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Extraction, Optimization and Characterization of Essential oil from Vetiver (vetiveria zizanioides) Grass Root by Steam Distillation for Perfume Application

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List of acronym

NEDFi	North Eastern Development Finance Corporation Ltd
INR	Institute of Natural Resources
ANOVA	Analysis of variance
R^2	Regression Coefficient
ASTM	American Society of Testing and Material
AOAC	Associates of Analytical Chemistry
GC	Gas chromatography
GC-MS	Gas chromatography- Mass-spectrometry
NRC	National Research Council
RT	Retention Time
EEORC	Ethiopian Essential Oils Research Center
SADC	Southern African Development Community
CI	Confidence interval
AOCS	American oil chemistry society
LIDI	Leather industry development institute

Abstract

The extraction of essential oils from natural sources is a highly profitable now a days. Vetiver grass is a viable vegetative absorbent and erosion barrier; in addition the valuable oils extracted from its roots are playing an increasing role in the perfume, food and pharmaceutical industries

The yield of essential oil that extracted from the vetiver grass root depends strongly on extraction parameters and method of extraction such as time, temperature and particle size are vital components for efficient processing of essential oil extraction. The experimental investigations undertaken using steam distillation has been determined a maximum average yield of (1.72%) after extraction time of 6 hours, particle size range of (0.425-0.75mm) and temperature of (95ºC) and the minimum average yield was obtained (0.15%) was found at extraction time of 2hr, temperature of (75ºC) and particle size of (1.075-1.4mm). From this statement, an increasing of extraction time, temperature and decreasing of particle size will increase the percentage yield of oil extract. The investigating of individual and interaction effect has been described by using excel graph and DESIGN EXPERT software (general full factorial) was applied to the extraction processes.

The composition of the vetiver oil extracts were analyzed using gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR) techniques and this showed that, a high percentage was obtained ((3S, 3aR, 6R, 8aS)-7, 7-dimethyl-8 methyleneoctahydro-1H-3a,6-methanoazulen-3-yl) methanol in a peak retention time of (16.780min) and high percentage of area was obtained (23.37%).

1. Introduction

1.1 Background

Recently there has been an increase in demand for essential oils extracted from plant material. This can be attributed to the fact that essential oils have multifunctional properties and hence are playing an increasing role in the food, fragrance, agricultural and pharmaceutical industries (Kim et al., 2005).

Vetiver grass also known by the Latin name Chrysopogon Zizanioides or its traditional name Khus, is a perennial grass which is part of the Poaceae family (Joy, 2009). The root is the most valuable part of the grass as it forms an intertwined network that stops erosion and it contains the majority of the essential oils which has valuable aromatic and biological properties (Danh, 2007).

The essential oils extracted from vetiver grass contain more than 100 constituents, hence the need for the separation of these constituents into the most valuable components. Essential oils are highly concentrated substances extracted from flowers, leaves, stems, roots, seeds, barks, resins, or fruit rinds. (Zhu et al, 2001).

Essential Oils are mixtures of organic compounds that are dominated by the Terpenes and the Terpenoids, oxygen containing derivatives of the terpenes. Essential oils are also complex mixtures of biologically active substances used as flavoring agents and constituents of number of commercial products. In developing countries, agricultural grasses root are very popular types of crops for rural outreach programmes due to the high value of the essential oils extracted from the grass root. Vetiver grass also known by the Latin name Chrysopogon Zizanioides or its traditional name Khus, is a perennial grass which is part of the Poaceae family (Joy, 2009).

The root is the most valuable part of the grass as it forms an intertwined network that stops erosion and it contains the majority of the essential oils which has valuable aromatic and biological properties (Danh, 2007).

Vetiver grass root has many uses; it is very easy to grow as it can withstand harsh environmental conditions and does not require large amounts of fertilizer or pruning and therefore it is easily maintained by the user at low cost. It has the unusual accolade of being both economically and ecologically important, which makes it one of the most versatile and exciting crops of today.

Its uses are not just limited to extracting the distinctive essential oil; the plant is used as a hedgerow barrier to control soil erosion and to stabilize crumbling rural roads, rivers and reservoirs, plus vetiver roots and grass are used to manufacture a vast range of handicrafts products. Accordingly, over 28,200 tons of essential oils are produced worldwide annually at an estimated value of $18 billion, of which China take the lead by producing about 16,600 tons per annum. (Trade and investment kwaZulu-natal, 2014).

The world production of vetiver oil is around 300 tons per annum of which India contributes about 20-25 tons. (NEDFi, 2005). Haiti, Indonesia (only Java), and Reunion produce most of the world's vetiver oil. (Cacadu, Trade and investment, 2009).

African countries collectively contribute less than 1% of global production. The major reasons for the difficulties on trade and market penetration of African essential oils are lack of quality standards, inability to consistently produce the critical mass of essential oil quantities, high shipping costs, lack of familiarity with market requirements, and the challenge of meeting product specifications of the international marketplace. (SADC, 2005). World Market, the demand for 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. It is used for Base-note in flavour and fragrance and the percent usage varies from 20-50% to 60-70 %. That means it has enough market potential and there is no doubt on its consumption. (NEDFi, 2005, hand book on medicinal & aromatic plants). The natural products sector of Ethiopia is dependent on international trade for its commercial success, as indicated above vetiver grass root oil have demand in different section in our country but the technology of vetiver grass root oil production is not introduced.

Apart from these, the Ethiopian essential oils research center (EEORC) sometimes extracts essential oils from different essential oil yielding plant spices for research purposes. Otherwise, no company in the region or at the country level has engaged in practical vetiver processing activity.

1.2 Statement of the problem

The natural products sector of Ethiopia is dependent on international trade for its commercial success, as indicated in literature part essential oil demand in different section in our country.

One of major problems of essential oil industry in the country, unavailability of small scale adapted technology based on the socio-economic level of the rural community, lack information on the social and economic benefits to be derived from the industrial utilization of aromatic plants, and the low priority given by the government have been a major factors impeding the development of the essential oils extraction industry in Ethiopia like other small-scale industries, is scarcity of adapted technological input in the required level.

The product from vetiver grass root from small farmers generally does not enter the market, or enters in a very limited quantity for the purpose of soil erosion protection, but when we process vetiver grass root in oil form is advantages to preserve it and use the essential constituents within it.

The availability of raw material in our country, as Ethiopia is importing essential oil value added products from international market at a high price; there is an opportunity to substitute the imports. The increasing importance of essential oils as pharmaceutical and aromatherapy aid besides their traditional role in cosmetics not only as potent ingredient but also as a fragrance donor has opened up wide opportunities for global marketing. This suggests the existence of huge demand for essential oils in the country, introducing value added processing of resources would produce greater benefits and offers viable investment opportunities.

1.3 Objectives

1.3.1 General objective

The aim of this thesis is to extract essential oil from vetiver grass root by steam distillation.

1.3.2 Specific objective

The specific objectives of this study are;

- To characterize raw vetiver grass root in terms of, protein content, ash content, fat content and moisture content by proximate analysis
- To investigate the main effects and interaction effect of the three operating conditions of steam extraction condition, namely time, temperature and particle size at the same levels numbers and replicates.
- To optimize these conditions for the highest oil yield by using the general full factorial design experiment.
- To characterize vetiver grass root essential oil that produced at the optimum condition using Gas chromatography mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR) analysis.

1.4 Significance of the study

As we know the essential oil industry is an agro based industry, which involves cultivation and distillation activities. It can involve the rural population by having them produce raw materials and by creating job opportunities in the processing of the oils.

It will minimize the foreign currency that will cost for import of the oil and maximize the income of the farmers that they will harvest more for the production of the oil as raw material with the growing awareness of the many uses and advantageous characteristics of the vetiver grass. The high world market demand of essential oils can also be an attractive drive to introduce small-scale essential oil industry as a source of foreign currency.

The government is starting to appreciate its advantages and this in turn has lead many research groups around the country to study its uses and value. It also will give a chance of employment opportunity in the processing area and in the bulk harvesting area of the vetiver grass root.

It strongly help to maximize the exported gain of vetiver grass root that the loss due to import of the oil will compensate by both processing the oil and exporting the vetiver grass root in bulk. Also will have a great chance to introduce and distribute to the society, for those who apply the use of the oil industrially and cosmetics purpose consumptions in appropriate price.

Initiate investors to pay capital for the extraction of essential oil from vetiver grass root and others on such kind of research, do further scientific investigation on the essential oils of different plants.

It will provide the transformation of technology and initiation to develop the country's capacity in operating and processing ourselves. It will satisfy the agricultural lead industry of our county strategy. According to different data's a single processing center can provide employment. Most of the workers in such processing centers are found to be women or the poor. Establishment of such processing centers within Ethiopia would generate employment opportunities for thousands of women and poor.

2. Literature Review

2.1 Essential oils

Essential oils are frequently referred to as the “life force” of plants. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total. That's why tons of plant material is required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Essential oils cannot be substituted with synthetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate.

Research has confirmed centuries of practical use of essential oils, and we now know that the 'fragrant pharmacy' contains compounds with an extremely broad range of biochemical effects. Essential oils are so termed as they are believed to represent the very essence of odor and flavor. (Virendre P.S.Rao, Diwaker pandey 2006-2007).

2.2 Factors affecting the yield and quality of the essential oil

The yield and quality of essential oils have been known to vary due to a number of factors,

Mode of distillation: technique for the distillation should be chosen on basis of oil boiling point and nature of the herbs as the heat content and temperature of steam can be alter distillation characteristics.

Proper design of equipment: Improper designing of tank, condenser, or separators can lead to loss of oils and high capital investment.

Material of construction of equipment: Essential oils which are corrosive in nature should be preferably distilled in stills made of resistant material like copper or stainless steel.

Condition of raw material: Condition of raw material is important because some materials like roots and seeds will not yield essential oil easily if distilled in their natural state. These materials have to be crushed, powdered or soaked in water to expose their oil cells.

Filling raw material /steam distribution: Improper loading of the herb may result in steam channeling causing incomplete distillation.

Operating parameters: Proper control of injection rates and pressure in boiler operated units is necessary to optimize the temperature of extraction and for maximal yield temperature of condensate should not be too high as this can result in oil los to evaporation (Aggarwal, K.K., 1998).

Pre-condition of equipment: Equipment should be well steamed for multiple crop distillation. It should not be rusted for quality oil (Dey,1998).

Particle size: plays a determining role in extraction processes controlled by internal mass transfer resistances, since a smaller mean particle size reduces the length of diffusion of the solvent. As a rule, particles with mean diameters ranging approximately between 0.25 and 2.0 mm are used (Reverchon and Marco, 2006).

2.3 Essential oil in Ethiopia

Currently the industrial demand for essential oils and extracts in the country is met through imports. Exact data on the imports of essential oils extracted from natural vetiver grass root is not available. There is also Essential Oils Research Centre that has its head office and the main laboratory in Addis Ababa and an agricultural research station, laboratory and a small-scale production unit at Wondo Genet, (about 267 km south of

Addis Ababa). In addition to this, some firms are engaged in processing and export of some specific types of essential oil.

This includes Ariti Herbal Private Company, Aromabyssinia Plc. and Cheleleka Edible Oil Factory. Apart from these, the Ethiopian Essential Oils Research Center sometimes extracts essential oils from different essential oil yielding plant spices for research purposes. Otherwise, no company in the region or at the country level has engaged in practical vetiver grass processing activity (Development Studies Associates, 2008).

On the other hand various types of essential oil are imported to Ethiopia which can be categorized under some major groups according to their application. These are for Perfumery, Pharmaceutical, Cosmetics, Toiletries, And Confectionery industry. However, detail data of essential oil for each sub section could not be obtained. But it can be asserted that much of the demand is derived from the toiletries, and confectionery industries given the relatively more production in these sectors.

Nonetheless, according to the Ethiopian Revenue and customs Authority (ERCA) data the import volume of various type of essential oil and extracts has increased almost four fold within the last fifteen years (see table 2.1).such huge increase is result of expansion of the industrial sector that makes use of essential oil.

