ISSN (Online): 2349-1183; ISSN (Print): 2349-9265
TROPICAL PLANT RESEARCH
The Journal of the Society for Tropical Plant Research

8(3): 186–190, 2021
DOI: 10.22271/tpr.2021.v8.i3.023

Research article

Isolation and chromatographic fingerprint analysis of resinoids from de-oiled material of Vetiver [*Chrysopogon zizanioides* (L.) Roberty] for value addition

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Abstract: This analysis aims to determine the presence of active constituents of *Chrysopogon zizanioides* (*Khus*) from refuge material (De-oiled material) obtained from the essential oil industry which is considered as waste or utilized in the preparation of cheap products like incense stick (*agarbatti, dhoopbatti*), incense burner (*havansamigri*), etc. The same refuge can be utilized for the preparation of good quality products for pharmaceuticals, chewing confectionery, cosmetics, toiletries, *attars*, good quality fragrances and many highly valuable products. For this study, the resinoid is extracted by using the soxhlet apparatus and analysed by HPTLC for the presence of active constituents.
Keywords: Essential oil - Attars - De-oiled - Fingerprint.

[Cite as: Srivastava SK, Akbar N, Yadav SK & Saxena BK (2021) Isolation and chromatographic fingerprint analysis of resinoids from de-oiled material of Vetiver [*Chrysopogon zizanioides* (L.) Roberty] for value addition. *Tropical Plant Research* 8(3): 186–190]

INTRODUCTION

For centuries *attars* and essential oils are used as fragrance materials in India as well as around the world. Kannauj, a place in Northern India (Uttar Pradesh) is famous for its perfumes and *attars* from decades. There are about 700–800 families directly or indirectly involved with this industry either as a cultivator of aromatic plants, in the distillation of aromatic plants yielding essential oil and manufacturing of *attars* by the traditional *Deg & Bhapka* method (Singh *et al*. 1997). Floral essential oils, *attars* and hydrosols, are produced using the *Deg & Bhapka* method. This traditional method is still popular. The old system appears a craft rather than a processing industry (Husain *et al*.1988).

A survey of the different aroma chemicals used in the flavour and fragrance industry, from the simplest materials to the most complex ones is presented including alcohols (saturated alkenes, unsaturated alkenes, complex fragrance, aromatic and alkyl alcohols and phenols); acids (saturated aliphatic, unsaturated and aromatic acids); esters (saturated, unsaturated, and lactones); aldehydes (aliphatic, unsaturated), acetals, aromatics Nitriles); ketones, carotenoids, Ionones, irons, damascones and hydrocarbons (Chauhan 1999).

The major portion of *attars* & essential oil is consumed by betel nuts (*supari*), processed betel nuts (*pan masala*, *gutka*) and tobacco industry. After the extraction of essential oil from the plant material, the de-oiled (refuse) material is used in the production of cheap products like incense stick (*agarbatti*, *dhoopbatti*), incense burner (*havansamigri*) and cosmetic items. The estimated Sandalwood and Vetiver production in India is 1400–1600 and 1000–1500 tons per annum respectively with respect to this estimated refuse is found to be 1300–1500 and 1000–1400 tons per annum. Hence by doing value addition of this refuge material industrialists can fetch good prices from the products obtained from refuge material.

The aromatic roots of *Chrysopogon zizanioides* (L.) Roberty [= *Vetiveria zizanioides* (L.) Nash] commonly known as *Khus* yield the well-known oil of Vetiver, which has been highly valued by the perfumery industry since ancient times. The plant is a widely distributed native perennial grass of the Indian Sub-continent. Besides

Received: 01 June 2021 Published: 31 December 2021
https://doi.org/10.22271/tpr.2021.v8.i3.023

India, *Khus* is commonly cultivated in Sri Lanka, Indonesia, Haiti, Vietnam and Brazil for its sweet aromatic roots which yield a fragrant volatile oil (Guenther *et al*. 1948). The various plant types grown at various geographical locations are known to vary with respect to oil content and oil quality. The South Indian types are rich in oil percent but contain more hydrocarbons and less oxygenated constituents and are therefore inferior to North Indian types, which has higher percentage of oxygenated compound and possess a very high odour value.

