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Research Paper

Extraction of Ambrette seed oil and isolation of Ambrettolide with its Characterization by ^{1}H NMR

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ABSTRACT

An attempt was made to extract the ambrette seed oil by solid-liquid extraction process using benzene and was compared with Soxhlet extraction. Thus the oil obtained was containing fatty acids. The fatty acids were separated through freezing technique. The phase containing soluble ambrette seed oil was separated and recovered by concentrating under reduced pressure. Ambrettolide was isolated and purified using column chromatography and was characterized using ^{1}H NMR. An FTIR spectrum was scanned for ambrette seed oil and isolated ambrettolide and found to shown a marked difference in the spectra. Yield of oil, concrete, farnesol and ambrettolide was comparatively better as that of reported.

Keywords: *Abelmoschus moschatus;* Solid-liquid extraction; ^{1}H NMR; FTIR spectrum; Ambrettolide; GC analysis.

INTRODUCTION

Abelmoschus moschatus (L.) Medic, (Syn. *Hibiscus abelmoschus* L.) Family-*Malvaceae* is a tropical weedy shrub native to India valued for its scented seed. The area under ambrette is presently low in India but is increasing rapidly with seed exports to France, Germany, Japan, Singapore and Spain for its use as aromatic oil. Indian drug manufacturers are introducing new herbal drugs containing ambrette for medicinal use (Duke, 1985; Lawrence, et al., 1996).

The bitter, sweet, acrid, aromatic seeds are used as tonic and considered as cooling, aphrodisiac, ophthalmic, cardio tonic, digestive, stomachic, constipating, carminative, pectoral, diuretic, stimulant, antispasmodic, deodorant (Cravo, et al., 1992).

The main constituents of the oil are a sesquiterpne alcohol, farnesol ($C_{15}H_{26}O$, 12% on the weight of the seed). The characteristic musk like odour is due mainly to the presence of a lactone ambrettolide ($C_{16}H_{28}O_2$, B.P. 154-56^{o}C/mm, specific gravity 0.9580, n_d^{20}1.4815), a lactone of ambrettolic acid (16-hydroxy-7-hexadecenoic acid, $C_{26}H_{30}O_3$, m.p. α-isomer 53-55^{o}C, β-isomer 26-27^{o}C); on hydrogenation ambrettolic acid is converted into dihydroambrettolic acid ($C_{16}H_{32}O_3$, m.p. 92-93^{o}C) which is

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identical with juniperic acid. Ambrettolide is present in the crude oil to the extent of 0.3% and is colourless viscous liquid; the presence of acetic acid and ambrettolic acids in ester form has been reported. Furfural is present in the water condensate by steam-distillation.

Garner and Buil (1978) used a combination of techniques to examine the chemical compositions of ambrette seed concrete. They found that the concrete contained the following components: octanoic acid (0.07%), nonoic acid (0.10%), hexadecanoic acid(19.08%), octadec-9-enoic acid (5.29%), octadeca-9,12-dienoic cid, decanol(trace), dodecanol (90.13%), (E) nerolidol (0.22%), (ZE)-farnesol (0.22%), (EE)-farnesol (2.16%), decyl acetate (1.21%), dodecyl acetate (1.35%), (ZE) farnesyl acetate (2.25%), (EE)-farnesyl acetate (30.90%), (EE)-farnesyl myristate (0.54%), (EE) farnesyl palmitate (2.54%), (EE)-farnesyl stearate (6.84%), (EE)-farnesyl oleate (4.00%), (EE)-farnesyl linoleate(0.32%), (EE)-farnesyl-16-hydroxy-hexadec-7-enoate (1.72%) and ambrettolide (5.10%). The authors also showed that the composition of neutralized oil produced from the concrete could differ drastically depending upon the method of neutralization.

