, 1979) [4, 14].

Fatty oils possessing an exorbitant amount of unsaponifiable matter are commercially disadvantageous. Sandalwood seed oil contains 55-60% of a drying oil highly rich in unsaponifiable matter (7-17%). The separation of unsaponifiable matter (8.8%) in the seed oil has been estimated and separation procedure has been standardised (Shankaranarayana, 1988) [15]. The researcher has also attempted to estimate the fatty oil composition in sandalwood seed coat. It is reported that the seed coat of sandalwood contains 16% liquid fatty oil composed of palmitic acid (34%), oleic acid (44%) and Santalbic (20.3%) in benzene elute of petroleum ether extract (Shankaranarayana, 1988) [15]. It is reported that the sandalwood seeds from young trees (aged around 10yrs) are as much potentially rich in fatty oil, proteins and minerals as the seeds from the mature trees (age more than 30yrs). Deoiled seed mean, made from even young trees could be of utility as animal feed stuff (Shankaranarayana, *et al.,* 1990) [16].

Seed oils of five species of the family Santalaceae, not studied previously, were examined to determine the nature of the fatty acid components. Ximenynic acid was the main component of *Santalum yasi* oil. Infrared spectra and other data were obtained for six additional species of Santalaceae. The distribution pattern of acetylenic and other fatty acids in this family is discussed in relation to the classification of the genera (Hopkins *et al*. 1969) [4].

Materials and Methods

Seed materials

Sandalwood seeds were procured from Dhabadi village of Malegaon taluk in Nasik district of Maharashtra state from a progressive farmer. They were freshly harvested in 2016 from a 6 year old tree and were procured for the study purpose.

Seed Index evaluation

Three sets each containing 100 seeds were selected and analysed for their seed index evaluation. Each seed has been measured for their weight and size. Descriptive statistical analysis was done to evaluate and understand the seed yield and other seed parameters as it's the final product and many traits contribute to its performance.

Extraction of sandalwood seed oil

The classical Soxhlet extraction method provides the fundamental basis for a modern-day solvent extraction system. Normally crushed seeds are placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent i.e. Petroleum ether (60-80). The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of sample. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material to complete the extraction process (Anderson, 2004) [1].

Physico-Chemical Analysis

The oil is viscous, slimy pale greenish yellow and very smooth in nature. Sandalwood seeds could be of significant utility in cosmetic and pharmaceutical industries. The oil for a particular purpose is however determined by its characteristics and fatty acid composition. The physical and chemical parameters of the oil were analysed.

Refractive Index

Refractive Index is the ratio of the velocity of light (of specific wavelength) in air to the velocity in the substance of sample. Refractive Index may also be defined as the Sine of the angle of incidence divided by the Sine of the angle of refraction, as light passes from air into the sample. Refractive Index is a fundamental property used in conjunction with other properties to characterize hydrocarbons and their mixtures. To determine refractive index of the oil, accurate to one unit in the fourth decimal place, ABBE's Refract meter

with a monochromatic (sodium) light source is used. This method applies to refractive indices in the range between 1.33 and 1.60.

Specific gravity

Estimation of specific gravity of oil is very important quality criterion for its assessment of purity. The specific gravity of a substance is a comparison of its density to that of water. The Specific Gravity (SG) - is a dimensionless unit defined as the ratio of density of the material to the density of water at a specified temperature. The actual weight of the empty specific gravity bottle is determined and followed by weight of water and the sample i.e. sandalwood seed oil. It is expressed as the ratio of density of the sample to the density of water at the specified temperature.

Acid value

The acid value (AV) *is* the number that expresses, in milligrams the quantity of potassium hydroxide (KOH) required to neutralize the free acids present in 1 g of the substance. The acid value may be overestimated if other acid components are present in the system, *e.g.* amino acids or acid phosphates. The acid value is often a good measure of the breakdown of the triacylglycrols into free fatty acids, which has an adverse effect on the quality of many lipids.

Significance

Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid.

