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Analytical and Fourier transform infra red spectroscopy evaluation of sandalwood oil extracted with various process techniques

Omprakash H. Nautiyal
Institute of Chemical Technology (UDCT) Deemed University, NM Parekh Road, Matunga (E), Mumbai-400019, India
* Corresponding Author
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ABSTARCT

Sandalwood oil analytically examined as extracted by various techniques like hydro distillation, steam distillation, solvent extraction and subcritical carbon dioxide were evaluated for their quality parameters as business need for which the project was investigated. Steam distillation yielded the oil in 10h, hydro distillation yielded the oil in 30h, solvent extraction yielded the oil in 16h and subcritical carbon dioxide yielded the oil in 1h. Santalol content in the sandalwood oil extracted by the subcritical CO_2 was the highest. Organoleptic properties evaluated were remarkable in case of subcritical $CO_{2.}$ FTIR analysis has shown the sharp peaks for santalol and santalene in the oil extracted by subcritical $CO_{2.}$ Steam distillation and hydro distillations suffered the loss in yields for the reason of longer processing and solublization of major constituents in the distilled water.

Keywords: Sandalwood oil; Analysis; FTIR; Extraction technologies.

INTRODUCTION

The sandalwood is one of the oldest known perfumery materials and it has over 2000 years of uninterrupted history. A tract of sandal forests running from Kolhapur (Maharashtra) to Bellom (Mysore) an on to Chittoor (Andhra Pradesh) forms the northern fringe of this region, Chittoor to Erode and on to Mettuplayam (Tamil Nadu) forms the eastern and southern boundaries while the approximate western sandal border runs along from Mettupalayam through Kerala to Mercara and Mangalore (Mysore) then extending to Sagra and on to Kolhapur in the north to join the starting point (Salom, et al., 2003; Sedgley, 1982).

The quality and value of sandalwood oil is commercially determined by the level of α- and β-santalol. There are various methods of extraction currently used such as solvent extraction, Supercritical fluid extraction, water distillation and steam distillation. Making direct comparisons between studies using different means of oil extraction can be problematic given the variable results between them. Since steam distillation is the primary method used by industry using bulk wood samples, there is a need for a standardized ‘desk top’ method that gives equivalent results. In this study author compares the relative merits of four widely used methods of extraction and proposes a standardized method that may be developed for use across industry to allow for more accurate comparison between studies (Salom, et al., 2003; Sedgley, 1982).

With interest in improving product quality across all sandalwood markets there is also a need for rapid determination of α- and β-santalol in the extracted sandalwood oil. There are many potential applications of this technology for both sandalwood science and industry alike (Salom, et al., 2003; Sedgley, 1982).

Subcritical carbon dioxide extraction of sandalwood oil gave most interesting oil yields and since it was operated below its critical temperature (31.1^{o}C) and pressure (71.3 bars) that has brought the solvent density much higher as compare to Supercritical carbon dioxide. In the study the main emphasis was given to technologies that enabled to yield high quality oil with respect to snatalol and santalene contents. Hence to assess the quality physical parameters like acid value, SOR, and refractive index were utmost important. They were successfully determined for the various extracted oil and found to conform the commercial specifications.

Sandalwood Oil is an active substance of agreeable odor employed in the treatment of sub acute and chronic infections of mucous tissues, particularly gonorrhea after the active symptoms have been mitigated. Chronic bronchitis, with fetid expectoration, chronic mucous diarrhea, chronic inflammation of the bladder and pyelitis are also said to be benefited by it. It occasionally disturbs the gastro-intestinal tract, and, like copaiba, which it was introduced to supersede, it will occasion cutaneous eruptions. The dose ranges from 5 to 20 drops, in capsules or emulsion.

The aim of the present work was to ascertain the quality and physical properties of the extracted sandalwood oil by various techniques. Specifically subcritical carbon dioxide extraction a novel technology for obtaining the low volume high price products like sandalwood oil was exploited to investigate the yield and quality parameters. A sophisticated FTIR spectrum of sandalwood oil has shown the neat spectra. Though the more minor constituents of the oil could not be identified and hence the focus was centered on the yield of the santalol and santalene as they are the major constituents of the oil and decides the price of the oil in the national and/or international market for their usage in wide industrial applications. Organoleptic evaluations of the oil extracted by various techniques revealed that subcritical carbon dioxide extraction was far better than any of the other conventional techniques employed for the sandalwood oil recovery.