Rijke et al., (1980) reported that the following novel compounds have been found as constituents of ambrette seed oil: 1-(-acetoxyethyl)-1-hexylcyclopropane and 1-(4-acetoxybutyl)-2-hexacyclopropane. Sriniwas (1986) reported that ambrette seed oil contained the following constituents: cis-jasmone (0.78%), trans-jasmone (0.90%), a farnesol isomer 916.59%) and another farnesol isomer (51.52%). Sriniwas was unable to characterize which farnesol isomer was predominant and which was not.

Our aim was to develop such a process where utilities consumption would have been eliminated so that yield of the oil and constituents should have remained unaltered to retain the fragrance of the precious oil. Also while eliminating the fatty acids from the extracted oil it was carried out in such a way that selective recovery of the absolute could have been possible.

MATERIALS AND METHODS

Extraction of ambrette seed oil using Soxhlet apparatus: The powdered ambrette seed 200g of 40-60 µ was charged in thimble and placed in Soxhlet apparatus. The ratio of the solvent to material was 1:10. It was run for 12 hours until the powder was completely leached as monitored by the TLC. When the negligible amount of the extract was noticed it was stopped. The mass was filtered and concentrated under reduced pressure. The concrete obtained was 5.50g. It was processed for the recovery of the absolute as mentioned earlier and the oil obtained was 0.6wt% of the weight of the seed charged.

Solvent extraction of ambrette seed oil and isolation of absolute from concrete: Musk seed oil is prepared by extraction with benzene, petroleum ether and alcohol as solvent. The concrete obtained is of a resinous nature and an absolute with a remarkably persistent odour is prepared from it. Extraction with petroleum ether yields 10-14% of resinoid, of which 80% is soluble in alcohol.

The extraction of ambrette seed was carried out at room temperature ($30\pm2^{o}C$) using benzene as a solvent. The agitation was provided by using a high speed impellor (Remi stirrer) so that uniform mixing of the solid mass and the solvent was maintained. The experiments were conducted at different batch time to study the effect of batch time on the yield of ambrette seed oil and ambrettolide.

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After each run the extracting solvent was filtered and filtrate containing ambrette seed extract was concentrated under reduced pressure to obtain a viscous dark brown coloured concrete rich in fatty acids. Fats free ambrette seed oil was obtained by selective dissolution and Thereafter the mass was allowed to freeze so that the fats get solidified at the bottom and oil gets dissolved in the solvent.

Ambrette seed concrete was made to dissolve in 95% absolute alcohol in the ratio of 1:10 and was stirred thoroughly. It was then allowed to stand in chamber having the temperature of $10\pm5^{o}C$ until the waxes and/or fats get freeze at the bottom of the container. Then it was taken out and slowly poured out the portion containing essential oil. It was then concentrated under reduced pressure to recover the oil. Oil was added with pinch of anhydrous Na_2SO_4 to remove any traces of moisture present in it. Thus the fragrant absolute was recovered. In each run of the experiments the process was followed and results recorded in table1.

The ambrette seeds were obtained from the state of the Assam. The moisture content of the seed was determined to be 6% using Clevenger apparatus.

Analysis of the ambrette seed oil: The oil was analyzed by Perkin Elmer 8000 Gas chromatography. The analysis conditions were as follows: column: OV 17 (10%) on chromosorb W (HP), column material: S.S, column length: 4m, internal diameter: 1/8 inch, injector temperature: $300^{o}C$, FID temperature: $300^{o}C$, flow rate of N_2: 38ml/min., temperature programme: 180-$300^{o}C$ with $10^{o}C$/min. rise of temperature (Davies, 1990; Dung, 1999).

Isolation and analysis

Thin layer chromatography of ambrette seed oil: A plate was prepared by coating it with silica gel (TLC grade, gel prepared in acetone) dried and spotted with the oil and kept in the TLC jar containing solvent mixture of hexane and ethylene chloride (40:60) (Bernard, 1988; Nee, 1986). It was kept for half an hour to achieve the maximum travel, for calculating the Rf values. It was then taken out, dried and kept in the iodine chamber to identify the coloured spots. Ten spots were observed and Rf values for identified spots were calculated.