For instance, in 2011 the country paid out nearly 30 million USD to import 1,596 tons of various types of essential oils and mixtures of odoriferous substances from countries supply such as India Germany, Ireland, Italy, South Africa, Great Britain and China in order of their importance. This presents the existence of huge demand for the product and the burden it is exerting on the country's foreign exchange reserve.so, implementation of the production of the essential oil has great multi–direction economic advantages for the country (Investment Office, 2007).

Table 2.1 Import status of essential oil in Ethiopia (Ethiopian Revenue and customs Authority, 2007).

Year	Import volume (tons)	Value (USD)
1997	400.11	3,865,349.88
1998	423.99	4,461,726.80
1999	368.72	3,520,854.72
2000	375.83	4,535,625.86
2001	534.3	5,359,948.05
2002	577.65	6,358,126.20
2003	632.44	7,773,675.72
2004	728.53	9,615,956.76
2005	929.47	12,543,293.13
2006	1,106.77	14,667,401.40
2007	1,026.3	16,023,486.18
2008	1,420.47	23,396,98647
2009	1,428.53	27,482,015.22
2010	1,590.56	33,345,309.42
2011	1,596.48	29,896,818.62
Average	**876.02**	**13,522,971.62**

2.4 Source of essential oil for perfume

2.4.1 Vetiver Grass Overview

Vetiver grass is a perennial graminaceous plant (Gramineae) native to India, growing wild, half wild or cultivated in many tropical and subtropical areas (NRC, 1993).In particular, selected germlines of the species Vetiveria zizanioides (L.) Nash has long been. Cultivated for their odorous roots that contain the essential oil of Vetiver, used extensively in perfumery and cosmetics (Maffei, 2002).

Vetiver grass has a wide root system consisting of long, fibrous roots and rootlets forming a sort of fasciculate mass, extending 2-3 m deep. Root tissues contain oil producing cells, responsible for its characteristic odor (Peyron, 1989). Vetiver grass is native to India (National Research Council, 1993); however it is cultivated to a limited extent in South Africa and is used as a hedge plant.

Table 2.2 Countries where vetiver grass is currently known to exist (NRC, 1993).

Africa	Asia	Caribbean	America	Pacific	Others
Algeria	Bangladesh	Antigua	Argentina	Fiji	France
Angola	Burma	Barbados	Brazil	Cook Island	Italy
Burundi	China	Cuba	Columbia	New Caledo	Spain
Comoro	India	Haiti	Costa Rica	Wester	USA
Central Africa republic	Indonesia	Dominica	French	America	USSR
Ethiopia	Nepal	republic	Guiana	Samoa	
Gabon	Japan	Jamaica	Guatemala	New guinea	
Ghana	Malaysia	St. Lucia	Guyana	Tonga	
Kenya	Pakistan	St. Vincent	Honduras		
Madagascar	Philippines	Martinique	Paraguay		
Malawi	Singapore	Puerto Rico	Suriname		
Mauritius	Sir Lanka	Trinidad			
Nigeria	Thailand	Virgin Island			
Rwanda					
Reunion					
Seychelles					

Truong and Hart (2001) reported in the Pacific Rim Vetiver Network Technical Bulletin that in (1995) vetiver grass was first recognized for having very absorbent properties and by 1997 this was proven to be fact. Due to the scarce water problems we are facing in the 21^{st} century, more and more vetiver systems are being used for water treatment.

2.4.2 Description of vetiver grass

There are two types of vetiver grass (NRC, 1993) that we are aware of today; one originated from North India and one from South India. It is important to distinguish between the two types as the South Indian type is domesticated and is therefore found all over the world, whereas the North Indian type is wild and can become a weed.

Table 2.3 Comparison between South and North Indian Vetiver Grass (NRC, 1993).

South India	North India
• Domesticated • Non flowering • Non seeding (or at least non spreading infertile seeds) • Oil is dextrorotatory (rotate polarized light to the right) • Safe to use for erosion control • Thicker stem • Less branched roots • Wider leaves • Higher oil content and yield	• Wild • Flowering • Sets fertile seeds • Oil is levorotatory (rotates polarized light to the left) • Roots are shallow therefore not suitable for erosion control)

Vetiver grass can be described as large clumps of grass that consist of a crown, leaves, stems, roots and sometimes flowers (NRC, 1993). It can grow to a clump diameter of about 300 mm, an above ground height of 500 to 1500 mm and a root depth of 3000 mm. Vetiver leaves are long and narrow with a soft section on the top and a hard firm section at the bottom (NRC, 1993).

2.4.3 Growing of the vetiver grass

Vetiver grass is propagated mainly by root division or slips (NRC, 1993). These slips or tillers are cut off the main clump of the vetiver grass and planted as seedlings in the ground (50–80 mm deep, NEDFi, 2005). These slips are planted close together typically between 10 – 30 cm apart depending on whether hedge formation is an aim or not.

Figure 2.1 Bare root slips and tube Stock (Truong et al., 2008).

Vetiver grass will grow in any soil type but a rich and fairly well drained loam is considered best. However for fast growth a rich, well-drained soil with loose texture is recommended (NEDFi, 2005). The same applies for the climate; even though vetiver grass can withstand adverse weather conditions, it has an optimum growing climate. Vetiver grass grows best under warm and damp conditions (by the edge of water) and therefore prefers a warm summer climate with well distributed rainfall (NEDFi, 2005).

The microbes and bacteria present in the soil surrounding the vetiver roots react with the vetiver oil within the roots and hence sesquiterpenes undergo bio-conversion into oxygenated sesquiterpenes (NEDFi,2005).The oxygenated sesquiterpenes give the vetiver essential oil its unique odour. Therefore the odour in the vetiver oil produced is related to the soil conditions for growth and hence the location of growth.

Table 2.4 General tolerance range for growing vetiver grass (Truong and Hart, 2001).

Adverse soil condition	
Acidity	pH 4.2
Alkalinity	pH 10.5
Aluminum level (Al Sat. %)	80-87
Heavy metal / mg kg $^{-1}$.	
Cadmium	22
Copper	174
Lead	3123
Zinc	3418
Altitude /m	2800
Climate	
Annual rainfall /mm	250-5000
Forest (ground temperature)/°C	-22
(soil temperature)/°C	-10
Heat wave/°C	60
Fertilizer	Nitrogen and Phosphorous, Farm Manure
Palatability	Cows, cattle, goats, sheep, pigs and carp
Nutritional value %	Crude Protein 3.3 Crude Fat 0.4 Crude Fiber 7.1

For maximum growth it is advisable to trim the aerial portion of the grass at the start of winter; this encourages tiller and root growth. Once the vetiver grass is between 15-18 months old it is ready for harvesting. (NEDFi, 2005).

Between 15 and 18 months the roots of the grass are thicker and mature, therefore yielding more oils of a higher quality. If left longer than two years the roots become woody, hence loosing essential oil content.

Harvesting should be done during the dry season preferably at the beginning of the ground may become too dry and hard towards the end of the dry season making it difficult to dig up the roots (NEDFi, 2005 and Lavania, 2003).

After harvesting, the roots can either be extracted while they are still fresh or they can be left to mature for 12-24 months to increase the vetiver oil yield by enzymatic processes (Dowthwaite and Rajani, 2000). The vetiver roots grown on pontoons are shorter in length due to the fact that nutrients are readily available to the root and hence root spread downward is unnecessary (refer to figure 2.2). When designing a floating pontoon system one must take into account durability, functionality, environmental sensitivity, weight, buoyancy, anchoring, flexibility and cost (Headley and Tanner, 2006).

Generally a square, triangular or rectangular framed pontoon is used and the number of pontoons used depends on the size of the lake, pond or wastewater tank.

(a) (b)

Figure 2.2 Roots of the vetiver grass (a): grow in water and (b): grow in soil (Truong et al.,2008).

2.4.4 Roots of vetiver grass

The roots of Vetiver are the most useful and important part. Most grasses have fibrous toots, which spread out from the underground part of the culm and hold the soil in a horizontal pattern. The roots that penetrate vertically in to the soil are not deep.

In contrast, the root system of vetiver grass does not expand horizontally but penetrates vertically deep in to the soil, whether it is the main, secondary or fibrous roots. The horizontal expansion of the vetiver root system is limited up to only 50cm. The root vertical penetration up to 5 meters.

2.4.4.1 Physical description

Vetiveria zizanoides is a dense, erect, clumping and a perennial grass growing up to two meters high, and three meters deep in the ground. It has a strong dense and vertical root system. It grows both in hydrophilic and xerophytic conditions. (Alemu mekonnen, gtz ifsp s/gonder , ethiopia , june 2000).

(a) (b)

Figure 2.3 Physical description of vetiver, (a): vetiver grass, (b): vetiver root, from Bako (Anno agro industry farm plc.2017).

2.5 Vetiver grass in Ethiopia

The introduction of vetiver grass to Ethiopia is controversial, some say that it was introduced in the 1960's by a British scientist who was working in Jimma Research Centre as coffee intensification program, others say it was introduced by Ethiopian coffee researchers, who were once on educational tour in India, while other say, it was introduced by an Indian scientist, who was working as an expatriate in the Jimma research centre. On the other hand, the aim of the introduction of vetiver to Ethiopia is not well recorded (Tessema Chekun Awoke, 2017).

Mr. Fernie, a British agronomist arrived at the then Jimma Agricultural Research Center, and Mesfin Amha had traveled to Yamungi, Tanzania in 1971 and on the way back they brought with them Vetiver grass to Jimma Research station, Ethiopia for the first time.

Shortly after introduction of Vetiver grass to Jimma Research station, an observation trial was conducted and samples were sent to Tropical Institute, England for oil content analysis.(Tesfu Kebede and Tesfaye Yaekob 2006). The utilization of vetiver's potentials was started in early 1980's as barrier against the spread out of couch grass by state owned large coffee estates, in south-west Ethiopia.

Nowadays, In Ethiopia, few companies are engaged under two categories of vetiver processing activities. The first category is propagation of vetiver grass for agricultural lands erosion control and, the second is for demarcation of coffee research treatments. The former has been mainly performed by individual vetiver grass producing farmers with a negligible portion going to one private enterprise situated at Bako, (Anno agro industry farm).

2.6 Usage of vetiver grass root

Since ancient times in India, the versatile qualities of vetiver roots have been employed in a diverse range of household applications, and this practice is still expanding today.

Dried roots are woven into sandals, mats, screens; window blinds, hand fans and ornamental baskets, plus aromatic sachets are used to fragrance bed linen and clothing garments. Vetiver has been used since time immemorial in Ayurvedic medicine where it is considered to be antitoxic, astringent, bitter and cooling, and was used for bilious fevers and sweats, blood diseases, foul breath, headaches, infections, urinary ulcers, an antidote to poisoning as well as to strengthen the central nervous system and overcome depression, insomnia, anxiety and stress.

There are two ways of using vetiver grass (Chomchalow and Chapman, 2003): to make use of the planted vetiver grass or to utilize the harvested vetiver grass. When utilizing the live vetiver grass there are conventional and non-conventional uses, conventional obviously being the most popular uses.

2.6.1 Use of planted vetiver grass

Conventional uses: Soil and water conservation, Erosion control, Slope stabilization, absorption of heavy metals (utilization of vetiver grass in stabilizing slime dams in the mining industry) and Wastewater treatment.

Non-conventional uses: like Livestock grazing and Ornamentals.

Chomchalow and Chapman (2003) also state that every few months it is necessary to cut the leaves of the vetiver grass to promote growth and to prevent fire during the dry season. Hence the need to utilize the harvested vetiver leaves and culms to provide an extra income. The vetiver grass can also be grown specifically to harvest the roots of the grass that contain valuable essential oils. The roots, leaves and culms can be used in a processed, semi-processed or non-processed form depending on its application.

2.6.2 Use of harvested vetiver grass

Agricultural: Mulch (protective covering placed over soil), Compost (decomposed leaves and culms) and Animal feed (young vetiver leaves). Nootkatone, α-vetivone, β-vetivone, khusimone, zanal and epizizanal are components known to exist in vetiver oil which have insect repelling abilities and are non-toxic to humans due to their natural origin (Henderson et al. 2003). Handicrafts (known to have cooling properties), Construction (e.g. thatched roofs). Medicinal (traditional), Antifungal, anti-inflammatory and antioxidant (β and α - vetivones) (Danh, 2007).