MATERIALS AND METHOD

The carbon dioxide gas (99.9%) was purchased from Indian Oxygen Limited. The heart wood of sandalwoods and pure sandalwood oil were provided by the MPMC Chennai, the sponsor of the researched project. The SC-CO_2 pilot plant was imported from UHDE GmbH Germany of 1 litre capacity of extractor and separator each. Figure1.

SC-CO_2 pilot plant was sponsored by DST (Government of India) from UHDE GmbH Germany.

Acid value, Specific optical rotation and refractive index of extracted sandalwood oil**:** It was determined as per the procedure mentioned in Guenther and confirm the commercial sandalwood oil.

Gas chromatography analysis: The extracted oil by all techniques was analyzed by gas chromatography (Perkin-Elmer-8500). Column specification and temperature programme are described as: column SE30(10%) on chromosorb W, column material S.S, column length 4m, internal diameter 1/8 inch, injector temperature 300^oC, FID temperature 300^oC, Flow rate of N_2 38ml/min., temperature programming 100-250^oC with 6^oC/min. of temperature. Its physical properties were determined using Bausch Lomb refractometer for refractive index. Figure 2 and 3 is the chromatograph of sandalwood oil extracted by subcritical CO_2.

***Characterization*:** Pulverized sandalwood powder was checked for its pharmacognosy in the department of pharmaceutical of ICT Mumbai. Microscopically observed pharmacognosy shown to exhibit xylem and vessels containing oils.

Fourier transform infra red spectroscopy: FTIR scan of the sandalwood oil was done using Brookes IFS model. Characteristic transmittance of α, β-santalol and α, β-santalene is shown in the Figure 4. Region 2500-3500 cm^{-1} characterizes the presence of C-H and O-H stretching, 1400-1600 cm^{-1} the presence of C=O stretching. Figure 4 is the spectra of sandalwood oil extracted by subcritical CO_2.

RESULTS AND DISCUSSION

Subcritical CO_2 yielded oil determined to contain 82.5% santalol and 2.56% santalene, benzene extracted oil contained 42.99% santalol and 9.49% santalene, diethyl ether extracted oil contained 72.19% santalol and 2.04% santalene, ethyl alcohol extracted oil contained 83.56% santalol and 1.56% santalene, hydro distilled oil contained 52.59% santalol and 3.43% santalene, hydro distilled (alkaline) oil contained 56.79% santalol and 7.26% santalene, steam distilled oil contained 84.32% santalol and 2.57% of santalene. Table 1 shows the oil composition as analyzed for oil extracted by various techniques. Table 2 data are the determined values by the method mentioned in Guenther essential oil book (Sindhuveerendra, 1989; Van Tuyal, et al., 1989). Sandalwood heart wood and powder used for the studies provided by the company from Mysore, India, was quite fragrant and camphorous found rich in oil with good content of major constituents (santalol and santalene).

Interestingly subcritical CO_2 found to contain the oil yield recoverable only in four h as compared to other technologies. Oil recovered by other techniques was less superior in organoleptic properties. Although, hydro distillation with alkaline treatment, yielded the oil that was found to analyzed for containing the high content of sanatlene than those obtained by the other techniques. But quality of sandalwood oil fetches the market price for its snatalol contents. Subcritical CO_2 is safe and greener technology for the business and may be more economical for low volume high price business (Wise, et al., 2006; Jefferies, et al., 1982; Baldovini, 2011).

Non volatile compounds 9(*E*)-11-Hydroxy-α-santalol, (2*S*,7*R*)-2,12,13-Trihydroxy-10-campherene, Eugenol 4-*O*-rhamnosyl(1,2) glucoside, 7,8-*erythro*-4,9,9′-trihydroxy-3,3′-dimethoxy-8.*O*.4′-neolignan, (2*S*,4*S*)-hydroxyproline were identified in solvent extracts, and identified after isolation and structure determination by up-to-date NMR techniques. Some phenolic compounds identified in *S. album* essential oil may be artefacts generated during the processing of the wood by very long hydro distillation if the distillation vessel containing the biomass is heated with direct fire in the absence of agitation, inducing probably a pyrolytic degradation of lignin or even lignans and neolignans present in the wood (Nicolas, et al., 2011).