Column chromatography of Ambrette seed oil: Silica gel (mesh size 60-120, S.D. Fine Ltd.) was dried in the oven before use. 50gm silica gel was weighed and slurry prepared with solvent mixture (hexane: EDC, 40:60) and filled in the column. To form a uniform bed, the column was tapped several time, once bed formed uniformly cotton was placed above the bed (Sahoo, et al., 2003). 0.28g of oil was mixed with solvent mixture and poured into the column. To achieve better isolation, the polarity of the solvent was increased by using hexane to EDC ratio of 30:70 and 20:80.

Ambrettolide isolated and characterized by ^{1}HNMR was highly fragrant containing tenacious musky odour. Refractive index results of the oil, was also interesting to study the effect of batch time on their variations. FTIR scan, of ambrette seed oil and ambrettolide shown the marked qualitative difference at 1400, 1700 and 3000 cm^{-1} in their respective region of transmittance, with respect to ambrette seed oil. Figure 1, 2, and 3 may be referred.

RESULTS

Effect of batch time on extraction of ambrette seed oil: The batch time of extraction was varied from 1 to 3.5 hours under identical conditions of temperature and agitation. The experiments were also performed using Soxhlet apparatus with crushed ambrette seeds. The data are presented in table 1. Fresh solvent was charged each

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time. It was observed that the degree of extraction of concrete was increased up to 2 hours and remains nearly constant thereafter. The yield of the oil was found to increase with the batch time of extraction and was found to remain nearly constant after 2 hour.

Table -1: Effect of batch time on the extraction of ambrette seed oil.

Experiments	Time (hour)	% wt of concrete	% wt of oil	% wt of fatty acid
1	1	5.33	0.38	4.95
2	1.5	6.72	0.45	6.27
3	2	6.85	0.52	6.33
4	2.5	6.92	0.55	6.37
5	3	6.99	0.58	6.41
6	3.5	6.95	0.56	6.39

Table 2 shows the molecular weight, boiling point, retention time and composition of the major constituents in ambrette seed oil.

Table- 2: Data's and percent area composition of major constituents in ambrette seed oil.

Compound	Peak no.	Retention time	% area	Formula	Molecular weight	Boiling point
Farnesol	1	11.91	42.35	$C1_5H_{26}O$	222	149^oC
Unknown	2	13.05	10.74	-	-	-
ambrettolide	3	14.80	14.80	$C_{16}H_{28}O_2$	252	300^oC

Table 3 shows the analysis of the ambrette seed oil at every batch time with their percentage and retention time.

Table- 3: Chemical compositions of essential oil from ambrette seed (*Abelmoschus moschatus*).

Batch time (h)	Peak	Retention time (min.)	Percent composition	Constituents
1	1,2,3,4,5,6	6.55,7.29,8.23,9.30,10.60,11.37	2.66,1.60,6.78,1.73,4.04,6.50	Unidentified
	7	11.74	33.49	Farnesol
	8,9	12.77,13.65	10.01,1.63	Unidentified
	10	14.55	8.84	Ambrettolide
	11,12	15.75,17.87	9.92,3.48	Unidentified
1.5	1,2,3,4,5	6.19,6.65,8.32,10.70,11.50	3.16,2.15,3.32,2.80,5.36	Unidentified
	6	11.86	33.78	Farnesol
	7,8,9,10	13.02,13,84,13.93,14.24	9.67,3.87,2.22,2.33	Unidentified
	11	14.76	8.56	Ambrettolide
	12,13,14,15,16	15.57,15.75,15.98,16.17,18.14	2.00,1.70,5.51,2.12,2.55	Unidentified
2	1,2,3,4	6.22,8.42,10.80,11.61	2.43,3.94,4.82,33.35	Unidentified
	5	11.98	2.61	Farnesol
	6,7,9,10	12.94,13.22,16.01,18.30	3.52,13.56,5.15,2.18	Unidentified
	8	14.87	15.52	Ambrettolide
2.5	1,2,3,5	8.41,11.58,11.91,13.89	2.18.4.92,40.18,4.79	Unidentified
	4	13.05	10.82	Farnesol
	6	14.80	15.55	Ambrettolide
	7,8,9	16.08,18.20,6.10	22.25,4.99,1.22	Unidentified
3	1,2,3,4,5	6.70,6.94,7.42,8.35,9.65	2.09,1.51,2.20,3.59,1.37	Unidentified
	6,7,8,10,11	10.72,11.53,11.90,13.83,14.75	2.63,4.24,43.34,2.94,12.53	Unidentified
	9	12.97	10.74	Farnesol
	12	15.99	10.58	Ambrettolide
	13,14	18.14,6.08	3.24,1.60	Unidentified
3.5	1,2,3,4,5,6	6.68,7.39,8.33,10.69,11.50,11.86	1.89,1.46,2.75,2.87,4.92,41.05	Unidentified
	7	12.94	12.58	Farnesol
	8,9,11,12	13.78,14.71,17.08,18.08	3.30,11.92,2.07,3.11	Unidentified
	10	15.94	11.29	Ambrettolide

