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Technical Notes

A Novel Process for the Extraction of Fragrance Components from Ambrette (*Hibiscus abelmoschus* L.) Seeds

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Abstract:
The essential oil from *ambrette* seeds *(Hibiscus abelmoschus* L. syn. *Abelmoschus moschatus,* Moerich) has long been used in the perfumery industry. The essential oil is localized mainly in the seed coat that cannot be easily separated from the kernel. Different methods of separation of the seed coat have been attempted, and none of the methods has been found to be satisfactory. A method for its selective extraction with alcoholic solvents and purification is described. A fragrant extract free from fatty acids and fatty oil, which is superior to the steam-distilled product is obtained in improved yields.

Introduction

Hibiscus abelmoschus, syn. *Abelmoschus moschatus* of family *Malvaceae* known popularly as *ambrette*, is native to India.[1] It is cultivated in tropical regions of Asia, Africa, and South America for the seeds, which have a characteristic musklike odour.[2] The extract is widely used as a fixative in fragrance formulations.[3] The liquid oil is a valuable adjunct to high-grade perfume compositions to which it imparts a strong and characteristic musky note. It possesses a much smoother odor than synthetic musk compounds.[4] The liquid oil of commerce is noted for its rich, sweet, floral-musky, distinctly winelike odor with a bouquet and "roundness" rarely found in any other perfumery material and with a tenacity of odor that is almost incredible.[5] While some of the synthetic musk compounds have been shown to cause photosensitivity and dermatitis[6] in sensitive individuals, the *Abelmoschus* plant has been classified as "an herb of undefined safety" by the Food and Drug Administration (FDA), and the extracts are classified as generally recognized as safe (GRAS) for use in baked foods, candies, and alcoholic beverages.[7] Ambrettolide is reported to be nontoxic.[8] Kerschbaum[9] reported in 1927 the isolation of a lactone for the first time from *ambrette* seed oil by fractionation (after removing fatty acids with dilute alkali) and called it ambrettolide. It has been identified as (*Z*)-7-hexadecen-16-olide. However, it was only in 1973 that Paredes[10] reported that the *ambrette* seed essential oil contained farnesyl acetate[10] as the major constituent although ambrettolide was responsible for the typical and characteristic musky odor. Maurer and Grieder[11] reported isolation of (*Z*)-5-tetradecen-14-olide from ambrette seeds in addition to (*Z*)-5-dodecenyl acetate and (*Z*)-5-tetradecenyl acetate. In a classic work by Nee, Cartt, and Pollard[12] the *ambrette* seeds were carefully dissected into outer and inner seed coats, endosperm, and embryo, and each was analyzed for the fragrance components. The essential oil was found to be localized exclusively in the outer layer of the seed coat but not in the epicuticular layer. The seed coat constituted 30−33% of the weight of the whole seed. After an extensive chromatography and isolation of pure components, the principal components of the monoester fraction were identified through spectroscopic studies as (2*E*,6*E*)-farnesyl acetate (70%), (2*Z*,6*E*)-farnesyl acetate (6%), (*Z*)-7-hexadecen-16-olide, and (*Z*)-9-octadecen-18-olide (total 14%). Subsequently, Bernard et al.[13] obtained the essential oil by hydrodistillation of seeds in 0.16−0.27% yield, and the oil contained variable amounts of fatty acids. It is to be noted that the duration of distillation varied from 6 to 9 h. From a comparison of the composition of oils reported by Bernard et al., it can be seen that (2*E*,6*E*)-farnesol is formed in about 35% at the cost of farnesyl acetate, and ambrettolide content also is decreased to 8%, indicating considerable hydrolysis during hydrodistillation. These authors have also obtained an oleoresin by extracting the crushed seeds with 1,1,2-trichloro-1,2,2-trifluoroethane in 12.8% yield. The hydrodistillation of the oleoresin could be completed in less than an hour and an essential oil is obtained