Demole et al., 1976 identified the (*E*)-isomers of α-, β- and *epi*-b-santalals, but wisely suspected that they could be produced by the isomerization of their (*Z*)-analogues through the chemical treatment used for their isolation (Girard P reagent). One can speculate whether the thermal process during the hydro distillation and/or the

GC experiment was not able to induce (at least partly) this isomerization, which could then explain why the co-occurrence of (*E*) and (*Z*) was described in many studies. However, the four isomers were observed (by ^{1}H-NMR) in a solvent extract distilled and fractionated by PTLC (on silica gel), while the (*E*) isomers of α-and β-santalals were identified (^{1}H-NMR) in a cold methanolic extract fractionated by CC on silica gel and HPLC. This last experiment may support the natural occurrence of the (*E*) isomers, as the (*Z*)- santalals are usually sufficiently stable to be submitted to chromatographic separations on silica gel without the likely hood of isomerization (Nicolas, et al., 2011).

Quality Control**:** Today, quality control (QC) of sandalwood essential oils can rely on international norms, ISO-FDIS 3518 (2001) and ISO 22759 (2009), for *S. album* and *S. spicatum*, respectively. Indeed, due to the shortage of genuine East Indian *S. album* essential oil, not everything offered as sandalwood oil is necessarily'100% derived from the claimed botanical species'. The content of santalol isomers is one of the main criteria for quality assessment. Before the introduction of gas chromatography in analytical laboratories, this determination was performed by 'wet chemistry', almost exclusively by classical acetylation. In this way, the total alcohol content expressed as santalol should not be <90% in both *S. album* and *S. spicatum* essential oils. In routine QC, gas chromatography is today the method of choice for particularly when dealing with solvent extracts (Nicolas, et al., 2011).

Subcritical CO_2 extracts found to contain the maximum contents of santalols and sanatlenes but the oil could not be evaluated extensively as done by other authors due to lack of availability of standards for comparing the retention time indices either by capillary and/or GC-MS. Had these standards been availed the subcritical CO_2 extracted oil definitely would have been found to contain lot more of constituents mentioned by the authors and would have been great revealing in this invention.

CONCLUSIONS

Particle size plays an important role in the uniform extraction of oil and its compositions. It has been established by the extraction techniques and analysis that the compositions of the sandalwood oil were almost uniform throughout the experimental studies. FTIR qualitative analysis was found to be quite interpretative in establishing the sandalwood oil measurement. Organoleptic properties shown the subcritical CO_2 extracted sandalwood oil was very tenacious, camphourous and golden yellow in color specifically due to very mild experimental conditions and compatible contents of santalol and santalene. The pH was found to be under control during hydro distillation and even on hydro distilling for 48h yielded high content of β-santalol with 14.89% amongst all investigated techniques. Not only observed that but santalol contents were also the highest. It was very interesting to reveal the increase in the contents of santalene but how exactly it happened was unknown.

Steam distilled and hydro distilled sandalwood oil was light pale yellow in color and retained the maturity after a week time. Solvent extracted oil though had the color of the oil much better to distilled oil but the technique was tedious and involved various steps and solvents. This may make the process less cost incentive. In case of solvent extracted oil also it required almost a week for retaining its maturity and the tenacity was warmth unlike to distilled oil. Subcritical carbon dioxide technology was found to be most compatible processing of the oil from all the quality parameters that conforms the specification of the commercial oil. Perfumer from industries, on evaluating the oil gave good scales to extracted oil with subcritical CO_2. Organoleptically the oil was outstanding.

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Table- 1: Sandalwood oil compositions analyzed by GC and organoleptics.

