SEASONAL IMPACT ON YIELD AND MAJOR CONSTITUENT OF ESSENTIAL OIL OF *VETIVERIA ZIZANIOIDES* L. NASH GROWN UNDER JORHAT CONDITION, ASSAM, INDIA

Rumi Kotoky[1], S.C. Nath[1], H. Lekhak[1], J.C. Sarma[2] and S. Kalita[3]
1. Division of Medicinal, Aromatic and Economic Plants
2. Division of Natural Product Chemistry
North East Institute of Science & Technology, (CSIR) Jorhat, 785 006, Assam
e-mail: kotoky15@yahoo.co.in / rumikotoky18@gmail.com
3. Head, Department of Environmental Science, Guwahati University-781014, Assam

Abstract

Vetiveria zizanioides L. Nash, with high economic potential, known as Khas or Khus grass in trade, is a perennial plant species belonging to the family Poaceae. Studies on essential oil contents in vetiver species throughout the world have attributed their significance in pharmaceutical and perfumery industries. The thick fibrous adventitious roots are also used for economical importance. Numerous studies have been carried out to understand the variation of oil contents with time and space depending upon the agro- climatic conditions of different regions. Considering the importance and variability, an exploratory investigation has been made to understand the variation of *Vetiveria zizanioides* root oil contents under the agro - climatic condition of Jorhat, Assam, India. The roots of the propagated plant species of vetiver during the period from January to December 2010 were utilized for the present study and observed the major constituent of essential oil and seasonal impact on oil yield. The root of the vetiver grass was extracted by hydro-distillation using Cleavenger type apparatus. The yield of oil was varied in different seasons ranging from 0.2 to 0.7% indicating the influence of changing weather condition of Jorhat. GC and GC/MS analysis of oil showed the presence of 25 constituents where valerenol was the major compound which was however varied in different seasons ranging from 18-28% of the total oil. Valerenol was earlier reported as a minor compound in the essential oil of vetiver root. The present finding thus indicate the existence of a valerenol rich chemotype of *Vetiver zizanioides*. Seasonal variations in oil yield (0.2-0.7%) and major active ingredient valerenol (18-28%) were also found to be significant in the vetiver grass grown under the agro-climatic condition of Jorhat. Thus the present study will help to explore the feasibility of vetiver grass under the agro-climatic condition of Assam for promotional activity.

Key Words: oil yield, valerenol, seasonal variation, Jorhat

Introduction

The Vetiver or Khus plant (Vetiveria *zizanioides* L.Nash), an aromatic, perennial grass belonging to the family Poaceae is well known for its perfumery and flavour values. It is native to India and its roots yield an essential oil of considerable industrial demand which is mainly used as a fixative in perfumery and for blending in cosmetics and soap industry. The roots are also important in traditional medicine and the main action of vetiver oil is on the nervous system and it is both sedating and strengthening in effect (Wilson 1995). It stimulates the circulatory system and makes a useful massage oil for elderly or debilitated people with poor circulation (Chomchalow, 2001). In India, the demand of vetiver oil is increasing day by day due to its unique odour, for which it is used in both flavour and fragrance industries . One more reason for increase in demand is that this oil cannot be substituted with reconstituted oil and cannot be made synthetically. Vetiver grass is also known for its soil binding properties. It was first used for soil conservation and stabilization purposes in Fizi in the early 1950s and promoted by the World Bank for soil conservation in India in the 1980's (Dalton *et al* 1996). It possesses a lacework root system that is abundant, complex and extensive (Chomchalow, 2001), that can reach 3-4 meters in the first year of planting (Hengchaovanich, 1998) and acquires a total length of 7 meters after 36 months (Lavania,2003). Vetiver grass can tolerate extreme soil conditions (Troung, 1996; Troung and Baker 1998, Zheng *et al* 1998, Roongtanakian and Chairoj 2001a and 2001b, Chen *et al* 2004) and can grow on sites where annual rainfall ranges from 200 to 5000 mm (Rahman *et al* 1996). There are distinct geographical differences in quality and perfumery note of essential oil obtained from different regions of the world. Numerous studies have been carried out to understand the variation of vetiver oil contents with time and space depending upon the agro climatic conditions of different region. (Lemberg and Halley 1978, Ghani, 2003). The agro climatic condition of Assam is unique and composition of essential oil content of vetiver is still not precisely known. Therefore, considering the importance and variability an exploratory investigation has been made to understand the variation of *Vetiveria zizanioides* root oil contents under the agro climatic conditions of Jorhat, Assam