(1) *The Wealth of India: Raw Materials*; National Institute of Science Communication, Council of Scientific and Industrial Research: New Delhi 1959; Vol. 5, p 75.
(2) Misra, G.; Nigam, S. K.; Mitra, C. R. *Acta Phytother.* **1971**, *18*, 134.
(3) Bernard, T.; Perineau, F.; Braro, R.; Delmas, M. *Parfums, Cosmet., Aromes* **1988**, *83*, 65.
(4) Guenther, E. *Essent. Oils* **1963**, *II*, 689−690.
(5) Arctander, S. *Perfume and Flavor Materials of Natural Origin*; Arctander: Elizabeth, New Jersey, 1960; pp 58−60.
(6) Machet, L.; Vaillant, L.; Bensaid, P.; Muller, C.; Lorette, G. *Ann. Dermatol. Venerol* **1992**, *119*, 737 and described in Ambrette. *Lawrence Rev. Nat. Prod.* **1994**, May.
(7) Duke, J. A. *Handbook of Medicinal Herbs*; CRC Press: Boca Raton Florida, 1985.
(8) Leung, A. Y. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics*; John Wiley & Sons: New York, 1980.
(9) Kerschbaum, M. *Ber.* **1927**, *60B*, 902−905. *Chem. Abstr.* **1928**, *21*, 2118.
(10) Paredes, C. *Polytechnica* **1973**, *3*(*1*), 163−70.
(11) Maurer, B.; Grieder, A. *Helv. Chim. Acta* **1977**, *60*, 1155.
(12) Nee, T. Y.; Cartt, S.; Pollard, M. R. *Phytochemistry* **1986**, *25*, 2157.
(13) Bernard, T.; Perineau, F.; Branvo, R.; Delmas, M.; Gaset, A. *Parfums, Cosmet., Aromes* **1988**, *84*, 77.

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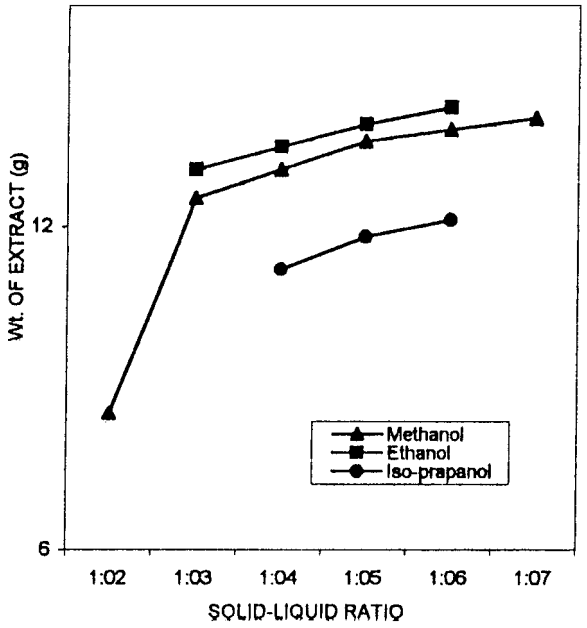

Figure 1. **Effect of solid−liquid ratio on extraction stirring time: 6 h.**

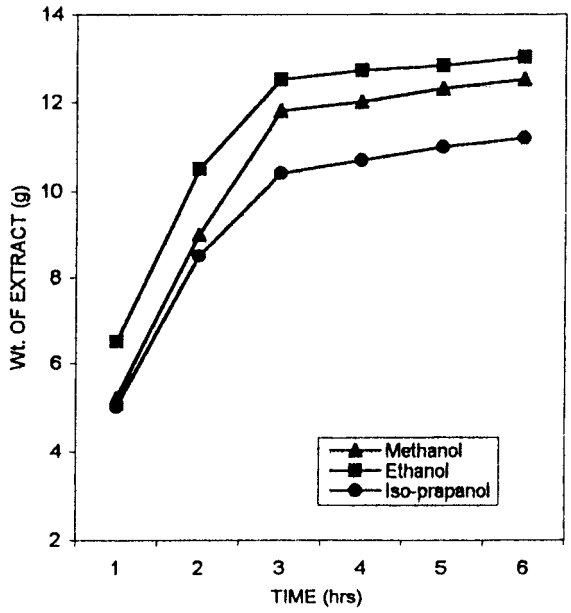

Figure 2. **Effect of stirring time on extraction solid−liquid ratio: 1:3.**

Table 1. **Separation of seed coat by different methods**

		results of sieving %		
			−8 mesh	
sl.no	method adopted	+8mesh (2 mm) predominantly seed coat	+16 mesh (1 mm) mixture	−16 mesh predominantly embryo
1.	soaking in water for 5 h and roll crushing	33.1	25.6	40.5
2.	soaking for 5 h and pounding	30.4	29.4	39.5
3.	grinding in domestic mixer with vegetable cutter	36.5	10.5	52.3

in 1.3−1.9% yield. Furthermore, the oil had as much as 65% farnesyl acetate content and 14.7% ambrettolide. The first detailed analysis of ambrette essential oil was reported by Buil et al.[14] In a more recent report Perineau et al.[15] have compared the chemical composition of a volatile product obtained by steam distillation of oleoresin with that of essential oil of ambrette seeds of Ecuadorian origin and reported 44 components. (*Z*)-9-Octadecen-18-olide appeared to have undergone considerable hydrolysis.