Fragrance: Perfume fixative or as a fragrance itself. e.g. Guerlains ,Vetiver, Chanels Coco, Diors, Miss Dior, Yves St.Laurents ,Opium and Givenchys,Ysatis (Dowthwaite and Rajani, 2000).

Aromatherapy: Vetiver oil is known to have several beauty, health and emotional benefits.

Flavour and preservatives in the food industry: for Ice cream, Beverages**,** Food preservative**,** Spices**,** Energy Source (Ethanol) and Raw material for pulp and paper industry **(**Lavania, 2003**).**

2.6.3 Vetiver use for wastewater treatment

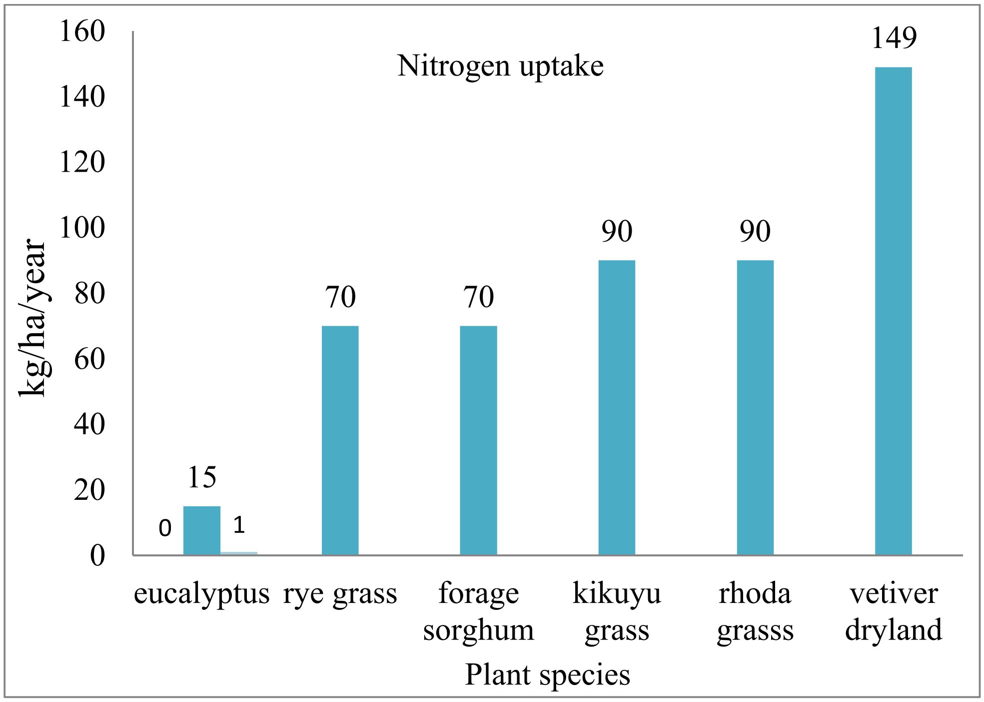
After primary treatment of wastewater there are still significant amounts of contaminants and nutrients in the water and therefore further treatment is required to reduce these contaminants and nutrients to an acceptable level. According to Peavy et al. (1985) constructed wetland can be used to remove these contaminants from wastewater. The vetiver grass roots provide a large surface area for colonization of wastewater by heterotrophic bacteria that degrade organics materials and at the same time the vetiver roots create a hostile environment for other pathogenic organisms in the wastewater (Chomchalow, 2001). Vetiver grass can serve as a sink for wastewater as it can be grown in pontoons on wastewater ponds; it can be grown in constructed wetlands; or used for irrigation of the vetiver crops. Either way a resource is produced from a waste product.

Truong and Hart (2001) discuss the suitability of using a vetiver grass system for wastewater treatment. They found that due to the following morphological and physiological features of the vetiver grass, that it is indeed highly suitable.

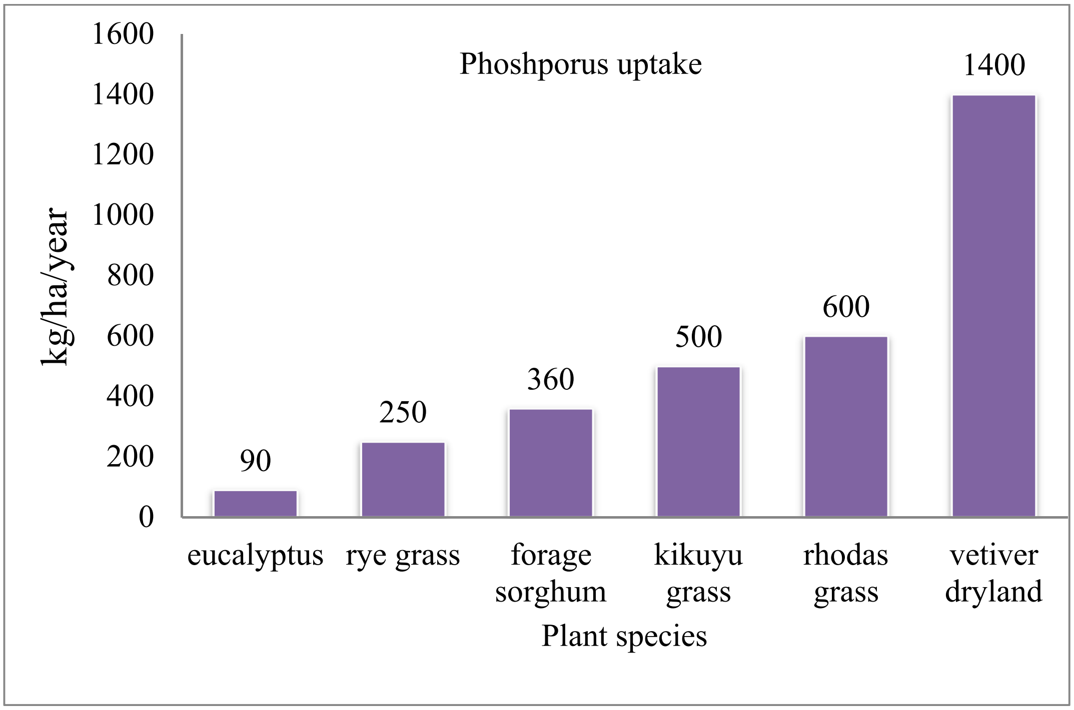
Morphological features: Stiff and erect stems which can withstand high velocity flows; therefore when planted close together can form a living porous barrier.

Its deep root system allows the plant to grip tightly into the ground and to withstand adverse weather conditions. It has many fine root branches which allows for a large surface area for absorption of contaminants and nutrients.

Physiological features: High tolerance to heavy metals, high tolerance to adverse weather conditions, high tolerance to adverse soil conditions, high absorption rate of nitrogen and phosphorous, highly tolerant to pesticides, Regenerates rapidly and high water use rate. According to Truong and Hart (2001) vegetative methods are the only feasible and practical methods available for large scale reduction or disposal of wastewater. Recently it has been found that using vetiver grass as a vegetative absorbent is highly effective; this is due to its ability to absorb high amounts of nitrogen, phosphorous and organic compounds, which are key elements in water pollution (see to Figure 2.4 a and b).



(a)



(b)

Figure 2.4 (a), Nitrogen uptake and (b), Phosphorous rates (Truong and Hart, 2001).

There are two common methods (Truong and Hart, 2001) for this treatment: land irrigation systems or wetlands. According to Headley and Tanner (2006) “Constructed treatment wetlands are engineered systems designed to enhance the process and interactions that occur in natural wetlands between water, plants, microorganisms, soils and the atmosphere in order to remove contaminants from polluted waters in a relatively passive and natural manner”.

2.7 Vetiver grass root essential oil

Essential oils are generally a mixture of organic compounds that are located throughout different plant material. This oil is essential to the plant hence the name essential oil and is found in many parts of the plant such as the sacs, cells, glands or even ducts inside the roots, stem, bark, flowers, seeds or leaves (Dowthwaite and Rajani, 2000).

Vetiver root essential oil is extracted from the roots of a grass known botanically as (vetiveria zizanoides). Roots contain the higher quantity of essential oils with high concentration of bicyclic and tricyclic sesquiterpenes, hydrocarbons, alcohol & rsquys and carboxylic acids.

Those that repel insects are minor constituents. Vetiver is mainly cultivated for the fragrant essential oil distilled from its roots. In perfumery, the older French spelling, vetyver, is often used. Due to its excellent fixative properties, vetiver is used widely in perfumes. Vetiver oil is the basis of the Indian perfume (Majmua) and is the major ingredient in some 36% of all western perfumes (e.g. Caleche, Chanel No.5, Dioressence, Parure, Opium) and 20% of all men's fragrance. Vetiver root essential oil is amber to dark brown in colour; it is one of the most viscous of the essential oils and therefore has a low evaporation rate and it is also soluble in alcohol (Lavania, 2003).

This allows the vetiver essential oil to be a perfect candidate for use in the perfume industry, especially as a fixative. Vetiver oil is known to blend well with patchouli, sandalwood, jasmine and many other essential oils; however it is often diluted to prevent the odour from dominating the blend (Bhatwadekar et al., 1982). The odour in most essential oils vary widely with the natural elements and hence location (Dowthwaite and Rajani, 2000).

Vetiver root essential oil is known for its earthy woody balsamic odour; however the odour can also be more sweet and roseate (Lavania, 2006). The vetiver root oil varies with the natural elements in which it grows because it is composed of sesquiterpenes and sesquiterpene derivatives which have a high chance of polymerisation and hence polymerise to different degrees depending on the natural elements that it is exposed to (Chowdhury et al., 2002). Like most essential oils the composition of the vetiver essential oil is extremely complex, it is known to contain more than 100 sesquiterpene compounds and their derivatives (Lavania, 2006). Vetiver oil is rich in C_{15} sesqui-terpeniods which can boil at over 200°C (Dowthwaite and Rajani, 2000).

The main constituent of the vetiver essential oil includes (Lavania, 2003): sesquiterpene hydrocarbons:e.g., cadenene, clovene,amorphine, aromadendrine,junipene, sesquiterpene alcohol derivatives e.g.,vetiverols-khusimol, epiglobulol, spathulenol, khusinol and sesquiterpene carbonyl derivatives: e.g., vetivones – vetivone, khusimone ,sesquiterpene ester derivatives, e.g., khusinol acetate.

Sesquiterpenes are a class of terpenes (organic compounds found in plants) that consist of three isoprene (C_5H_8) units and have the molecular form $C_{15}H_{24}$ (National Library of Medicine,2011). Sesquiterpenes have high molecular weights with low vapour pressure (Danh. 2007). The most valuable components found in the vetiver oil have the highest boiling points and therefore are not easily vaporised for collection (Chomchalow, 2001).

Generally, Vetiver grass root essential oils are important raw ingredients used in perfume industry, entering the formula of numerous modern fragrances. Vetiver oils are considered to be among the most complex essential oils, resulting most of the time in highly coeluted chromatograms whatever the analytical technique.

The top 5 major components identified in vetiver oil according to gas chromatography area percentage given in literature are as follows:

Table 2.5 Major components of vetiver essential oil (leite Bianca 2012).

No.	Major components of vetiver oil	Percentage
1	Zizanoic acid	15-32
2	Khusimol	7-15
3	Isovalencenol	5-9
4	α – Vetivone	4-8
5	β – Vetivone	0-3

The above conclusion was taken from papers by Danh et al. 2010 and Martinez et al. 2004, both of whom used vetiver grass grown in Brazil for their extractions.

2.7.1 Annual demand of vetiver grass roots essential oil

The statistics reported in Table 2.6 by the NRC in 1993 are outdated; however it shows a gap in the market for vetiver essential oil. According to the NRC (1993) these figures should have stayed roughly the same over the years due to the fact that vetiver oil is expensive and therefore cedar wood oil was being used as a substitute.