Materials and methods

Field study was conducted at the Experimental Farm of North East Institute of Science and Technology (NEIST), Jorhat (Longitude $94^{0}09'31''$ East and Latitude 26^{0} 44'19″ North) during the period 2007-2010. Planting materials of the vetiver grass were obtained from its germplasm maintained in the experimental farm of NEIST, Jorhat and transplanted in the field with 60 x 60 cm spacing. Field soil was sandy loam having pH 5.5, organic matter 0.86%, available nitrogen, phosphorus and potassium 0.075%, 0.0006% and 0.0034% respectively. Likewise the area witnessed average temperature, $16.72^{0}C$ to $31.17\ ^{0}C$ and rainfall 0.5 mm to 120.4 mm (Table 1). The two years old vetiver root samples were harvested every month during 2010 beginning from January up to December.

The root sample (300g) was collected and washed with water, removed the foreign particles and kept for over night in room temperature, hydro-distilled in a Clevenger type apparatus for 16 hours. The oils obtained were dried over anhydrous sodium sulphate; oil yields (v/w) were calculated and stored at 4^{0} C in sealed vials prior to analysis.

Analysis of oil was carried out by a combination of capillary GC and GC/MS using Shimadazu GC 17A and GC-MS- QP 5000 instruments. The capillary column used for analysis was a CP-Sil 5CB fused silica column, 25m x 0.25mm, film thickness 0.25µm. The initial oven temperature was held at $35^{0}C$ for 25 min, then programmed at $5^{0}C$/min to $28^{0}C$, Split ratio 50:1; carrier gas, Helium at a flow rate of 30 cm^{3}/s. The injector and detector (FID) temperature were maintained at $280^{0}C$. For GC/MS analysis, a quadruple mass analyzer with an electron ionization (EI) system was used. The mass spectra acquired were in the range 10 - 400 Da, with a scanning rate of 4 spectra/s. The transfer line temperature was kept at $280^{0}C$ at the helium flow rate of 40 cm^{3}/s.

The percentage composition of the oil constituent was calculated from electronic integration measurements using FID detection without response factor correction. Linear retention indices of the component were determined relative to n-alkanes. The constituents of the oil were identified by matching their mass spectra and retention indices using NIST library search facility available with the instrument. NIST library search data indicated that valerenol was the probable major component of the oil. For confirmation, the pure compound was isolated through preparative thin layer chromatography from the oil of the species with solvent system petroleum ether and ethyl acetate (8:2). After purifying, the compound was studied through NMR, IR and Mass spectra.

Results and discussion

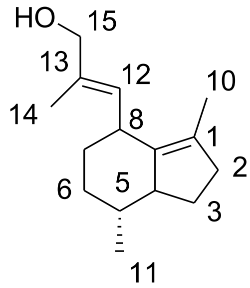
The essential oil content of vetiver obtained with respect of different times was presented in Table 2. The oil was a golden yellow coloured viscous liquid having pleasant smell with the yield ranged between 0.2 to 0.7% (v/w). Literature review also revealed the wide variation on the yield of root essential oil of vetiver grass (Pripdevech, et al 2006, Ramana, 2009, Lamberg and Hale 1978, Champagnat et al 2009). Essential oil of vetiver root is extremely complex. It contains more than 100 components that are mainly sesquiterpenes and their derivative (Aklhila and Rani, 2002). The main constituents of vetiver root oil comprise of khusimol, khusinol, vetivone, khusimone, α- vetivone, β – vetivone (Lavania, U.C.2003.). Adams et al 2003 reported that khusimol was the major component of vetiver oil among thirteen accessions of this species located in Nepal, Portugal and Florida with the concentrations ranging from 14.52 to 31.42%. The present work of GC and GC/MS analysis of the vetiver root oil showed the presence of 25 components , out of which valerenol (Fig.1) was the major component with the concentration ranging from 18 to 28%. The essential oil yield (0.7%) and valerenol percentage (28%) were highest in the month of March. However, the percentage recovery of oil in the month of July was although higher but with a comparatively lower concentration of valerenol (Table 2, Fig. 2). This might be related to high rainfall during this period (Table 1) which played a role affecting the concentration of specific component.