- Edible oil contain > 1%
- Pharmaceutical oil must not have any acidity.

For the determination of the acid value, 1.0gm of sandalwood seed oil was weighed accurately into a 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 colour. This was considered as the end point and the acid value was calculated using the following formula.

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

Saponification Number

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance. The saponification number is a measure of the average molecular weight of the triacylglycerols in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present i.e. Saponification value is inversely proportional to the mean molecular weight of fatty acids (or chain length).

$$\text{Saponification value} = \frac{56.1\ (B\text{-}S) \times N \text{ of HCl}}{\text{Weight of oil}}$$

B: ml of HCl required by Blank.
S: ml of HCl required by oil

Analysis of fatty acid methyl ester of sandalwood seeds

A simple one-step extraction and trans-methylation of lipids in sandalwood seed was attempted. 15 g of fresh flower sample was refluxed with 100 mL of a mixture containing Methanol: Benzene: Hexane: Aluminium chloride: Sulphuric acid in a ratio (37:20:36:5:2) for 30 minutes in a one neck-flask. The resulting mixture was allowed to cool and then filtered.

The supernatant was separated in a separating funnel and the organic layer washed with distilled water to neutrality. The organic layer was concentrated to obtain the trans methylated product: fatty acid methyl esters (FAMEs) and the yield noted.

Results and Discussions

In the current study, the seed index evaluation, the physico-chemical parameters and fatty acid composition for the seeds of *Santalum album* Linn procured from 6th year plants have been evaluated.

Physico chemical analysis

In this study, the outer husk of sandalwood seeds were removed from the seeds manually and the ratio of inner oil rich-kernel to husk was also estimated (Table. 3). The average inner oil rich kernel: shell (endocarp) ratio is 71:29.

Table 3: Estimation of kernel: husk ratio

Description	Set-1	Set-2	Set-3	Average values
Weight of 100 seeds (in gms)	22.94	24.34	23.22	23.5
Wt. of kernel obtained (in gms)	16.19	17.31	16.55	16.68
wt of seed shell (endocarp) obtained (in gms)	6.75	7.03	6.67	6.82
% of kernel (oil rich portion) ratio	70.58	71.12	71.28	70.99
%of seed shell (endocarp) ratio	29.43	28.88	28.73	29.01

The kernel portion of sandalwood seeds were further crushed manually using a pestle and mortar. The oil was extracted using a Soxhlet apparatus as described. 500 g of the crushed kernel material was mixed with Petroleum ether (60-80) and extracted for 6 hours. The solvent was evaporated to dryness using a rotary vacuum evaporator. The oil yield was noted and was refrigerated for further analysis. The average yield of oil was estimated on kernel basis and seed basis and it was 43.20%, 42.268 and 41.644% respectively (Table 4)

Table 4: The physico-chemical characteristics of the sandal wood seed oil

Sl. No.	Description	Characteristics features of the sample analysed
1.	Colour	Pale greenish yellow
2.	Appearance	Viscous, Thickens
3.	Specific gravity	0.93
4.	Yield of oil	The average yield of oil was estimated on kernel basis and seed basis and it was 43.20%, 42.268 % and 41.644% respectively.
5.	Refractive Index	1.4885 @ 25°C
6.	Acid value	3.35
7.	Saponification value	168
8.	Iodine value (Hanus)	132

The qualitative evaluation of sandalwood seed oil was examined in terms of physic-chemical parameters (Table 4) like specific gravity, refractive index, acid value, ester value and saponification value which serves to detect adulteration as well as for identification of valued oils (Vijayalakshmi *et al*., 2010) [22].

Refractive Index is a very important parameter of oil which 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 1.4885 @ 25°C and specific gravity was found to be 0.93.

The acid value indicates the freshness of fatty oil and also is indicative about its shelf life properties. Lower the acid value the quality of the oil will be fresher. 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 168.