Method of extraction	Batch time (hours)	Physical/pre-treatment	Oil composition Major constituents%	Color of oil	Odor of oil
Hydro distillation	36	Cold immersion 24h	1) traces 2) traces 3) 48.38 4) 28.73	Pale yellow	pleasant
Solvent extraction (toluene)	5.15	Post distillation	1) 0.36 2) 0.83 3) 39.71 4) 19.76	Pale yellow	Less pleasant
Hydro distillation of concrete	12	-	1) 3.98 2) 4.87 3) 38.47 4) 20.42	Pale yellow	Less pleasant
Hydro distillation	30	Pulverized coarse powder	1) 2.17 2) 1.26 3) 40.19 4) 20.42	Pale yellow	pleasant
Soxhlet Extraction (toluene)	10	Medium/coarse pulverizing	1) 3.98 2) 4.80 3) 29.22 4) 30.54	Pale yellow	Less pleasant
Hydro Distillation	48	0.3%alkaline water,	1) 4.25 2) 3.01 3) 41.90 4) 14.89	Pale yellow	Pleasant
Hydro distillation	38	whole chips immersed in hot water for 24 hours	1) 0.30 2) 0.91 3) 56.73 4) 27.10	Pale yellow	Pleasant
Steam distillation	10	Fine pulverized powder	1) 0.77 2) 1.80 3) 54.744) 29.58	Pale yellow	
Soxhlet Extraction (benzene)	3	Steam distilled powder	1) 0.85 2) 1.70 3) 42.22 4)23.26	Pale yellow	Pleasant
Soxhlet Extraction (ethyl alcohol)	6	Coarse pulverizing immersed	1) 0.96 2) 3.28 3) 50.03 4)27.87	Pale yellow	Less pleasant
Soxhlet Extraction (diethyl ether)	5	Coarse pulverizing immersed	1) 0.57 2) 1.47 3) 48.824) 14.89	Pale yellow	Less pleasant
Soxhlet Extraction (benzene)	5	Post hydro distilled	1) 3.42 2) 4.99 3) 38.21 4)23.37	Pale yellow	Pleasant
Soxhlet Extraction (toluene)	12	100 µ	1) 3.84 2) 4.03 3) 37.04 4)15.89	Pale yellow	Less pleasant
Soxhlet extraction (benzene)	5	40-60 µ	1) 7.79 2) 5.12 3) 30.54 4)15.98	Pale yellow	Pleasant

1) **α**-santalene 2) β -santalene 3) **α**-santalol 4) β –santalol

Table-2: Physical properties of sandalwood oil.

Method of extraction	Pre treatment	Refractive index	Optical rotation	Acid value
Hydro distillation	Whole chips	1.500	-22.97	-
Soxhlet extraction (TOLUENE)	Pulverized coarse size powder	1.503	-19.14	4.67
Hydro distillation of concrete	-	1.499	-	7.33
Hydro distillation	Pulverized coarse size powder	1.499	-14.46	5.58
Hydro distillation	Pulverized coarse size powder, 0.3% alkaline distilled water	1.502	-19.57	2.66
Steam distillation	Pulverized powder immersed in cold water (48 hours)	1.503	-24.67	6.39
Soxhlet extraction (ETHANOL)	Fine pulverized powder	1.504	-19.56	7.79
Soxhlet extraction (DIETHYL ETHER)	Fine pulverized powder	1.503	-14.46	7.79
Soxhlet extraction (BENZENE)	Pulverized fine powder	1.502	-28.07	6.95
Soxhlet extraction (TOLUENE)	Pulverized coarse size powder, immersed in hot water	1.501	-	6.71
Liquid CO_2 extracted	Fine pulverized powder	1.505	-22.97	4.10
Commercial sandalwood oil	-	1.504	-19.57	4.15

- **Required specification: Refractive index: 1.499-1.506; Acid value: 0.5-8: Optical rotation:-15^{o} to -19.**