Ambrette commercial products are (i) the essential oil obtained by steam distillation of crushed or uncrushed seeds and (ii) the steam volatile oil obtained by extraction of crushed seeds with nonpolar solvents followed by steam distillation of the oleoresin and purification..

A process for the preparation of a concrete from *ambrette* seeds of Indian origin has long been patented by Misra and Mitra.[16,17] It was obtained in 1.5% yield and is claimed to have good shelf life on the basis of olfactory evaluation. No analysis has been reported.

It may be noted that the essential oil is present exclusively in the seed coat that cannot be separated easily from the endosperm and embryo. Existing methods of extraction involve steam distillation at some stage of processing, a result of which is the deterioration of the quality of the oil.

We describe here a cold process for isolating the fragrance components from the ambrette seeds in a purity as close as possible to its natural occurrence by selectively extracting the seeds with alcoholic solvents followed by liquid−liquid extraction of the oleoresin with a nonpolar solvent. Since the fragrance components occur exclusively in the seed coat, attempts to separate the seed coat from the embryo have also been made, and we present here results of these trials also.

Materials and Methods

The *ambrette* seeds required for the present studies were obtained from the plants cultivated in the Aromatic and Medicinal Plants Division of our laboratory and also those supplied by M/S Global Herbs, New Delhi.

Different methods attempted to separate the seed coat from the embryo are (i) soaking in water and roll crushing, (ii) pounding, (iii) milling in a hammer mill, (iv) milling by a hand-operated roller mill, and (v) grinding in a domestic grinder fitted with a vegetable cutter. In the last method, the seed coat was shredded into bigger pieces while the embryo got pulverized, and they were separated by sieving on a BSS 8 mesh (2 mm) and BSS 16 mesh (1 mm) test sieves. The essential oil from the seed coat was obtained by steam distillation in a Clevenger-type apparatus.

Reagent-grade solvents used for both extraction and purification were distilled before use. The extraction studies were carried out on 1-kg scale for optimizing the parameters.

(14) Buil, J. P.; Laurent, R.; Fournil, J. Ph.; Joulain, D.; Hardy, P. Y. *Parfums, Cosmet., Aromes* **1989**, *89*, 95.

(15) Cravo, L.; Perineau, F.; Gaset, A.; Bessiere, J. M. *Flavour Fragrance J.* **1992**, *7*, 65−67 and reviewed in *Progr. Essent. Oils, Perfumer Flavourist* **1996**, *21*, 58.

(16) Misra, G.; Mitra, C. R. *Res. Ind.* **1964**, *9*, 325−327.

(17) Misra, G.; Sharma, V. N.; Mitra, C. R; Kaul, K. N. Indian Patent No. 72408, 1960.

Table 2. **Recovery of essential oil by different methods**

sl. no.	method adopted	% yield	remarks
1	steam distillation of whole seed	0.15−0.2	The essential oil has fatty odor and needs to be aged before use; low shelf life. Duration of distillation about 8−10 h.
2	extraction with hexane and steam distillation of oleoresin	0.2−0.23	Fatty acids present in the oil, and it needs rectification.
3	essential oil from seed coat	0.2	Sweet-smelling, mostly free of fatty bodies.
4	extraction of crushed seeds with alcohol and rectification by crystallization and fractionation	1.2−1.3	The concretion contains fatty bodies and other polar extractives.
5	extraction of whole seeds with alcohol and isolation of fragrance components by the present method	0.24−0.29%	Free fatty acids almost absent, and the oil provides sweet-smelling and lasting odour.