However, according to the NEDFi (2005) the above prediction was proved incorrect and the world market demand for vetiver essential oil is increasing day by day. This is due to the fact that vetiver has a unique odour, it cannot be substituted with reconstituted oil and it cannot be made synthetically. Vetiver essential oil is found in large proportions in 20% of all men‟s perfumes and 36% of western perfumes (Danh, 2007). Meschede (2009) states that, "Although vetiver essential oil has a good demand, it is still facing a tight supply". In the publication by Dowthwaite and Rajani (2000) these researchers label vetiver oil as being high priced oil. An investigation into the typical selling prices of vetiver oil from various suppliers was undertaken and from this it was reported that the selling price varies between 285.71 and 2857.14 USD per kg of vetiver oil (Average of latest trends). The large variation in the selling price is due to the large variations in the quality of vetiver oil produced from different regions and suppliers (leite Biaca 2012).

Table 2.6 Annual demand of vetiver essential oil (NRC, 1993)

No.	Countries	Percentage
1	United States	100
2	India	100*(of which 80% is met by import)
3	France	50
4	Switzer land	30
5	United kingdom	20-25
6	Japan	10
7	Germany	6
8	Netherland	5
9	Other	30-40

2.8 Extraction of vetiver grass roots essential oil

According to Luque de Castro et al. (1999) the techniques for extraction of essential oils from plant matter can be classified into three techniques: continuous conventional, discontinuous conventional and non-conventional. The type of extraction method used directly affects the quality, yield and odour of the essential oil. The steps below describe the mass transfer mechanism for the extraction of essential oils from plant material (Talansier et al., 2008):

Constant extraction rate: The external surfaces of the particles (plant material) are completely covered with oil.

Falling extraction rate: Phase where the external surface oil has been depleted by mass transfer into the extracting fluid and the surface area of the particles are only partially covered.

Diffusion period: No oil is present on the external surface of the particle and hence diffusion occurs. According to Talansier et al. (2008) 70% of extracted oils are extracted in the constant and falling rate extraction periods of which 50% is extracted in the constant rate period. Therefore the process can be modelled considering convective processes only (i.e. neglecting the diffusion process). However, Chomchalow (2001) states that vetiver roots do not easily yield oils as the oils are located in the inside root tissue and hence the slow physical process of diffusion must occur before oils are extracted. This and the fact that the vetiver oil consists of high molecular weight Sesquiterpene contributes to the long extraction times necessary for extraction of the vetiver oil from vetiver roots.

2.8.1 Distillation (continuous-conventional)

Distillation is the process in which the raw material (prepared vetiver roots) is heated in order to separate the volatile and non-volatile components by collecting both the top product (distillate) and the bottom product (bottoms), which is condensed and recycled respectively.

The type of distillation is defined by the heating medium used. There are four types of mediums employed when distilling the essential oils from the vetiver grass (Douglas et al., 2005) these include**:**

2.8.1.1 Hydro distillation

Also known as water distillation, it is the simplest and most common method of distillation. The raw material is mixed with water in a still pot and heated at the bottom which causes the water to vaporise and take with it the valuable oil extracts. The vaporised water and extracts are then condensed into an oil separator where the extracts can be separated. A perforated grid is used to prevent the raw material from settling to the bottom of the pot and becoming overheated. The raw material must also be agitated at all times to promote extraction. This method is not preferred due to the heat and water damage imposed on the extracts**.**

Hydro distillation on a laboratory scale, for the extraction of essential oils from plant material is commonly performed in a Clevenger apparatus. This apparatus is better suited for separation of the extracts from the water due to the fact that the small amount of extract can be collected easily in the collecting arm.   .

2.8.1.2 Steam distillation

Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. Steam distillation is the most widely accepted process for the production of essential oils on a large scale and is regarded as the standard practice throughout the flavour and fragrance industry. (African Business Access Cape Town, South Africa July 2006). The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s). Essential oils contain substances with boiling points up to 200°C or higher temperatures.

In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C, at atmospheric pressure (African business accesses Cape Town South Africa July 2006). In this technique the steam is provided by an external boiler source. The raw material is packed onto a perforated plate and the steam is passed over it in order to collect the valuable extracts which are then condensed and sent to the oil separator. Although the capital and operating cost will be more for this method, by supplying an external steam source one can control the amount of steam and the temperature of the steam passing over the raw materials and therefore thermal degradation can be controlled.

In this study, the separation process that has been chosen is steam distillation. Steam distillation is one of the separation processes that used solid-liquid extraction theory. It is most used to produce many types of essential oil such as from vetiver grass root.

The raw material is packed onto a perforated plate and the steam is passed over it in order to collect the valuable extracts which are then condensed and sent to the oil separator. Distilled water is used at its boiling point as steam to extract the essential oil from vetiver grass root. The steam will help to release the aromatic molecules from the vetiver grass root. The molecules of these volatile oils then escape from the vetiver grass root and evaporate into the hot steam. The temperature of the steam must be carefully controlled. It is because to control the vetiver grass root from burning and lost its purity. The process is cheaper than other extraction processes. In order to use the oil to medical purpose, this means for direct use (De Silva,1998).

Advantages of using steam distillation: Amount of steam can be readily controlled, No thermal decomposition of oil constituents and Most widely accepted process for large-scale oil production, superior to the other hydro distillation processes. Generally, steam distillation is that it is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are not altered. The only drawback of steam extraction is the high capital expenditure needed to build a system (MBB consulting services south (Pty) Ltd. July, 2006).

The main difference between this technique and others involving steam is that a separate steam boiler is used to generate the steam, before it is let into the charge vessel, containing the plant material from which the oil is to be extracted.

2.8.1.3 Water and steam distillation

The raw material is packed into the still pot on a perforated plate and water is boiled beneath the plate (refer to Figure 2.8). Therefore the water vapours pass though the raw material while extracting the valuable extracts. The vapours are also condensed and passed to an oil separator.

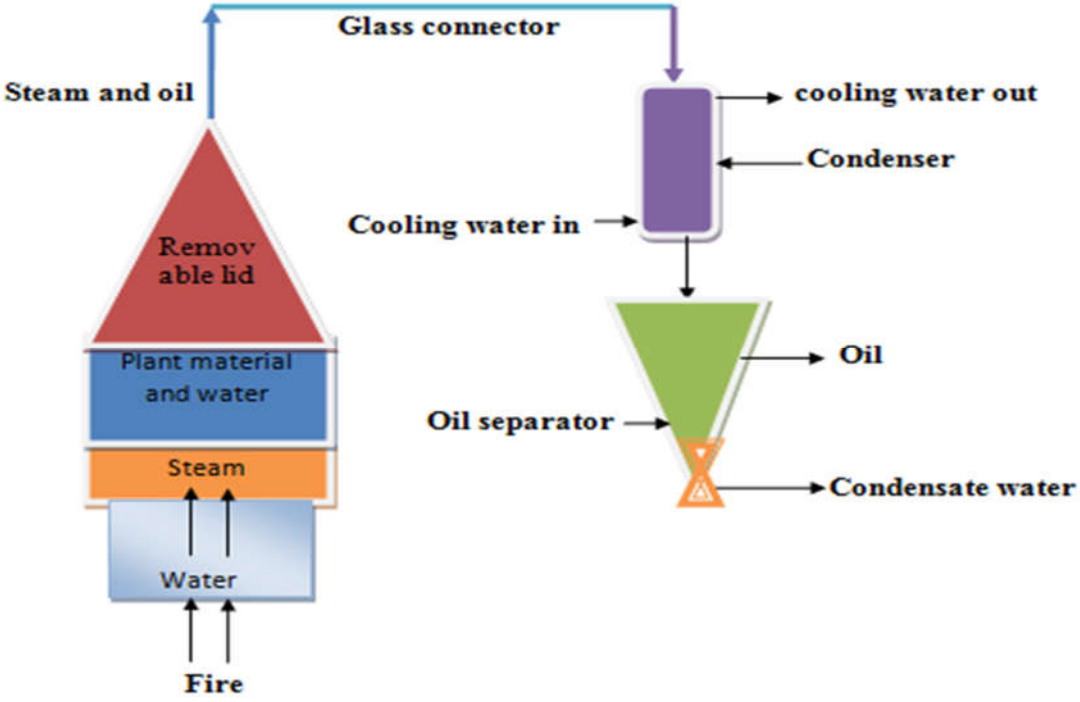


Figure 2.5 Schematic of a water and steam distillation setup (Douglas et al., 2005).

2.8.1.4 Vacuum distillation

In vacuum distillation the pressure above the raw material is reduced to less than the vapour pressure of the raw material causing the least volatile components to evaporate at lower temperatures.

According to Douglas et al. (2005) distillation is the most economical extraction method for essential oils from plant material. This is due to the simplicity of the process, it is affordable and can be done close to where the plant is being harvested. However this method has many disadvantages such as incomplete extraction of oils from the plant material; requirement of a post-extraction separation technique from water, as well as high operating temperatures which cause losses of thermo labile components and promotion of hydration reactions of chemical constituents (Danh et al., 2009 and Danh, 2007).

According to Luque de Castro et al. (1999) further disadvantages include low selectivity and long extraction times. Steam distillation is still the most commercially accepted method of extraction of vetiver oil. Although steam distillation is expensive, it operates at high temperatures and pressures which are needed to rupture the cells of the vetiver roots and hence remove the heavier components of the vetiver oil. In order to remove the vetiver oil compounds (Sesquiterpenes, > 200°C boiling point) it is recommended to use 3 bar steam pressure for 18-24 hrs (Dowthwaite and Rajani, 2000).

2.8.2 Solvent extraction (continuous or discontinuous-conventional)

Common solvent extraction uses a pure organic or mixed organics to extract the valuable extracts from the plant material. Typical solvents include ethyl acetate, diethyl ether, methanol, ethanol and hexane. This procedure is normally done in a Soxhlet extractor (Danh et al., 2009) in which the solvent is continuously refluxed though the raw material to collect the extracts. The extracts are removed from the flask and left to evaporate until no solvent is present and the essential oil can be analysed. According to Luque de Castro et al. (1999) the disadvantages of solvent extraction are the long extraction times, low selectivity, unfeasibility for automation and the presence of toxic residues in the extract. Therefore solvent extraction is often undertaken on fragile plant material that could be destroyed by the high temperatures used during distillation. Hexane is the common choice of solvent as it has a low miscibility in water and after evaporation it leaves insignificant amounts of residues.

2.8.3 Mechanical expression (discontinuous-conventional)

Prior to the discovery of distillation most essential oils were expressed mechanically or cold pressed (Sellar, 2001). It is the simple process of heating the plant material to low temperatures and then physically pressing the essential oil out. Today mechanical expression is used mainly for citrus peels and is unpopular due to the low extraction yield.

2.8.4 Microwave assisted techniques (non-tonventional technique)

The microwave assisted extraction technique is essentially solvent extraction which utilizes microwave energy to heat the solvent and raw material, thereby increasing the mass transfer rate of solute into the solvent. According to Mandal et al. (2007) a microwave is used to heat the microscopic traces of moisture found inside the plant cell therefore causing it to swell and burst allowing valuable extract to mix with the solvent. The process can be enhanced further by impregnating the raw material with the solvent to increase its liquid content.

2.8.5 Supercritical carbon dioxide extraction (non-conventional technique)

Supercritical carbon dioxide extraction is solvent extraction using a supercritical fluid as a solvent. According to Luque de Castro et al. (1999) supercritical fluid extraction (SFE) is based on the enhanced solvating power of fluids above their critical points. Supercritical fluid extraction is becoming increasingly more popular due to the fact that the world is becoming more and more environmentally orientated. SFE is a less energy intensive process and it produces a cleaner product that has a higher quality which adheres to the stricter regulations now in place. According to Danh et al. (2007 and 2009) supercritical carbon dioxide extraction is advantageous over conventional techniques as it operates at a lower temperature, therefore reducing thermal degradation and it eliminates the problem of residual solvents in the extracts.

The SCE method also allows the extraction to retain the organoleptic characteristics of the starting plant material. It can be said that SCE is a clean technology and therefore is very popular for extraction of oils for the use in the food industry (Martinez et al., 2004).