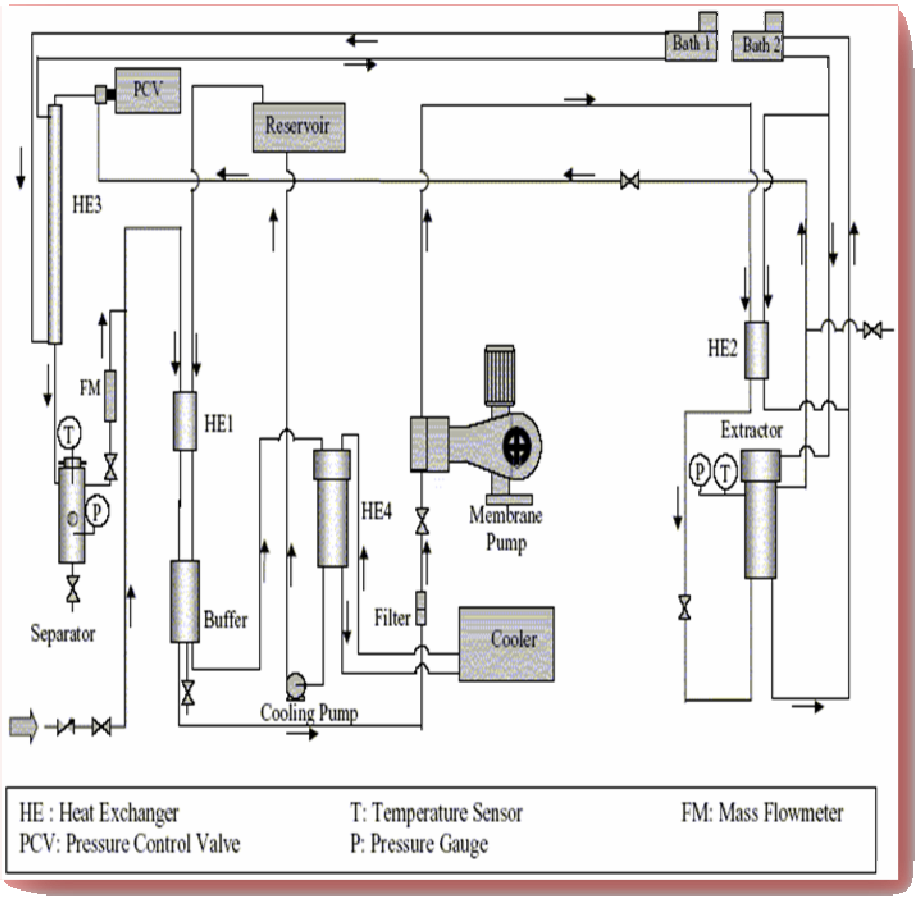

Figure- 1: Schematic flow diagram of SC-CO_2 pilot plant.

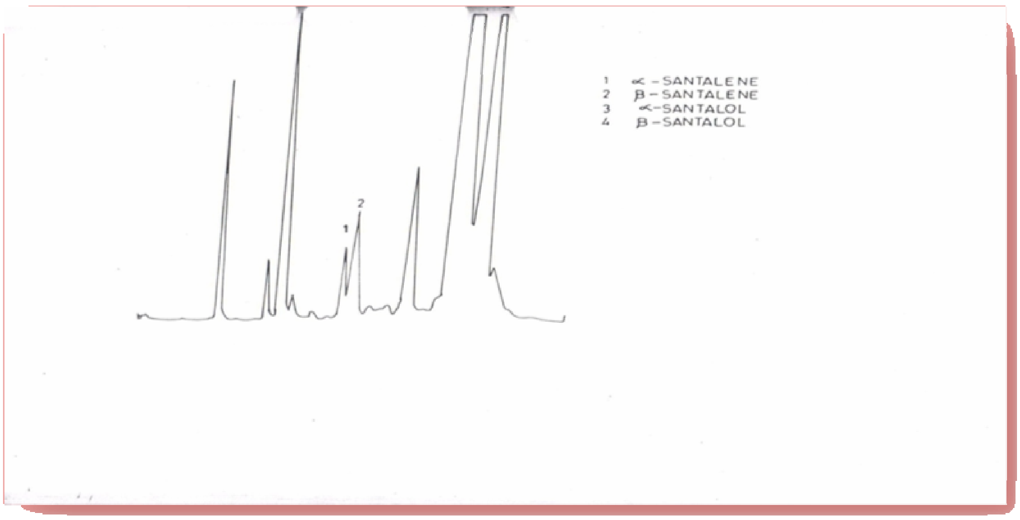

Figure- 2: Gas chromatograph of sandalwood oil.