Table 3. **Major constituents of ambrette seed essential oil by different methods**

sl.no	retention time	constituent	steam distilled oil	hexane extraction followed by steam distillation	methanol extraction and present method
1	16.2	decyl acetate	7.2	2.3	5.7
2	17.7	β-farnesene	9.7	13.6	9.6
3	21.2	nerolidol	2.2	–	–
4	22.4	dodecyl acetate	4.5	4.7	5.1
5.	26.2	(*Z*)-5-tetradecen-14-olide	1.2	1.5	1.3
5	27.6	(*Z*)-5-tetradecenyl acetate	0.9	1.3	1.2
7	28.6	(2*Z*,6*E*)-farnesyl acetate	3.4	3.7	3.8
8	29.6	(2*E*,6*E*)-farnesyl acetate	47.6	43.7	50.4
9	32.3	(*Z*)-7-hexadecen-16-olide	9.0	9.3	9.3
10	37.3	(*Z*)-9-octadecen-18-olide	0.6	1.3	1.2

Larger-scale work using 5 kg of seeds per batch were carried out in a stirred tank reactor using methanol. GC analysis was carried out using a Shimadzu GC-17A equipped with an FID and a WCOT column 25 m × 0.25 mm o.d. × 0.25 μm coated with 5% diphenyl dimethyl silicone (DB-5) using helium as the carrier gas at a flow rate of 1.2 mL/min and a linear temperature program of 80−280 °C (4 °C/min). The GC−MS was run on a Shimadzu QP-5000 quadruple mass spectrometer, and compound identification was carried out by comparison of the mass spectra with those of NIST and Adams libraries and confirmed by relative retention indices.[18] Component qantification was carried out by peak area normalization.

Results and Discussion

Grinding the ambrette seeds by pounding in a mortar, milling in a hammer mill or hand-operated roller mill resulted in powdering of both the endosperm/embryo and seed coat equally. These methods were not useful. After soaking in water for 3−8 h, the seed coat and embryo have shown different degrees of swelling, and it appeared possible to separate them. Roll crushing or pounding the swollen seeds resulted in flattening and opening of the endosperm/embryo. Sieving the air-dried rolled seeds resulted in partial separation only. Using a domestic grinder fitted with a vegetable cutter allowed preferential shredding of the hard seed coat while the more brittle embryo got pulverized. Moisture content of the seed is critical and is determined by ISS method;[19] about 8−10% moisture in the seed was ideal. It may be seen from Table 1 that both the fractions contained the other and that separation is not perfect. When the separated seed coat was distilled in a Clevenger apparatus, the yield of the essential oil was only about 0.2%. Apparently, considerable losses of the volatile constituents occurred during grinding and sieving. These results are summarized in Table 1.

The rate of extraction of the oleoresin from the uncrushed seeds with methanol, ethanol, and 2-propanol increases with time but reaches a plateau after 3−4 h of stirring. Methanol was found to be more suitable due to its easy removal at low temperature. In addition, extraction of fatty oil is found to be negligible with methanol as seen on TLC. These data are depicted in Figures 1 and 2. A two-stage counter-current extraction was found to be adequate for completely extracting the fragrance components.

Liquid−liquid extraction of the oleoresin obtained after removal of the alcoholic solvent with a hydrocarbon solvent such as pentane or hexane transferred the fragrance components into the nonpolar layer, leaving the polar glycosides and pectins in the aqueous alcoholic layer. Fatty acids and other polar impurities co-extracted are removed by passing over a short column of silica gel. Evaporation of the solvent provided the desired volatile oil as a sweet-smelling liquid with a lasting characteristic musk odor. Detailed chemical composition of the oil has been reported elsewhere.[20] A comparison of the different methods used for extraction of ambrette seeds for its essential oil is presented in Table 2, and the composition of major constituents of the essential oil obtained from the same quality seeds by these methods is presented in Table 3.

(18) Adams, R. P. *Identification of Essential Oils by Ion Trap Mass Spectroscopy*; Academic Press: San Diego, 1989; pp 17−28.
(19) *Methods of sampling and test for oilseeds*; Bureau of Indian Standards: New Delhi,1967; IS: 3579, pp 5−6.
(20) Rout, P. K.; Rao, Y. R.; Jena, K. S.; Sahoo, D.; Misra, B. C. *J. Essent. Oil Res.* **2002**. In press.

The method thus provides an efficient low-temperature process for extraction of ambrette oil which is close in composition to that occurring in nature with a recovery of 0.3% based on the weight of seeds, while steam distillation yields only 0.15−0.2%. TLC of the oil on silica gel plates showed it to be free of triacyl glycerols, but GC analysis showed the presence of a maximum of 0.5% methyl linoleate and no free fatty acids. The process was found to be easily reproduced on 5-kg scale with improved recoveries up to 0.35%.

Conclusions

The hard seed coat, which constitutes at least 33% of the weight of the seed, is difficult to be separated. The fragrance components are lost to a considerable extent during the separation methods. Cold extraction of whole seeds with an alcoholic solvent under stirring followed by a short-path chromatographic separation on silica gel gives a sweet-smelling product in higher yields.

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