- Retention time determined of experimental constituents using standards.

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Table 4 contains the data of physical properties of the ambrette seed oil determined as per Guenther E. (1963).

Table- 4: Physical properties of extracted ambrette seed oil.

Physical property	Values
Colour of the oil	Pale yellow
Refractive index$[n]_d^{30}$	1.468
Optical rotation	+30.40”
Acid value	2.38

- Physical properties determined as per method mentioned in E. Guenther Book of essential oils.

Effect of batch time on refractive index of oil: As the extraction progresses the recovery of the oil has an impact on refractive index of oil. Table 5 has shown that refractive index was high in the batch time of 1.5 hour, and subsequently slight decrease was observed. At 2.5 hour it was again found to decrease and thereafter again found to increase. This indicated the change in the density of the extracted essential oil.

Table -5: Effect of batch time on refractive index of ambrette seed oil.

Batch time (hour)	Refractive index
1	1.467
1.5	1.472
2	1.470
2.5	1.467
3	1.469
3.5	1.470

- Physical properties determined as per method mentioned in E. Guenther Book of essential oils

On TLC separated constituents were identified against the standards. Identified ambrettolide was successfully scratched, extracted with methanol and vacuum dried. It was latter used as standard for isolated ambrettolide through column chromatography, while characterization using ^{1}H NMR.

Claude Delphis (2000) in his patent described the process for manufacturing Cis-isoambrettolide and characterizing its structure employing ^{1}H NMR.

DISCUSSION

Batch time variation resulted in extracting 5.33-6.95 wt% of concrete, 0.38-0.55wt% of absolute and 4.95-6.39wt% of fatty acids. As far as absolute was concerned so the yield was high against reported in the literature by Sahoo, 2003; Nee, 1986.

In the first hour of extraction 11 constituents were extracted out of which farnesol and Ambrettolide were analyzed to be 33.49 and 8.84%, confirmed by using the standards and confirming their retention time under identical conditions of analysis. Second hour 16 constituents were extracted. Farnesol and ambrettolide were 33.78 and 8.56%. Third hour 10 constituents were extracted and farnesol and ambrettolide were analysed to be 2.61 and 15.52%. Fourth hour 9 constituents were extracted; farnesol and ambrettolide were 10.82 and 15.55%. Fifth hour 14 constituents were extracted; farnesol and ambrettolide were 10.74 and 10.58%. In the final hour of extraction 12 constituents were extracted and farnesol and ambrettolide were 12.58 and 11.29% (Leung, 1980).

Thus it was found that major constituents were extracted in the high percentage as compare to progressing batch time.

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CONCLUSIONS

This process may be promising as it was performed at the ambient temperature so as to minimize the loss of the solvent as well utilities. The progress of the ingredient extraction was monitored using gas chromatography. As it is clear from the table that the progress of the ingredient extraction was improving as the batch time was found to progress. Farnesol and Ambrettolide were analyzed to be appreciably high as that reported in the literature.

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