The Vetiver is an obligate cross-pollinated species. It has 84% hermaphrodite florets which facilitates cross-pollination. It is found that selfed inflorescence does not produce seed but the puff collected from open-pollinated panicle contains viable seeds. Pollen fertility is very high in North Indian types. All the collection reveals the somatic chromosome 2n = 20. In general, the karyotypes are nearly symmetrical with chromosomes having median to the sub-median centromere. The total haploid chromatin length varied from 25.6 µm to 38.7 µm. A considerable amount of natural variability morphometric traits exists in North Indian types (0.6–0.9%) and South Indian types (0.8–1.1%). A significant correlation of oil yield with plant height, root length and oil content has been reported (Gildmeister *et al*. 1913).

The economic value of Vetiver culture meant for commercial cultivation depends upon high root production and a higher percentage of essential oil present in roots. Improvement in the root yield and essential oil is of prime importance that can be achieved by adopting the following breeding strategies: 1. Clonal selection, 2. Polyploidy and 3. Interspecific hybridization (Upton *et al*. 1999).

The Vetiver oil is viscous, light brown to dark brown liquid, having a characteristic sweet, earthy, woody, odour. The oil of Vetiver contains a total of 150 sesquiterpene compounds. It is evaluated for its total alcohol content, calculated as Vetiverol, which ranges from 45 to 85 percent in different cultivars and hybrids (Guenther *et al*. 1948). Because of the high boiling points of its chief volatile constituents, and their high viscosity, the distillation of Vetiver roots is somewhat difficult. Therefore, the distillation of roots is carried out with live steam of 4 to 5 atmosphere pressure (Teja & Eckert 2000).

The spongy aromatic Vetiver roots are traditionally employed for making fanny household goods like mats, fans, door-screens, hats, and ropes covering the walls of coolers that emit sweet fragrance imparting a cooling effect in the hot summer season when sprinkled with water. The aerial parts are commonly used in the thatching of roofs (Truong & Baker 1998, Truong *et al*. 1999).

Some of the active chemical constituents in *Chrysopogon zizanioides* oil are Khusimol (13.4–27.9%), Vetiselinenol (10.3–19.5%), Vetiverol (6.1–7%), β–eudesmol (5.5–6%), α-vetiver (1.5–2.5%) and β-vetiver (1.5–1.8%), others minor constituents are Khusilol, Khusene, Khushol, Elemol and Vetiverene etc.

Therapeutically it is used for circulatory & nervous problems, used to create tonic bath that is the reason, why it is often included in quality soaps. This could be use to combat lice as well. The earthy smell of Vetiver essential oil will ease tension and stress & stop you from being over sensitive, bringing a feeling of quiet calm & while balancing estrogen & progesterone will help PMS as well as menopausal ailments, It alleviates muscular aches & pains. On the skin it has a nourishing and moisturizing effect and is useful or dry irritated & dehydrated skin. Other properties are antiseptic, aphrodisiac, cicatrisant, nervine, sedative, tonic and vulnerary (Zhu *et al*. 2001).

The de-oiled material is obtained after steam distillation of aromatic plant materials. This material is utilized presently in the preparation of cheap products like incense stick (*agarbatti, dhoopbatti*), incense burner (*havansamigri*), etc. From this study, the resinoid can be utilized for the preparation of good quality products like pharmaceuticals, chewing/confectionery, cosmetics, toiletries, *attars*, good quality fragrances and many highly valuable products (Reinoso *et al*. 2006, Reverchon & Marco 2006).