In spectroscopic studies of the pure isolated compound, NMR spectrum shows a dublet with J=7H_z at 0.78 ppm corresponding to methyl group no.11 at C-5.Two methyl groups 10 and 14 at C-1 and C-13 displayed two singlets at 1.0456 ppm and 1.0737 ppm. The olefinic proton at C-12 was visible as overlapping signal with two protons of C-15 in the range 4.73 to 4.56 ppm. OH proton was also coming as overlapping multiplet at 3.47 ppm. Mass spectrum of the compound showed its molecular ion peak at 220. 18 loss due to hydroxyl group was clearly evident with a peak at 202. Loss of CH_2OH (C-15) was given a peak at 189. Base peak at 162 is due to loss of 58 mass unit corresponding to cleavage of double bond between C-12 and C-13. Other major fragmentations of 150, 133, 119, 105, 91, 79 etc. mass units clearly supports the proposed structure of valerenol. Library search in GC-MS also support valerenol as a major compound with other fraction in it.

NMR (300 MHz, $CDCl_3$, δ ppm) 4.73 to 4.56 (3H, overlapping signal), 3.47 (2H, overlapping signal), 1.50 to 2.40 (overlapping multiplet), 1.07 (3H, s), 1.05 (3H, s), 0.78 (3H, d, J=7 Hz); (Fig. 3)

IR ($CHCl_3$) cm^{-1} 3374, 2926, 2865, 1638, 1456, 1378, 1219, 1026, 888, 772. (Fig. 4)

MS m/z at 220 (M+, 15%), 205 (8%), 202 (15%), 189 (58%), 187 (30%), 162 (100%), 161 (70%), 150 (58%), 145 (32%), 133 (56%), 119 (86%), 105 (70%), 91 (74%), 79 (38%). (Fig. 5).



VALERENOL

Fig. 1 $C_{15}H_{24}O$ Mol Wt. 220

The present findings thus clearly indicated the existence of a valerenol rich chemotype of *Vetiveria zizanioides*. Variation of valerenol up to 3.5% in vetiver root oil was reported previously (Thubthimthed et al 2003). Literature survey on the essential oil components of vetiver reported from different geographical regions of the world also evidenced the occurrence of valerenol content of the oil in the state of either negligible amount or un-traceable condition. These results suggested that the quality of vetiver essential oil is closely related to the metabolism of its roots which is affected by the agro climatic conditions under which it is grown.

Conclusion

That yield attributing characters of the root essential oil of *Vetiveria zizanioides* grown under the agro climatic condition of Jorhat (Assam) were studied taking into account of their seasonal variations. Valerenol was identified at the major component of the root essential oil of the crop grown under the condition. Seasonal variations on the percentage of oil yield and valerenol content of the crop under the condition were however observed. Oil yield (0.7%) and valerenol content (28%) were found maximum during the month of March while minimum in the months of May (0.2%) and July (18%) respectively. Quality of vetiver oil is seemed to be closely related to the metabolism of its roots which is affected by the agro climatic conditions.

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Table 1. Monthly average temperature and rainfall condition of Jorhat during 2007 - 2010

Month	Highest Temp. (^oC)	Lowest Temp. (^oC)	Rainfall (mm)
January	25.9	7.3	6.6
February	27.1	8.1	18.5
March	33.8	14.3	74.5
April	31.2	17.2	147.0
May	31.9	20.4	228.0
June	33.3	21.2	311.5
July	34.6	23.9	312.5
August	34.8	24.3	166.0
September	33.8	23.0	171.5
October	32.7	18.8	0.5
November	29.5	13.4	5.0
December	25.5	8.8	3.5

Table 2. Monthly variations on yield and valerenol content of vetiver root oil under Jorhat condition.

Month	Percentage of Oil (%)	Valerenol component (%)
January	0.40	27.0
February	0.57	26.4
March	0.70	28.0
April	0.30	23.0
May	0.20	21.7
June	0.48	19.2
July	0.65	18.0
August	0.50	20.5
September	0.40	24.1
October	0.36	22.0
November	0.50	25.2
December	0.53	25.6

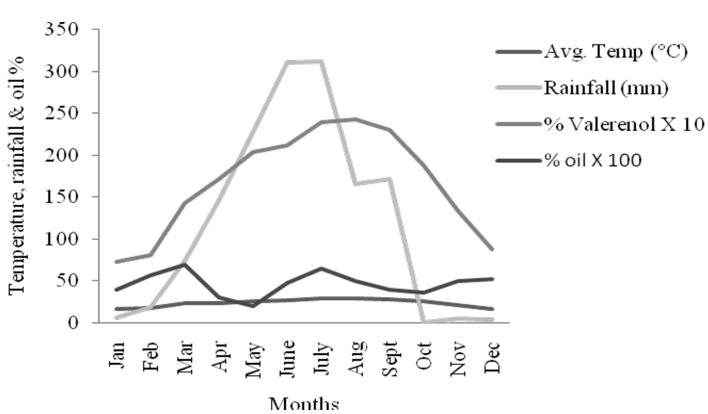

Fig. 2. Month-wise variations of temperature, rainfall, oil yield and valerenol content