Fatty acid analysis

The methyl ester conversion of sandalwood seed oil has been done with a different method which afforded the extraction, hydrolysis and methylation of the lipid in a single procedure is a slight modification of the method previously described (Olubunmi *et al.*, 2015; Garces and Mancha, 1993) [13, 6]. The chromatographic analysis (Fig.1) of methyl esters of sandalwood seed oil reveal 6 peaks with two major peaks at 15.01 and 31.21 minutes which were identified as oleic acid and xymenynic acid respectively.

The seeds collected from the 6 year old tree and the oil extracted was subjected to one step direct conversion. The methyl ester of the seed oil obtained contained 9.51% oleic acid (C18:1; Mono unsaturated fatty acid) and 84.91% of Santalbic acid (Xymenynic acid) (C18:2; Poly unsaturated fatty acid) which represented about 94.42% of the relative percentage of the oil. Xymenynic acid is an effective anti-inflammatory agent, which attributes to the indigenous use of the sandalwood seed kernel.

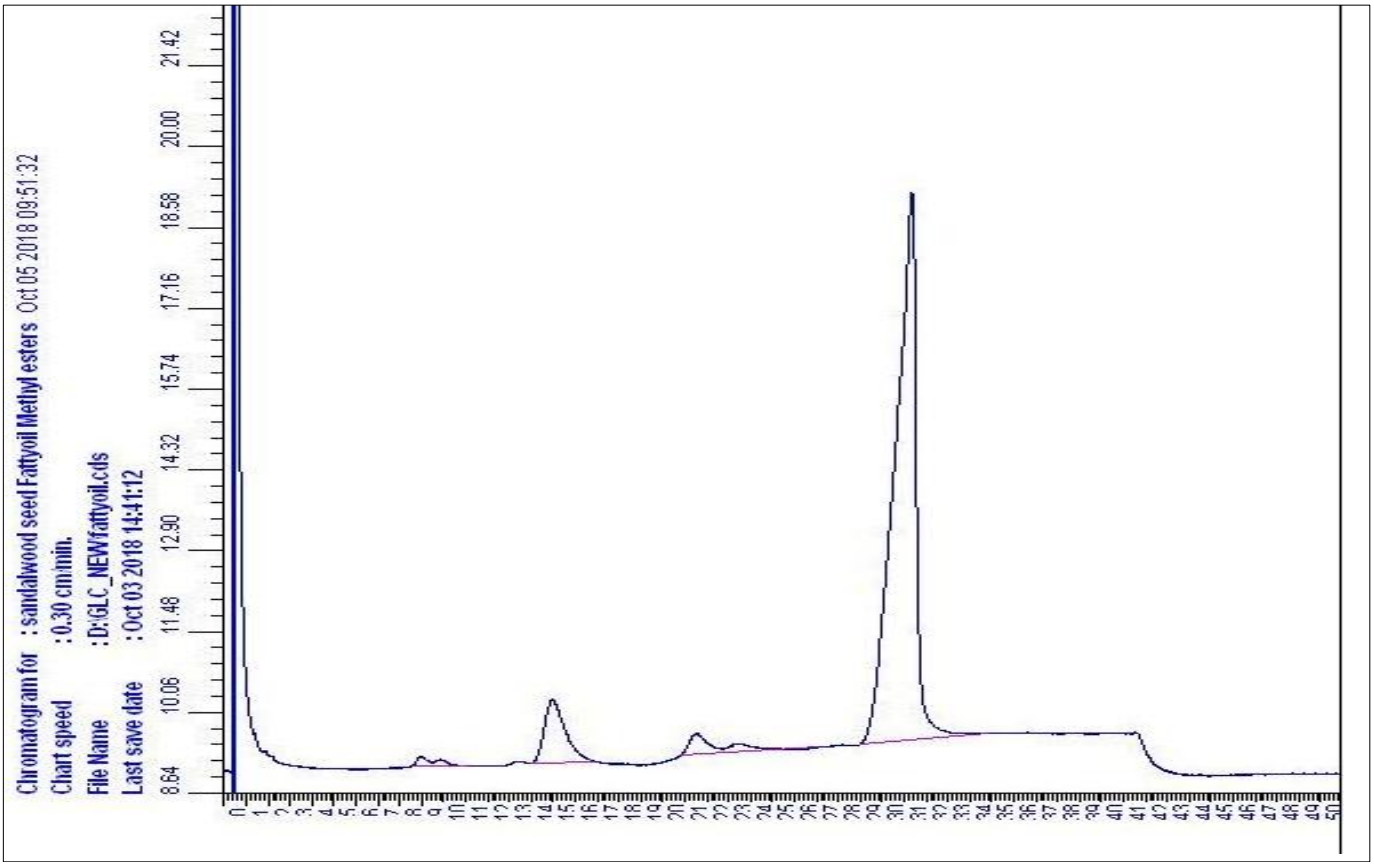

Fig 1: Chromatogram of Methyl esters of fatty oil of sandalwood seed oil

Seed weight and size estimation

The information on seeds per plant and seed weight exerts maximum positive indirect effect on biological yield per plant and harvest index (Heitholt *et al*., 1985) [8]. It is well established that seed yield is the final product and many traits contribute to its performance.

Descriptive analysis is utilized to depict the fundamental features of the data in the study. The descriptive analysis of data provides the primary evaluations and outlines the data arranged in seed size and provides information about the variability or uncertainty in the data. It also expresses indications of unexpected patterns and perceptions that should be considered while doing formal analysis.