The engineering design of a supercritical extraction setup requires the knowledge of the thermodynamics and kinetic constraints that apply to the system. According to Ferreira et al. (2002) the mass transfer mechanism for SFE extraction is not fully understood due to the complex flow patterns within the bed and even more so when extracting essential oils as the essential oils consist of many components. Hence the interactions between the solvent and the solute are difficult to predict. In the perfume industry, most modern essential oil production is accomplished by extraction, using volatile solvents such as petroleum ether and hexane.

The chief advantages of extraction over distillation is that uniform temperature can be maintained during the process, As a result, extracted oils have a more natural odour that is unmatched by distilled oils, which may have undergone chemical alteration by the high temperature. This feature is of considerable importance to the perfume industry; however, the established distillation method is of lower cost than the extraction process.

2.9 Comparison of the extraction techniques

Table 2.7 Optimum operating parameters and yields for the extraction of vetiver oil using various techniques found in literature. (Leite Bianca, 2012).

	Danh et al. 2009	Danh et al. 2010	Talansier et al. 2008	Martinez et al. 2004
Extraction time/ hr.	12	12	3	16
No. Extractioons	3	3	-	-
Operating Temp/ °C	100	100	100	100
Operating pressure	Ambient	Ambient	Ambient	Ambient
Yield /%	0.31	1.69	1.4	1.8
Solvent extraction:				
Apparatus	Soxhlet	Soxhlet		
Weight of roots/ g	20	30		
Solvent	Hexane	Ethanol		
Volume of solvent/ml	300	500		
Extraction time/ hr.	5	5		
Yield	1.91	1.5		
Supercritical fluid extraction				
Size of Column (SS) /ml	50	50	5	100
Weight of roots /g	10	10	Packed full	30
Flow rate of CO_2	2ml/min	2ml/min	0.09-0.12g/s	0.069g/s
CO_2 pump temp /°C	4	4	-	-
Static time / min	30	15	5	3
Dynamic time /min	100	105	300	60
Operating Temp/°C	50	50	40	40
Operating pressure /bar	190	190	200	200
Yield /%	1.38	3.74	2.9	3.2

In 2009 Danh et al. used roots that where only 7 months old whereas in 2010 Danh et al. used roots that where older than 4 years.

2.10 Composition analysis of vetiver grass root essential oil

Gas chromatography-mass spectrometry (GC-MS): is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples, including that of material samples obtained from planet Mars during probe missions as early as the 1970s (Cazaussus, A,1988). The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer.

The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g.5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

Once the essential oils have been extracted it is necessary to determine which components are present in the oils and their quantity to see if the essential oils are valuable. This analysis can be done using a Gas Chromatography (GC) or a Gas Chromatography-Mass Spectrometry (GC-MS). The GC is used to separate the components of a sample by passing the sample through a long column containing a stationary phase (Hites, Ronald A. 2016).

Depending on the size of column and the type of stationary phase used, each component will be absorbed and desorbed on the surface of the column packing at different rates therefore allowing the components to elute from the column at different times. Essential oils consist of a complex mixture of monoterpene and sesquiterpene hydrocarbons as well as their derivatives which leads to components which exhibit similar mass spectra.

Hence an analysis technique is created based on the identification of components by the comparison of their retention data (Hochmuth, 2011 and Van Iterson, 2011) and mass spectra with those found in library data banks as well as in literature. For the analysis of essential oils such as vetiver oil an alkane range of C_8-C_{30} is recommended (Kim et al., 2005).

Fourier Transform Infrared Spectroscopy (FT-IR): Fourier Transform infrared (FT-IR) spectroscopy is a particularly useful analytical technique because of its versatility. Its distinct advantage over other forms of spectral data acquisition is that it has the ability to look at all the wavelengths of a spectral region simultaneously, rather than one wavelength at a time. Such an approach saves time dramatically and utilizes light more efficiently. Recently, essential oils extracted from various herbs and spices have been a subject of intensive research partially due to the continuous discoveries of their multifunctional properties other than their classical roles as food additives and/or fragrances. For example, antibacterial, antifungal, antioxidant and anti-inflammatory activities of many essential oil have been investigated and confirmed (Grifiths,P: deHasseth, 2007).

2.11 Physico-chemical property of vetiver grass root essential oil

Vetiver oil is a light to dark brown, olive, or amber viscous oil having a deep smoky, earthy-woody odor with a sweet persistent undertone. The color and scent can vary according to the source. Poorer grades with darker color and have smoky back notes are also produced in China and Java by subsistent farmers with primitive equipment (Dowthwaite and Rajani 2000).

Table 2.8 Physico-chemical property of vetiver grass root oil (Leite Bianca 2012).

Specific gravity 20ºc	0.980-1.003
Refraction index 20ºc	1.520-1.530
Foreign matters	Negative
Ester value	5-26
Ester value after actylating	100-500
Total vetiverol	50% min
Acid value	10-35
Solubility	Insoluble in water

Vetiver oil has a rather powerful smell but is very pleasant when diluted (Curtis 1996). It blends well with oils of sandalwood, rose, violet, jasmine, opopanax, patchouli, oakmoss, lavender, clary sage, mimosa, cassia, and ylang ylang (Lawless, 1995).

It is high-priced oil as it is used extensively in fine perfumery and cosmetic products. In dilute state, it smells like sandalwood oil (Georgi, 1924). It is used exclusively in the preparation of compound perfumes, in which the oil, on account of its low volatility, is normally used as a base to fix other high-value volatile oils like rose oil, lavender oil, and jasmine oil (Chomchalow, 2001).

2.12 Uses of vetiver grass root essential oils

Vetiver grass root oil has been utilized as raw materials for various fragrant products such as perfumes, deodorants, lotions, soaps, etc. In addition, vetiver oil plays an important role in aromatherapy. Furthermore, vetiver oil is shown to have insecticidal activity. Currently, vetiver oil is proved to have antioxidant and anticancer activities

2.12.1 Aromatherapy

Vetiver oil owes several beauty benefits and emotional effects. It balances the activity of the sebaceous oil glands, has deodorizing properties, and helps normalize oily skin and clear acne. It replenishes moisture in dry and dehydrated skin and has a rejuvenation effect on mature skin, as well as cuts, wounds, irritated and inflamed skin, when used regularly during pregnancy; Vetiver oil reportedly prevents stretch marks. The oil strengthens the central nervous system and is helpful in overcoming depressions insomnia, anxiety, stress, tension and nervousness (Curtis 1996).

2.12.2 Perfumery

Vetiver is known for its perfumery value since ancient times. On account of its pleasing aroma and slow evaporation rate falling under the category of lower 'base note' vetiver oil as such is a 'perfume in its own right' for which no synthetic substitute is yet available. Vetiver oil is the basis of the Indian perfume 'Majmua' and is the major ingredient in some 36% of all western quality perfumes, such as Caleche, Chanel, Dioressence, Parure, Opium, Guerlain, Christian Dior, Givenchy (Dowthwaite and Rajani 2000) and 20 % of all men's fragrances. A 15–30 % dilution of vetiver oil in alcohol is good enough to make true vetiver perfume. Its further dilutions have value as vetiver 'eau de cologne' and 'eau de toilette'. 'Vetiver pour Homme' by Carven 1957, and 'Vetivert' by Guerlain 1961, are the two famous 'eau de toilette' for men prepared from vetiver oil (Groom 1992).

Furthermore, the vetiver oil is one of the finest fixatives known (Lavania, 2003). Its complex chemical composition and oil odor, high solubility in alcohol that improves its miscibility with other perfumery material, makes it a unique perfumery resource for which no synthetic substitute is yet available.

In addition to its own perfumery value on account of vetiver hydrocarbons and carbonyl compounds, their alcohol derivatives i.e. vetiverols lend unique position to vetiver oil for perfumery applications as a valuable resource. Because of clear-cut differences in boiling point of the various constituents of vetiver oil, its vetiverol fraction could be easily separated by fractional distillation of oil under high vacuum. Also, vetiverol could be acetylated with acetic anhydride to produce vetiveryl acetate.

Both vetiverols and acetates have softer odors and fixative qualities, and are used as blender with high-class perfumery products. They blend well with ionone, linalool, cinnamic alcohol, oakmoss, vanila, sandalwood, patchouli and rose bases, and are frequently used in western 6 types of fragrances having chypre, fougere, rose, violet and amber aldehyde base, and oriental fragrances and floral compounds (Lavania, 2003).

In addition to its direct perfumery applications, vetiver oil in its diluted form is extensively used in after-shave lotions, air freshners and bathing purposes, as well as flavoring syrups, ice cream, cosmetic and food preservation. Khus essence is used in cool drinks, and for reducing pungency of chewing tobacco preparations, providing sweet note to other masticatories and incense sticks (National Research Council 1993).

2.13 The three main odour influencing components

Chemical composition of vetiver oil is extremely complex, containing over 100 sesquiterpene-type compounds and their derivatives, belonging to 11 structural classes (Akhila and Rani, 2002).

The main constituents of vetiver oil comprise of: sesquiterpene hydrocarbons (γ-cadenene, clovene, α-amorphine, aromadendrine, junipene and their alcohol derivatives), vetiverols (khusimol, epiglobulol, spathulenol, khusinol; carbonyl derivatives), vetivones (α- vetivone, β-vetivone, khusimone; and ester derivative). Among of them, three carbonyl compounds, α-vetivone, β-vetivone and khusimone, are considered the primary odor influencing components; β-vetivone has the better odor, and is considered the most important, while its major isomer nordihydro β-vetivone has a strong, rich, woody-peppery note (Lavania, 2003). All these components individually and collectively contribute to the characteristic odor of the vetiver. Therefore, they, α-vetivone, β-vetivone and khusimol, can be considered as the ‘finger print’ of vetver oil (Demole et al. 1995). The three main odour influencing constituents are known to be α -vetivone, β -vetivone and khusinol (Bhatwadehar et al., 1982).

There are distinct geographical differences in quality and perfumery note of essential oil obtained from different geographic regions of the world. In a broad sense, the essential oil of vetiver having high specific gravity, negative optical rotation, high vetiverol concentration and higher ester value is considered superior from perfumery view point (Lavania, 2003). Reunion oil with roseate note is highly regarded in perfumery industry, but the vetiver oil (khus oil) obtained from the roots occurring in wild state in north Indian plains, commonly known as 'khus' is considered to be the best for its balsamic woody note. Lately, vetiver genotypes producing vetiver oil with roseate and saffron note have also been identified from north Indian plains (Lal et al. 1998).

2.14 Valorisation of the vetiver essential oils

The vetiver essential oil is said to be more valuable from a perfumery point of view if it has a high specific gravity, negative optical rotation, high vetiverol concentration, no residues and a high ester value (Lavania, 2003). Generally vetiver oil is considered to be of a high quality if the oil is viscous and dark brown in colour. For the use in the perfume and food industry the vetiver essential oil must be free from all toxins that may cause harm to the consumer.

The vetiver grass consist of two types of roots (Lavania, 2003), the main smooth roots and the secondary hairy roots. These secondary roots contain unwanted non-polar compounds that reduce the value of the essential oils. Hence it is necessary to reduce these non-polar compounds by one of the following methods. The harvested roots are dried in a cool dry place to allow natural evaporation of the unwanted lighter fraction (Danh, 2007). And the essential oil extracted in the first 15 - 30 minutes of extraction can be discarded (Lavania, 2003).

One can say that the vetiver oils are valuable for the perfume industry if they contain large amounts of odour influencing alcohols such as khusimol, hence the need to convert undesirable acids and hydrocarbons into valuable alcohol (leite Bianca 2012).

Therefore other valorisation techniques include the removal of the acids from the vetiver oil or the chemical conversion of the acids into more valuable components such as khusimol (Martinez et al., 2004). Another dominant alcohol found in vetiver oil is vetiverol which gives the oil a cleaner note; therefore for a slightly fruity-woody note one can acetylate this alcohol to vetiveryl acetate (Dowthwaite and Rajani, 2000). Fractionation of the vetiver oil extracts is another technique used to increase the value of the essential oils.

Fractionation into more specific groups of components such as hydrocarbons, alcohols, ethers, esters etc., can increase the value of the essential oil by allowing the fractions to be used for a more specific function.