MATERIAL AND METHODS

Isolation

i. Refuge material: The refuge of Vetiver was collected from authentic perfume manufacturing houses and essential oil distilleries of Kannauj. The identification was confirmed by experts of the essential oil industry from the fragrance and flavour development centre, Kannauj, Uttar Pradesh.

ii. Extraction: The refuse material was sun-dried and finally pulverized into fine powder. The powdered refuge of the Vetiver was further subjected to the process of solvent extraction with the help of the Soxhlet apparatus. For this extraction process hexane was used due to its higher yield of resinoid with this solvent. Firstly a thimble, made up of filter paper was used for holding the refuse material, was prepared. Then the refuse material was accurately weighed and filled into the thimble. A thimble containing the refuse material was kept inside the extractor. Then from the top of the extractor, the solvent (hexane) was poured over the thimble till it was completely dipped into the solvent. After setting the whole apparatus it was left overnight to remain dipped (refuse) in the solvent (Fig. 1 & 2).

Figure 1. Vetiver [*Chrysopogon zizanioides* (L.) Roberty]: **A**, Vetiver plant; **B**, Vetiver roots.

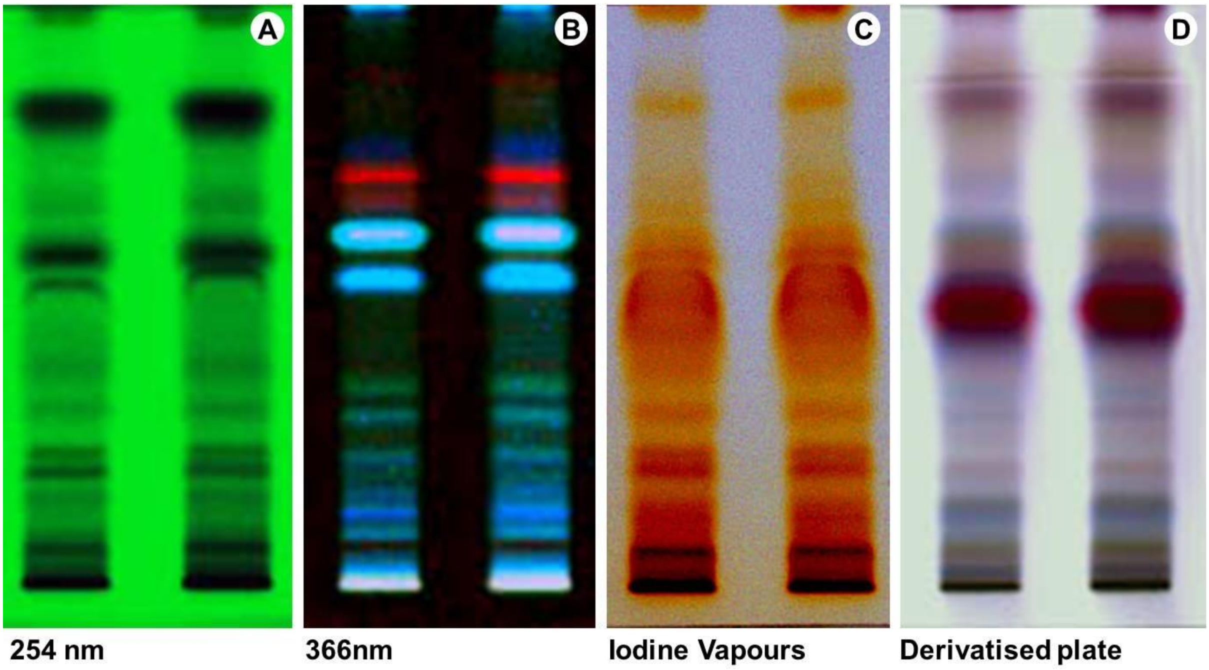

Figure 2. HP-TLC finger prints.