The focal tendency of a distribution is an estimate of the "middle" of a distribution value. These are represented as Mean value. Each score in a distribution contributes to the determination of mean. It is otherwise known as arithmetic average. Mean is the average of all values in a distribution. The average or mean estimation of sandalwood seed is 7.4, 7.01 and 6.91mm respectively. The average or mean seed weights are estimated to be 0.229, 0.243 and 0.232 mg respectively (Table 5 & 6).

The median is the positional average that divides a distribution into two equal parts so that one half of items falls above it and the other half below it. In other words, the midpoint of a distribution of values is called the median. It is the point, below and above which 50% of the population lies. The Median is the score found in the exact middle of the set of values.

Table 5: Descriptive statistics analysis for seed size (in mm)

1st Set		2nd Set		3rd Set	
Mean	7.43	Mean	7.00	Mean	6.99
Standard Error	0.02098	Standard Error	0.02155	Standard Error	0.04183
Median	7.4	Median	7.01	Median	6.91
Mode	7.26	Mode	7.23	Mode	6.99
Standard Deviation	0.209809534	Standard Deviation	0.215535884	Standard Deviation	0.418377771
Sample Variance	0.04402004	Sample Variance	0.046455717	Sample Variance	0.17503996
Kurtosis	-0.42282759	Kurtosis	0.500208258	Kurtosis	-0.571175895
Skewness	0.225660853	Skewness	-0.373974563	Skewness	0.214372401
Range	1.05	Range	1.21	Range	2.02
Minimum	6.85	Minimum	6.24	Minimum	5.86
Maximum	7.9	Maximum	7.45	Maximum	7.88
Sum	742.96	Sum	700.22	Sum	699.38
Count	100	Count	100	Count	100
Largest(1)	7.9	Largest(1)	7.45	Largest(1)	7.88
Smallest(1)	6.85	Smallest(1)	6.24	Smallest(1)	5.86
Confidence Level (95.0%)	0.041630762	Confidence Level (95.0%)	0.042766994	Confidence Level (95.0%)	0.083015224
Average	7.43		7.00		6.99

Table 6: Descriptive statistics analysis for seed weight (in mg)