3. Materials and Methods

3.1 Materials and equipments

Dried vetiver grass root was the main raw material used in this study. Equipment and instruments used in this study were, glass beakers (different size), mixing glass rod, measuring cylinder, pipettes, electronic balance, vials, round bottom flask with a refluxing condenser, screen sieves, oven, ceramic crucible, thermometer, pH meter, grinder, heater, two way round flask, glass connecter, separating funnel or arm , GC-MS and FT-IR.

The chemicals and reagents to use in this study were; vetiver grass root which cultivated with sandy soil type and 5th year roots, Sodium sulfate anhydrous and distilled water.

3.2 Methods

3.2.1 Raw material collection, pretreatment and characterization of vetiver grass roots.

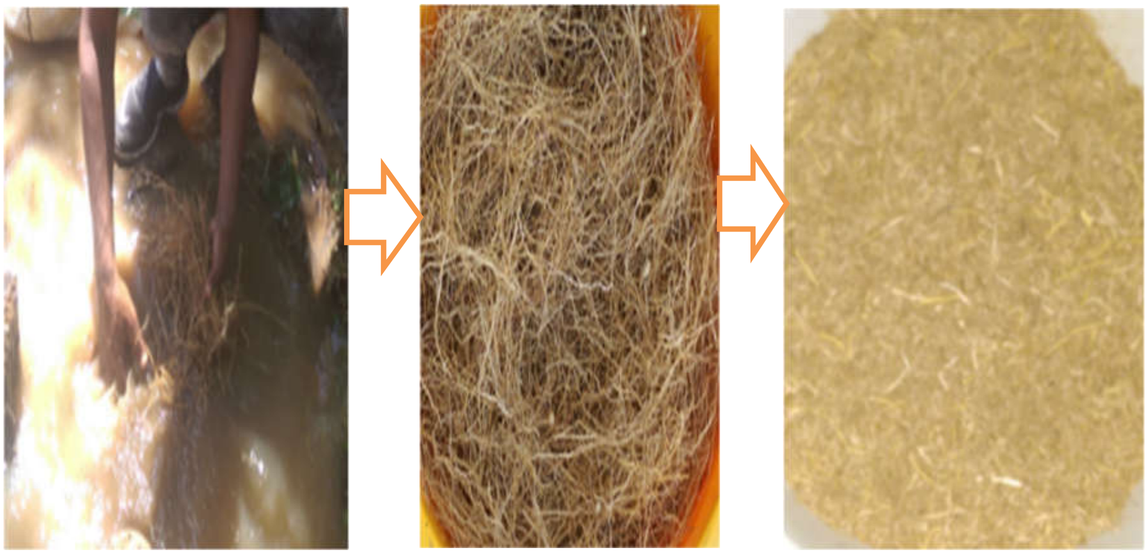
Raw vetiver grass root was collected from Bako,(Anno agro industry farm plc.) based on information of: root age, type of soil that growth vetiver grass and quality of vetiver grass root essential oil yield.

(a) (b)

Figure 3.1 Vetiver grass collection process from bakko (anno agro industry farm plc.2017), Oromia region, (a): vetiver grass and (b): outing roots of vetiver from ground.

After the vetiver grass roots have been collected, it must be prepared for extraction**.** The roots were first soaked and washed to remove any unwanted soil contaminants from the ground (see to figure 3.2). The roots were then dried in a cool dry place for 48-72 hours at room temperature to allow all the low value, non-polar, low boiling components of the oil to evaporate naturally. The dried roots are milled in attrition mill (Retsch GmbH) with sieve size 1mm and 2mm. The material into smaller sizes in order to increase the surface area for maximum extraction of the oil.

The particle size was then determined after milling by a 10 minute vibrating shaker (Retsch) with set of sieves sizes arranged in descending order 1.8mm, 1.4mm, 1mm, 0.85mm, 0.71mm and 0.425mm to obtain particle size of 0.425-1.4 mm. The roots were stored in a sealed bag in deep freezer (up to -20^{o}c) until extraction to avoid any further loss of volatile component at room temperature.



(a) (b) (c)

Figure 3.2 Vetiver grass roots preparation process, (a): washing, (b): washed and (c): after milling.

3.2.2 Characterization of raw vetiver grass roots

Proximate analysis was carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1990) to determine the moisture content, ash content protein content and crude fat content as follows:

3.2.2.1 Moisture content determination

One gram of the sample was weighed into a previously weighed crucible. The crucible and sample taken were then transferred into the oven set at 105 ^{0}C and allowed to dry overnight. At the end of the 24 hours the crucible plus sample were removed from the oven and transferred to the desiccator and cooled for 15 minutes and weighed (ASTM D4442).

$$\text{\% Moisture} = \frac{w_2 - w_3}{w_2 - w_1} * 100 \qquad (3.1)$$

Where, W_1=Weight of crucible

W_2=Weight of crucible + Weight of sample as received

W_3=Weight of crucible + Weight of dried sample

3.2.2.2 Ash content determination

According to (AOAC, 1990), the Ash content was determined by weighing one gram of finely ground, dried sample into a pre-ignited and previously weighed ceramic crucible, placed in a muffle furnace and ignited for 3 hrs at 575^{0}C. After ashing, the crucible and its contents were cooled to room temperature in a desiccator. The crucible and its contents were re-weighed and the weight was reported as percentage ash content by using the next formula**:**

$$\text{Ash content (\%)} = \frac{w_3 - w_1}{w_2 - w_1} * 100 \qquad (3.2)$$

Where, W1 = Weight of crucible

W_2 = Weight of crucible Weight of sample before ashing

W_3 = Weight of crucible + ash

3.2.2.3 Protein content determination

Kjeldahl method was used for the determination of Protein Content.1g of the sample was weighed into a Kjeldahl flask and 20ml of concentrated sulphuric acid (98% wt/wt) was added and the setup was swirled under tap water for proper mixing. Three grams of the Kjeldahl catalyst (a mixture of 10g of $Na2SO_4$ and 1g of $CuSO_4$) were added to the flask. To the mixture was also added anti-bumping chips and the whole set up was boiled in a fume cupboard until the charred particles disappeared and a clear green solution was obtained. Ten ml of 2% boric acid was measured into a 250ml beaker and 3 drops of methyl orange indicator was added. Ten ml of the digest was placed in a distillation flask and 30ml of 40% (w/v) NaOH was added to the digest and the mixture was heated for 25minutes. The distillate was then titrated with 0.1N HCl to violet end point. Blank determinations were made, using boric acid and indicator. The values obtained were used to calculate the total nitrogen and the percentage crude protein obtained by multiplying with 6.25.

$$\% \text{ N} = \frac{[milliliters\ H_2so_4 * NH_2SO_4 - millilitersNaOH]*14.007}{sample\ weight(milligrams)} * 10 \qquad (3.3)$$

$$\% \text{ Protein} = 6.25* \qquad (3.4)$$

3.2.2.4 Fat content determination

Five grams of the dried vetiver grass root flour was subjected to fat estimation by refluxing for 3 hours using Soxlet extractor and 200ml of petroleum ether as the extracting solvent.

A round-bottom flask containing a mixture of fat and petroleum ether solvent was detached from the Soxlet extractor and petroleum ether solvent was evaporated on a rotary evaporator.

The round-bottom flask and its content were heated to 105 ^{0}C in an oven for 30 minutes and later cooled in a desiccator. The weight of the extracted fat was determined and expressed as percentage fat using the formula:

$$\%\ \text{Fat} = \frac{weight\ of\ fat}{weight\ of\ dried\ sample} * 100 \qquad (3.5)$$

The activity was done in Addis Ababa University College of natural science department of food and nutrition**.**

3.2.3 Extraction of vetiver grass roots essential oil

3.2.3.1 Steam distillation

Vétiver oil is an essential oil extracted from the roots of vetiveria zizanioides. It is of interest to the cosmetic and perfumery industry, not only due to its scent, but also due to its ability to fix some more volatile essential oils (Anonymous,1976).

Vetiver grass root oil was extracted from pretreated raw vetiver root by Steam distillation. The yield of oil (up to 3 % (dw) (de Guzman and Oyen,1999) depends not only on the vétiver type cultivated, but also on the climate, the soil, frequent cutting of the grass, the harvesting time, methods of distillation, time of distillation are vital components for efficient processing of essential oil distillation.

In this work the steam distillation unit consists of five parts (see to figure 3.2), the round bottom flask of 1000 ml capacity, the separating arm with 10 ml graduations, the condenser which is water solution inside the coils and a height of 60 mm, Two bottom round flask of 500 ml capacity which was the raw material loaded.

The fifth piece is the heating mantle (Glas Col STM1001 230 V/600 W) which is used to heat the round bottom flask which loaded water during extraction and it creates steam.

In these cases oil extraction was performed at atmospheric pressure and 5th year root of vetiver grass. The experimental set up of steam distillation which was used in this research is shown below.

Figure 3.3 Steam distillation laboratory scale setup at LIDI, (2017).

The experimental activity was done at leather industry development institute (LIDI) Ethiopia. The pretreated raw vetiver grass root was loaded into contactor for distillation and steam from round bottom flask passed through loaded veiver grass root. The steam containing volatile components, then condensed with cold water and collected in separating arm. The experiment was carried for different temperatures (75-95°C) and extraction time of (2-6hrs). Particle size of pretreated raw vetiver grass root used was (0.425-1.4mm). Extracted oil was separated by gravitational separation from the separatory arm and then kept in airtight vials for future experimental analysis.

3.2.3.2 Steam distillation experimental procedure

A 1000 ml round bottom flask was mantled a heating mantle that was clamped a ring stand. 40 gram of grinded vetiver grass root was transfer to the two rounded bottom flask. 950 ml distilled water filed in to round bottom flask for each experiment. The separating arm was connected to the connecting glass tube to collect the distillate a condenser was clamped to the connecting glass tube and the flask. After completing this, open the cooling water inlet valve fully. The cooling water starts to flow in to the condenser, make sure that water is flowing through the condenser.

Finally the heating mantle was turn on the setting temperature and stayed until the time specified (using stopwatch) and the yield collected at the separating arm was separated using gravitational separation and Measure using measuring cylinder. Watch the run by continuously replacing the condensate collecting container, once full or to take cuts.

So that distillate drips at a rate of one drop every 2-5 seconds. Once the specified distillation time is completed switch off the main steam supply (heating mantel), wait a few minutes to clear. Then close the cooling water supply. All condensate (vetiver oil and water) and the vetiver extracts separated from the water to be weighed for calculation of the yield. Finally remove the two rounded bottom flask and remove the spent veiver grass roots by lifting the sieve plate up and out of the round bottom flask.

3.2.4 Determination of essential oil yield

The percentage oil yield was calculated by using following relation (AOAC, 2000)

$$\text{Percentage oil yield (w/w)} = \frac{mass\ of\ oil}{mass\ of\ samlple} * 100 \qquad (3.6)$$

3.2.5 Characterization of essential oil

The physico-chemical properties of extracted vetiver grass root essential oil were characterized based on the procedure coved in several standard analyses methods.

The Organoleptic profile and physico-chemical properties of vetiver grass root oil were analyzed with instruments and by titration.

3.2.5.1 Physical properties determination

Determination of Specific gravity

For the determination of specific gravity of oils, the density of the oil was determined by using density bottle. A clean and dry bottle of 25ml capacity was weighed (W_0) and then the bottle was filled with the oil, stopper inserted and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2) (Middle-East J. Med. Pl. Res. 2012).

$$\text{sp.gr} = \frac{W_2 - W_0}{W_1 - W_0} = \frac{\text{mass of substance}}{\text{mass of an equal volume of water}} \tag{3.7}$$

Determination of pH value

2g of the sample was taken and putted in a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. And then it was cooled in cold water bath to 25^0c.The pH electrode was standardized with a buffer solution first and then immersed in the sample and the pH was read (AOAC official method analysis 960.19, 2000).