The next day the heating was started and 80^{o}C temperature was maintained. Simultaneously the water was also supplied to the condenser. After 10 minutes the solvent started forming vapours which went into the extractor containing the thimble. The vapours then started filling the extractor. After about 45 minutes the extractor was filled and then the solvent was automatically siphoned back into the flask through the siphon tube. This completed one siphoning. Like this six siphoning was done with both the set ups. The whole process of Solvent extraction took five hours. The big advantage of this process is that majority of the solvent is recovered. After that heating was stopped and the flasks from both the apparatus were removed. These flasks were then kept on the water bath for the removal of the traces of solvent. This was checked by just smelling the resinoid over the smelling strip. After completely removing the solvent, the resinoid is washed with absolute alcohol and filtered to prepare the alcoholic extract or to separate alcohol soluble part from the resinoid (Table 1).

iii. Analysis: After extraction of the materials, the resinoid wereanalysed for their physicochemical properties. The physicochemical data of analysis of resinoid is given in table 1.

Table1. Physical analysis of Vetiver resinoid.

Yield of resinoid	Optical rotation at 20°C	Refractive index at 20^0C	Specific gravity at 20°C	Acid value	Ester value
0.15%	-7^0	1.5185	1.0081	13	35

Aroma chemicals screening through high-performance thin layer chromatography (HPTLC)

i. Sample preparation: 1 g resinoid of each sample was soaked in 10 ml of rectified spirit (90%) with occasional shaking for 15 minutes and filtered through filter paper (What man No. 1). The filtrate was concentrated and made up to 10 ml in a standard volumetric flask.

ii. HPTLC method: 7 µl of ethanolic extract of each sample was applied on aluminum plate pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness using Linomat IV applicator respectively. The plate was developed in appropriate solvent system (Toluene: Ethyl acetate *i.e.* 8:2 v/v) for Sandalwood and Vetiver roots and for Nagarmotha root tubers (Toluene: Ethyl acetate: Chloroform *i.e.* 8:2:0.5 v/v). After air drying the plate was visualized under UV 254 nm , UV 366 nm 12 & 15, 10 & 10, 11 & 11 spots were observed respectively, the plate was then kept in iodine vapour for about 20 minutes 12, 8 & 7 spot each were observed and the same plate was derivatised with anisaldehyde -sulphuric acid reagent and heated in oven at 105°C temp. up to visualization of spots. Twelve spots each were appeared on the plate respectively (Table 2).

Table 2. High performance thin layer chromatography (HPTLC).

S.N.	RF value of vetiver			
	254 nm	366 nm	Iodine vapours	After derivatisation
1.	0.08	0.11	0.07	0.09
2.	0.15	0.14	0.13	0.14
3.	0.21	0.30	0.20	0.21
4.	0.24	0.35	0.24	0.29
5.	0.31	0.54	0.31	0.34
6.	0.38	0.62	0.55	0.49
7.	0.56	0.67	0.58	0.53
8.	0.59	0.72	0.83	0.59
9.	0.66	0.77		0.69
10.	0.83	0.89		0.75
11				0.84
12				0.90

RESULT AND DISCUSSION

The pure, as well as refuse material of Vetiver, was processed at lab scale to find out the maximum percentages of resinoid using a suitable solvent. There have been occurred slightly differences in the yield of resinoids in both cases. Resinoids are more viscous than their oils but have a woody character due to its high boiling components. These high boiling components account for physicochemical properties like optical rotation, refractive index, specific gravity, acid value ester value etc. There are no standards/specifications of resinoids from refuse materials. In this regard this study may helpful for making specifications for valuable products.

After extraction of the essential oil from the pure materials generally, it was assumed that all the constituents are extracted. But the analysis of resinoids of the above-studied materials by high-performance thin-layer chromatography reveals the presence and confirmation of major as well as minor constituents. From the Rf values, it was observed that there are 8 active constituents present in resinoids of Vetiver. Out of them one or two were more active. Therefore, this study may help in the value addition of resinoids for proper utilization in pharmaceutical industries. It will render a new dimension to the pharmaceutical and essential oil industries enabling them to utilize the material in a better way.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal, D.A-V. P.G. College, Kanpur and Head, Department of Chemistry, D.A-V. P.G. College, Kanpur for supporting this research.

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