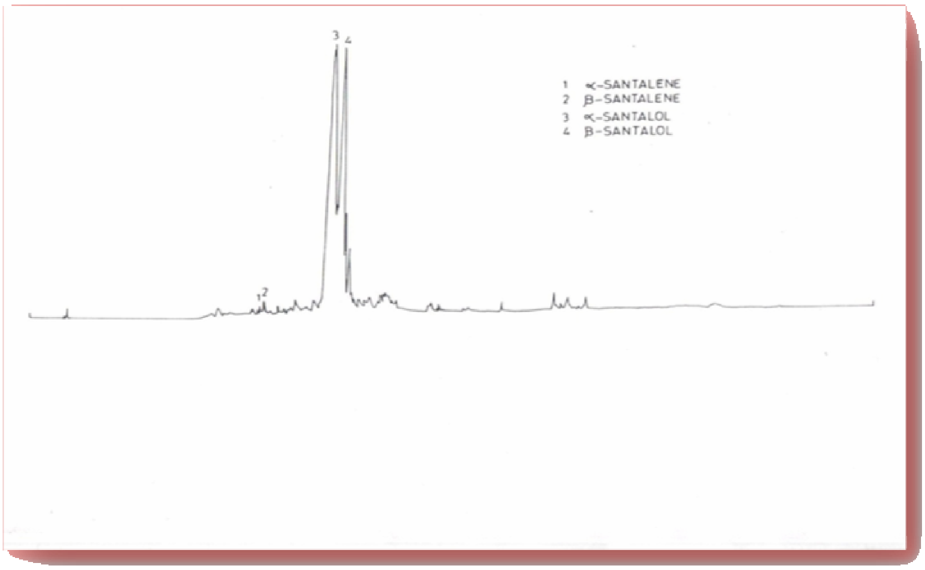

Figure- 3: Capillary gas chromatograph of sandalwood oil of subcritical CO_2.

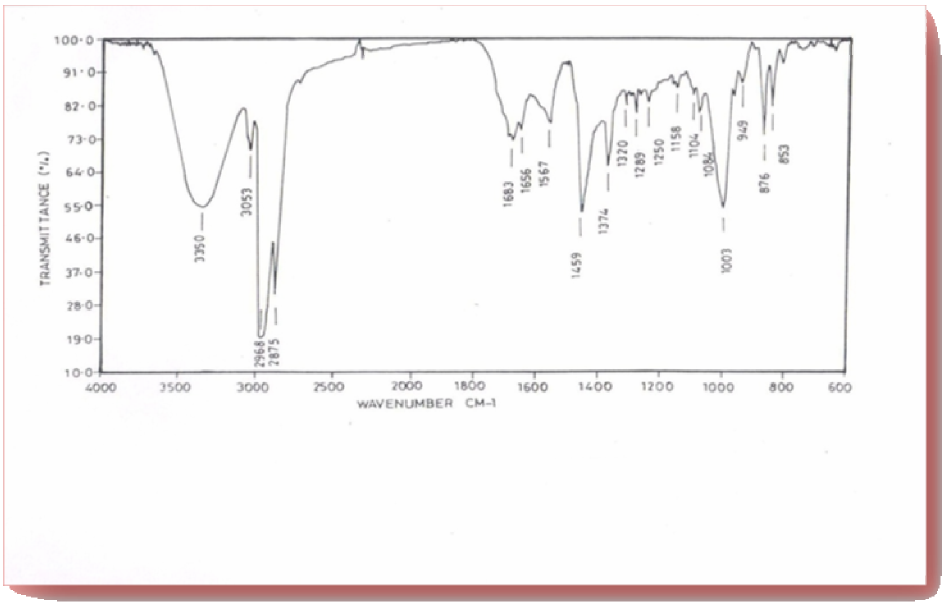

Figure-4: Qualitative analysis of sandalwood oil (subcritical-CO_2) by FTIR.