Determination of kinematic viscosity

A15m1 of oil was poured into a test tube and a viscometer was used to measure the viscosity at a temperature of 20^oc. A dynamic viscosity of the essential oil was measured directly using rotary-viscometer which was available in laboratory of school of Chemical and Bio Engineering. Then the kinematic viscometer was calculated.

$$\text{Kinematic viscosity} = \frac{Dynamic\ viscosity\ of\ the\ sample}{Desnsity\ of\ the\ sample} \tag{3.8}$$

3.2.5.2 Chemical properties determination

Acid value determination

Acid value is an important physicochemical property index of oil which is used to determine the quality, age, edibility and suitability of oil for industrial. 25ml of Toluene and 25ml of ethanol was mixed in a 250ml beaker. The resulting mixture was added to 2g of oil in a 250ml conical flask and few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1M KOH to the end point with consistent shaking for which a dark pink colour was observed and the volume of 0.1M KOH (V_0) was noted (AOAC 2006).

$$\text{Acid value} = \frac{V * C * 56.11}{M} \tag{3.9}$$

Where V = Volume of potassium hydroxide (ml), C = Concentration of potassium hydroxide, 56.11 = Molecular weight of potassium hydroxide, M = sample weight (Laboratory Handbook, 1997).

Determination of saponification value

Saponification value is an index of average molecular mass of various fatty acids in oil samples. The lower value of saponification means molecular weight of fatty acids is lower and has lower limit of use in industry. Indicator method was use as specified by ISO 3657 (1988) and AOCS Method cd 3-25 (1993).

Two gram of the sample was weighed into a conical flask; 25ml of 0.5N Ethanolic potassium hydroxide was then added.

The content which was constantly stirred was allowed to boil gently for 30min. A reflux condenser was placed on the flask containing the mixture. Few drops of phenolphthalein indicator was added to the warm solution and then titrated with 0.5M HCl to the endpoint. Until the pink colour of the indicator just disappeared.

The same procedure was used for other samples and blank. The expression for saponification value (S.V.) is given by:

$$\text{Saponification value} = \frac{56.1 * N * (V2 - V1)}{W} \qquad (3.10)$$

Where, N = normally of hydrochloric acid used for titration

V_1 = Volume in ml. of hydrochloric acid used for the blank

V_2 = Volume in ml. of hydrochloric acid used in determination

W = weight of sample (gm) (General et al., 2005).

3.2.6 Compositional Analysis of essential oil

3.2.6.1 Gas chromatography-mass spectrometry (GC-MS)

The identification and analysis of the vetiver grass root essential oil components were carried out by gas chromatographymass spectrometry (GC-MS). The ooperating conditions (refer to table 3.1) were applied to identify the components in the vetiver grass root oil extracted by steam distillation method for this thesis.

Table 3.1 Gas chromatography-mass spectrometry method for chemical analysis of the vetiver grass root oil samples

Method	
Type	Agilent 7820A
Column	HP-5 capillary(30*0.25,coating thickness 0.25µm and Agilent technology 5977E mass spectroscopy ion trap detector)
Carrier Gas	Helium
Linear Velocity/ $mL.min^{-1}$	25
Detector Type	Mass spectroscopy
Injection Volume/µL	5
Injection Type	Split Ratio 1:30
Detector Temp./°C	250
Injector Temp./°C	220-240

Table 3.1 continued

Oven Temp.	60-240ºC @ 3ºC.min^{-1}
Method Based on.	Martinez et al. (2004)
Comparison of.	Retention Indices

3.2.6.2 Fourier transform infrared spectroscopy (FT-IR)

Fourier Transform Infrared (FT-IR) spectroscopy is a dynamical instrumentation technique that is useful for examination of various forms of matter. When a sample is analyzed using infrared radiation, the FT-IR spectra provide information about the vibrational and rotational energies of a molecule. The infrared radiation is absorbed at specific frequencies characteristic of the molecular species, whereby the FT-IR spectrum is a true signature of the molecule. Knowing the radiation intensity as a function of the infrared frequency, one can specify the types of chemical functional groups.

Spectra can be obtained of samples in all three states of matter, and in most cases nondestructively. One of the most distinct and easily recognizable peaks in an IR spectrum is the broad O-H absorption of alcohols and phenols.

Its distinct advantage over other forms of spectral data acquisition is that it has the ability to look at all the wavelengths of a spectral region simultaneously, rather than one wavelength at a time. The IR spectra of the essential oil were recorded in transmittance (%) and the wave number region for the analysis were (4000 - 400 cm^{-1}) spectrophotometer in Addis Ababa University College of Natural Science Chemistry Department using prinks Elmer spectrum 65.

3.2.7 Design of the experiment

3.2.7.1 General full factorial designs

Data analysis has performed by design expert software using general factorial design Method. Experiments with two or more factors are encountered frequently.

The best way to carry out such experiments is by using full factorial experiments. These are experiments in which all combinations of factors are investigated in each replicate of the experiment. Full factorial experiments are the only means to completely and systematically study interactions between factors in addition to identifying significant factors. (http:/ / reliawiki. org/ index. php/ General Full Factorial Designs). For steam distillation extraction we had three factors, namely time, particle size and temperature with three levels and two replicates for each factors.

This design of the experiment helps us to differentiate the significance of the main and the interaction factors. This program software also used to develop the mathematical model that will describe the effects of the main and interaction factors on the response.

4. Results and Discussion

4.1 Characterization of raw vetiver grass roots

Some experimental values of various physico-chemical characteristics of the raw vetiver grass root are given in table 4.1below.

For this present study the raw material moisture content of average sample was obtained (5.8%) which is slightly higher than the moisture content reported in literature of approximately (4.25%) (Leite Bianca 2012).

Ash content indicates the level of essential or non-essential mineral elements in the sample. The average ash content of the present study obtained was (1.6%), which is lower than the literature reported value of (5.8%) (Guangdong, 2002)

The average crude protein content found in present study (2.8%) is slightly lower than the value reported by an earlier researcher (Zhong and Lin 2002) which is (3.6%) and the crude fat content was obtained (1.5 %).

Table 4.1 Laboratory result of raw vetiver grass root characterization

No.	Properties	Average value	Other literatures values
1	Moisture content (% dry weight)	5.8	4.25
2	Ash content (% dry weight)	1.6	5.8
3	Protein content (% dry weight)	2.8	3.6
4	Fat content (% dry weight)	1.5	-

Table 4.1 shows some of the phsico-chemical characteristics of raw vetiver obtained from the analysis of two samples and their average. These values are compared with corresponding values reported in literature.

4.2 Essential oil extraction

4.2.1 Steam distillation extracted results

Table 4.2 reports the results of essential oil yield from vetiver grass roots obtained using steam extraction at different extraction conditions. The percentage extraction yield was calculated by using equation (3.6) and the result is summarized as follows.

Table 4.2 Yields obtained by steam extraction (5 years old roots and sandy soil type growth).

Temperature (^{o}c)	Time (hrs.)	Particle size range (mm)	Test - 1 (g)	Test - 2 (g)	Average (g)	Yield (%)
75	2	0.425-0.75	0.16	0.14	0.15	0.37
		0.75-1.075	0.08	0.084	0.082	0.2
		1.075-1.4	0.06	0.075	0.068	0.17
	4	0.425-0.75	0.2	0.32	0.26	0.65
		0.75-1.075	0.14	0.17	0.16	0.38
		1.075-1.4	0.1	0.12	0.125	0.31
	6	0.425-0.75	0.43	0.28	0.36	0.89
		0.75-1.075	0.26	0.29	0.23	0.57
		1.075-1.4	0.17	0.23	0.2	0.5
85	2	0.425-0.75	0.18	0.16	0.17	0.42
		0.75-1.075	0.09	0.12	0.1	0.26
		1.075-1.4	0.083	0.108	0.095	0.24
	4	0.425-0.75	0.326	0.47	0.4	0.99
		0.75-1.075	0.306	0.34	0.323	0.8
		1.075-1.4	0.17	0.14	0.16	0.38
	6	0.425-0.75	0.54	0.56	0.55	1.37
		0.75-1.075	0.52	0.48	0.5	1.25
		1.075-1.4	0.37	0.3	0.335	0.84

Table 4.2 Continued

95	2	0.425-0.75	0.25	0.23	0.24	0.6
		0.75-1.075	0.23	0.19	0.21	0.52
		1.075-1.4	0.15	0.12	0.14	0.34
	4	0.425-0.75	0.57	0.61	0.59	1.47
		0.75-1.075	0.41	0.45	0.43	1.07
		1.075-1.4	0.23	0.35	0.29	0.72
	6	0.425-0.75	0.65	0.69	0.67	1.7
		0.75-1.075	0.58	0.52	0.55	1.37
		1.075-1.4	0.41	0.38	0.39	0.98

As we can see from the table (4.2), the maximum extraction of vetiver grass roots oil an average value of (1.7%) was obtained at particle size range from (0.425-0.75mm) after the extraction time of (6 hr) and temperature of (95ºC), and the minimum average yield obtained was (0.15%) at the maximum particle size range of (1.075-1.4mm), at minimum extraction time of (2hr.) and minimum temperature of (75ºC). The yield of oil extracted by steam extraction process obtained up to (3%). (de Guzman and Oyen,1999).

4.3 Characterization of extracted essential oil

4.3.1 Physical properties

4.3.1.1 Specific gravity

Specific gravity is the ratio of the density of respective substance to the density of water at 4°C. Density bottle method was used to determine the specific gravity of oil as the detail experimental procedure were stated in equation (3.7)

Therefore, specific gravity (Sp.gr) =. $\frac{W_2-W_0}{w_1-w_0} = \frac{42.135-1936}{44.57-19.36}$ = 0.9034

The specific gravity of vetiver grass root oil obtained in the present study found to be 0.9034, which is very close to the specific gravity of the standard range (0.978 – 1.038) (Chowdhury et al., 2002).

4.3.1.2 pH value

Table 4.3 pH value

Product	Test -1	Test -2	Average pH value
Essential oil	5.8	6.4	6.1

From Table 4.3, the pH value of vetiver grass root oil 6.1, which is the range of recommended value of 3.5 - 6.5 (Mueller et al, 2000).

4.3.1.3 Kinematic viscosity

Dynamic viscosity of oil which was determined by using rotary-viscometer was obtained 3.75mPa.s at a temperature of 20^0C, substituting the viscosity oil = 3.75mPa.s = 3.75×10^{-3} kg.m^{-1}.s^{-1} and density of vetiver grass root essential oil = 901.5kg/m^3.

Therefore the kinematic viscosity of vetiver grass root essential oil was calculated by using equation (3.8) as follows:

$$\text{Kinematic viscosity} = \frac{3.75\times10^{-3}\,\frac{kg}{m.s}}{901.5\,\frac{\text{kg}}{\text{m3}}} = 4.15*10^{-6}\text{m}^2/\text{s},$$

As we see the results, the kinematical viscosity of vetiver grass root essential oil is slightly higher than the relating with other essential oils.

4.3.2 Chemical properties determination

4.3.2.1 Acid value

Acid value is the measure of total acidity of the lipid involving contributions from all the constituent fatty acids that make up the glyceride molecule (Ekpa and Ekpe, 1995).

Table 4.4 Acid value

Raw material condition	The volume of KOH for the sample (ml)	Mass of sample (gm)	Acid
Dry	7.4	2	20.75

The acid number measures the amount of acids present in oil. Acid value is an indirect method for determination of free fatty acid of amount in oil samples and its edibility. Therefore, the value of acid in this present work is an agreement with that of different literature value (refer to table 2.8).

4.3.2.2 Saponification values

According to Codex Alimentarius (2005) and the APCC Standards, the saponification values (SV) range between 250-260 mg KOH/g oil and 248-268 mg KOH/g oil respectively. The saponification values gives information concerning the character of the fatty acid present in the oil.