Column1		Column2		Column3	
Mean	0.22935	Mean	0.24339	Mean	0.23216
Standard Error	0.001906521	Standard Error	0.002635419	Standard Error	0.002212714
Median	0.2245	Median	0.238	Median	0.2315
Mode	0.216	Mode	0.218	Mode	0.216
Standard Deviation	0.019065212	Standard Deviation	0.02635419	Standard Deviation	0.022127144
Sample Variance	0.000363482	Sample Variance	0.000694543	Sample Variance	0.000489611
Kurtosis	3.210905927	Kurtosis	-0.800038212	Kurtosis	8.645289839
Skewness	1.431042014	Skewness	0.498751546	Skewness	2.113155205
Range	0.101	Range	0.101	Range	0.152
Minimum	0.197	Minimum	0.198	Minimum	0.201
Maximum	0.298	Maximum	0.299	Maximum	0.353
Sum	22.935	Sum	24.339	Sum	23.216
Count	100	Count	100	Count	100
Largest(1)	0.298	Largest(1)	0.299	Largest(1)	0.353
Smallest(1)	0.197	Smallest(1)	0.198	Smallest(1)	0.201
Confidence Level (95.0%)	0.003782952	Confidence Level (95.0%)	0.005229243	Confidence Level (95.0%)	0.004390505

Conclusion

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 (Hettiarachchi *et al.*, 2013) [9]. Acetylenic acids such as ximenynic 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 ximenynic acid for cyclo-oxygenase and lipoxygenase enzyme studies (Butaud *et al.*, 2008) [3]. Hence, an attempt is made to assess the fatty acid methyl esters of Indian sandalwood seed oil.

Indian Sandalwood Seed Oil is a reliable source of rich Santalbic acid (Ximenynic Acid) which is well known source of C18:2 Poly unsaturated anti-inflammatory lipid. Seed oil of Indian sandalwood (*Santalum album)* possesses excellent stability and favourable physicochemical characteristics and also pharmacological values with great potential for various applications in cosmetics and others anti-inflamatory creams (Li *et al*., 2013; Liu *et al*., 1996) [10, 11]. Sandal seed oil finds use in treatment of skin diseases. It exhibit diuretic activity, antitremorogenic activity and antiviral activity.

Partial hydrogenated sandal seed oil can also be reliable source for various surface active products having excellent foaming capacity and foam suitability products which are having commercial value as foam boosters, germicides plasticizers emulsifying, thickening and wetting agents and for use in liquid detergents.

Research interest to evaluate its pharmacological and cosmetic potential has developed recently perhaps on realization that sandalwood seed oil may contain novel chemicals that exhibit a wide range of pharmacological cosmetic effects (Mohammad *et al*., 2015) [12]. Hence, more studies are required on phytochemical, cosmetic and pharmacological/ biological importance of seed oil of this important species.

The seed oil possesses diuretic, anti-tremorogenic, antiviral and hypotensive activities, lymphatic, decongestant, treatment of skin conditions such as eczema, psoriasis, rash or other inflammation. Sandalwood Seed Oil is a rich source of a natural and highly stable, acetylenic fatty acid called Ximenynic Acid, with well documented pharmacological results with its anti-aging property.

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 ximenynic acid has been associated with micro-vascular kinetic properties which could be beneficial in treatment of cellulitis, hair loss and varicose

veins (Hettiarachchi, *et al.*, 2013) [9]. Studies have also shown that a highly purified ximenynic 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 (Vasundhara *et al.*, 2015) [20].

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, which are unexploited rather useful source to be taped for its value addition in the pharmaceutical and cosmetic industry. The seed index analysis experimented shows that on an average there are around 4000 - 4500 nos of seeds in one Kg of seeds and each seed weigh around 0.23gms. The average yield of oil is around 35-40%. It is highlighted about the potential availability of seed oil of *S. album* to be a good source of xymenymic acid and also additional income generation to the sandalwood growers as the seeds sets in right at 4th years onwards.

Acknowledgement

The authors are thankful to Director, Group Coordinator (Research) and other researchers of Institute of Wood Science & Technology for their kind guidance and help in this regard. The authors are also thankful to Maharastra forest department and farmers for their help to collection of *Santalum album* seed.

Abbreviation: gms – grams; % - percent; mm – millimetre; g- gram; C – Celsius; ml – millilitre; HCl - Hydrochloric acid.

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