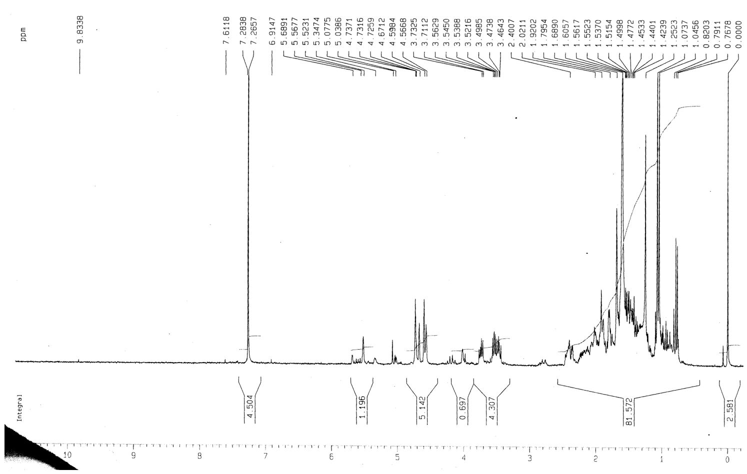

Fig. 3. Nuclear Magnetic Resonance (NMR) image of valerenol

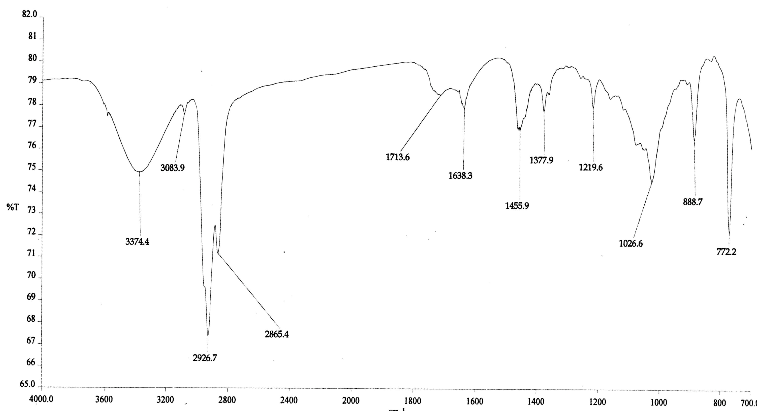

Fig. 4. IR image of valerenol

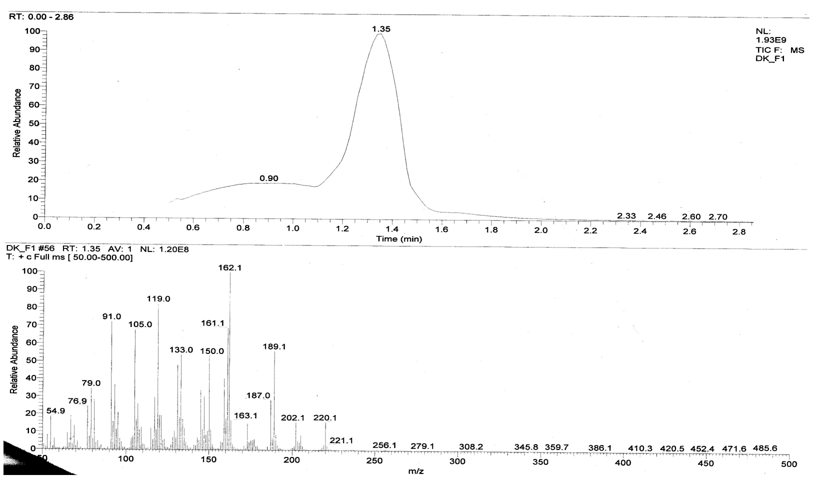

Fig. 5. Mass Spectroscopy (MS) of valerenol

Bio-data:

Name : Mrs. Rumi Kotoky
Designation : Technical officer
Qualification : M. Sc. in Ecology and Environment
Division : Medicinal, Aromatics and Economic Plants
North East Institute of Science and Technology, Jorhat, Assam

Having 15 years of research experience

During the span of fifteen years at NEIST, Jorhat , actively engaged in several national and international projects, acquired experience concerning extraction of essential oil from different aromatic plants, plant microbe interaction, isolation and identification of vesicular – arbuscular mycorrhizal fungi, biochemical estimation and separation of compounds from plant sources. Also, engaged in agro technology development of different aromatic and medicinal plants.

During this period published 24 research papers, attend several national seminars and have one US patent (Patent no. 6,160139).