Table 4.5 Saponification values

Raw material condition	The Volume of HCl for blank(ml)	The volume of HCI for the sample (ml)	Mass of sample (gm)	SVs
Dry	30.5	16	2	203.36

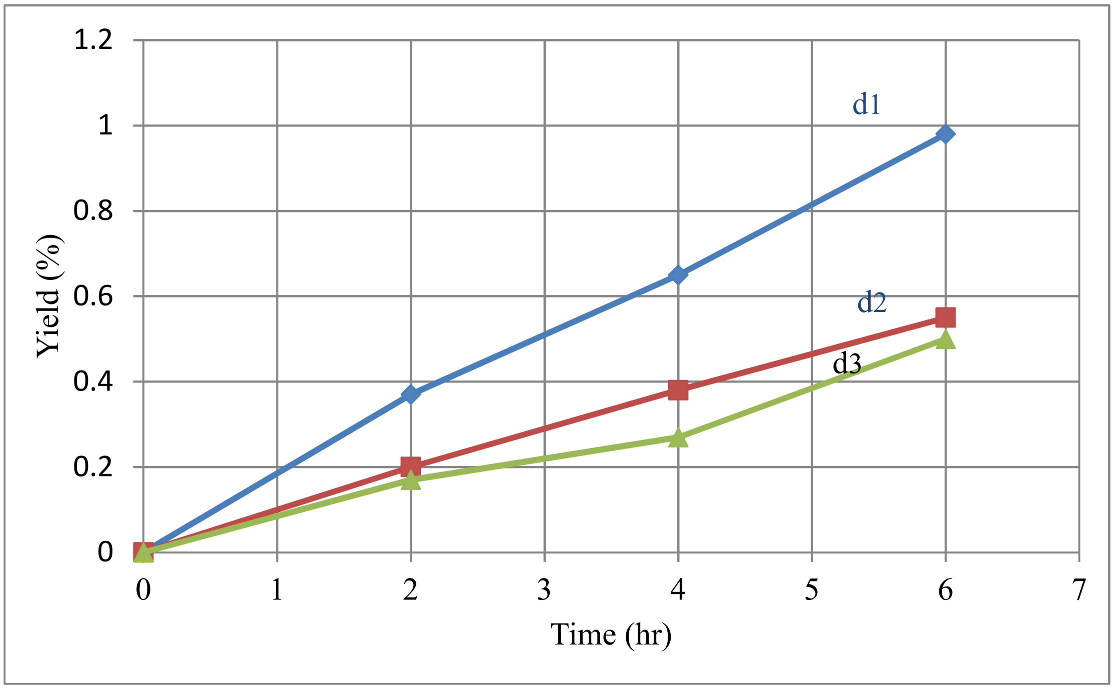
The result of Table 4.5 shows that the saponification values of vetiver grass roots essential oil 203.36 which are lower than the standard value of (APCC Standard and Codex Alimentarius 2005).

4.4 Effects of operating parameter on percent yield of essential oil

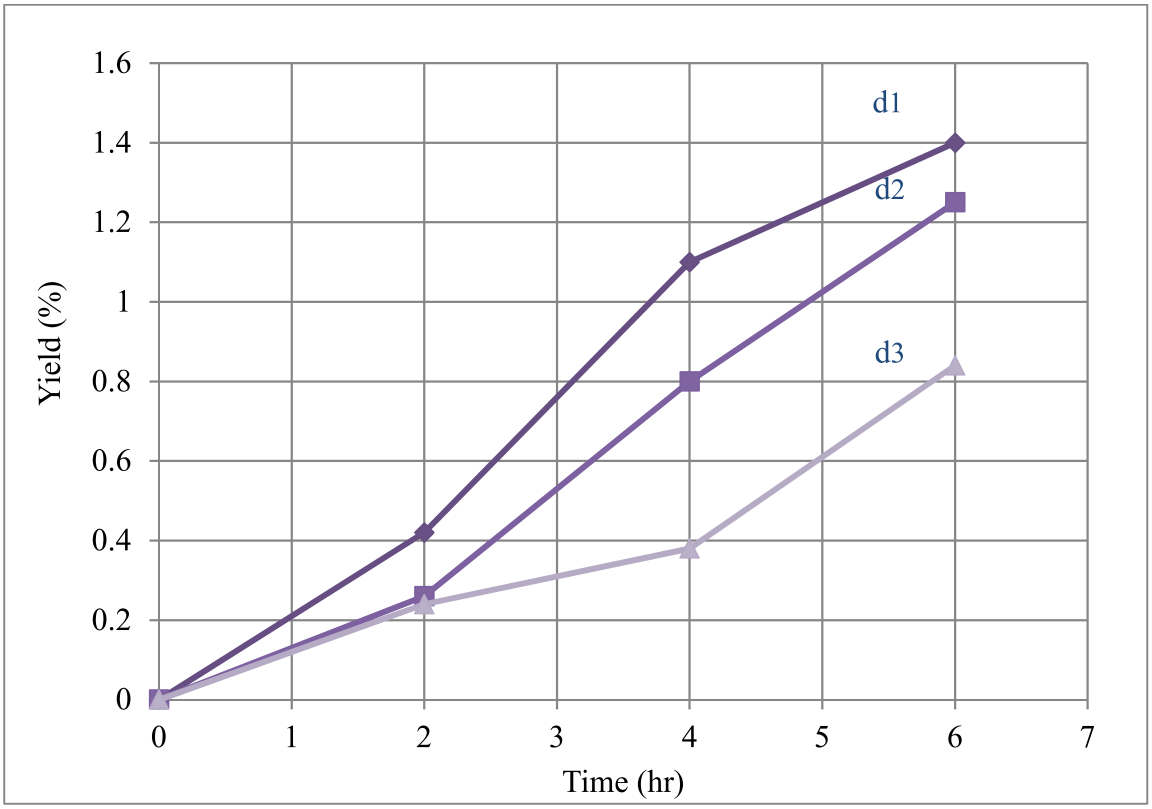
4.4.1 Effect of extraction time

Extraction time plays a great role on the percentage yield of essential oil using steam distillation. There was a strong correlation between the steam extraction time and the yield of essential oil.

As expected for present study, the yield of vetiver grass root essential oil increases with increasing extraction time which means that an increasing in the steam distillation time contributes to a longer contact between steam and raw material for providing larger mass transfer. As we can see in Figure (4.1) most of the oil was being extracted within 120 to 360 minutes and the amount of yield obtained up to this period (0.15% to 1.72%). However, further increase in extraction time resulted is no significant improvement in the extraction performance. In general, the rate of extraction was high at the beginning of the extraction but get slow gradually by time. (john tshilenge , kabuba, February 2009).

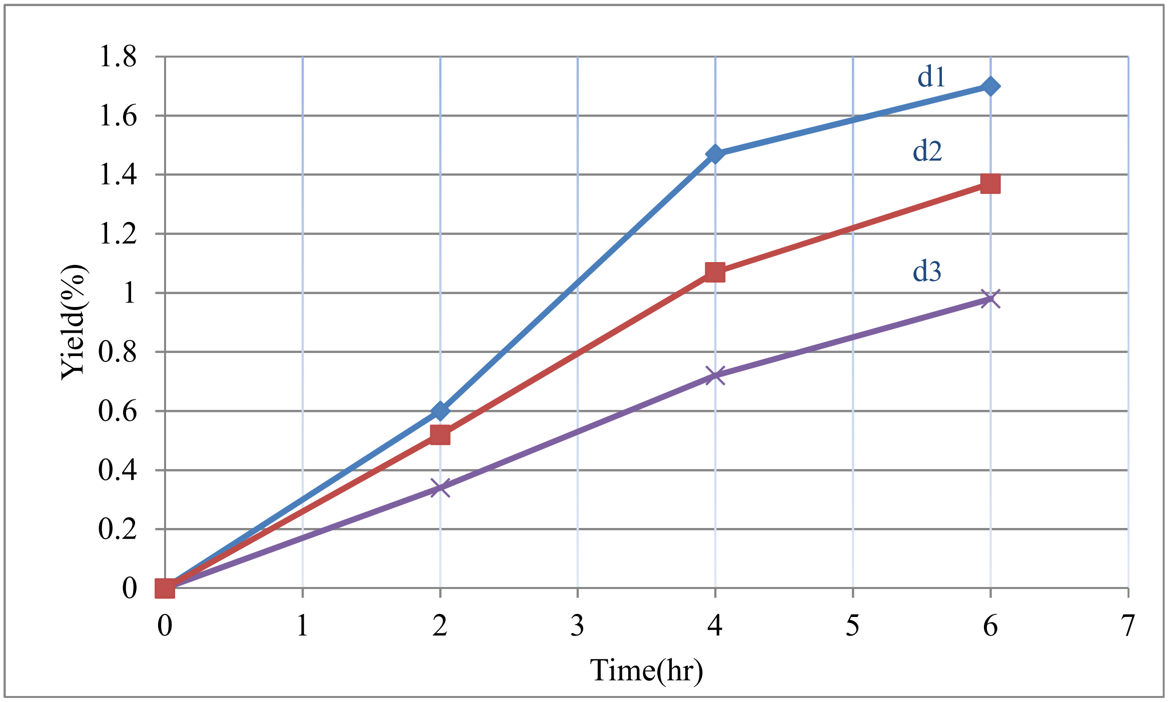


(a)



1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Yield (%)
0
1
2
3
4
5
6
7
Time (hr)
d1
d2
d3

(b)



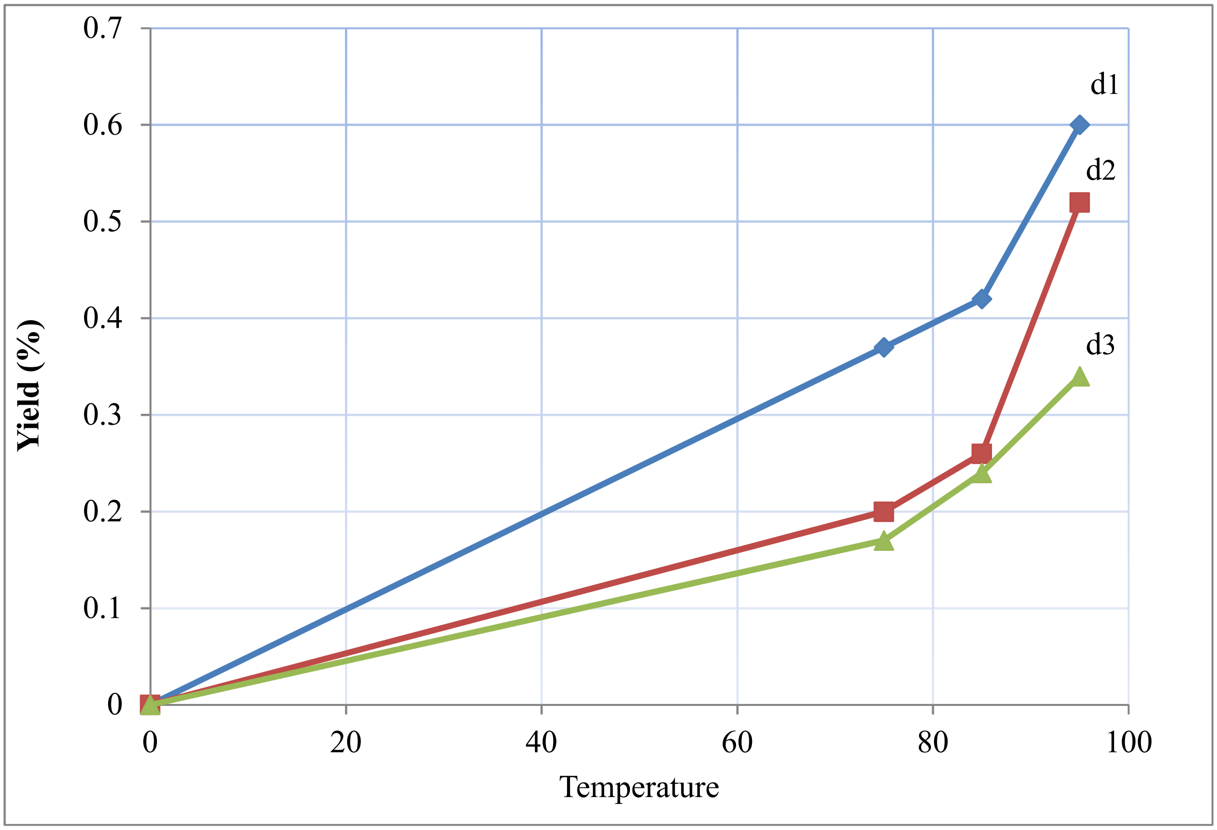
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Yield(%)
0
1
2
3
4
5
6
7
Time(hr)
d1
d2
d3

(c)

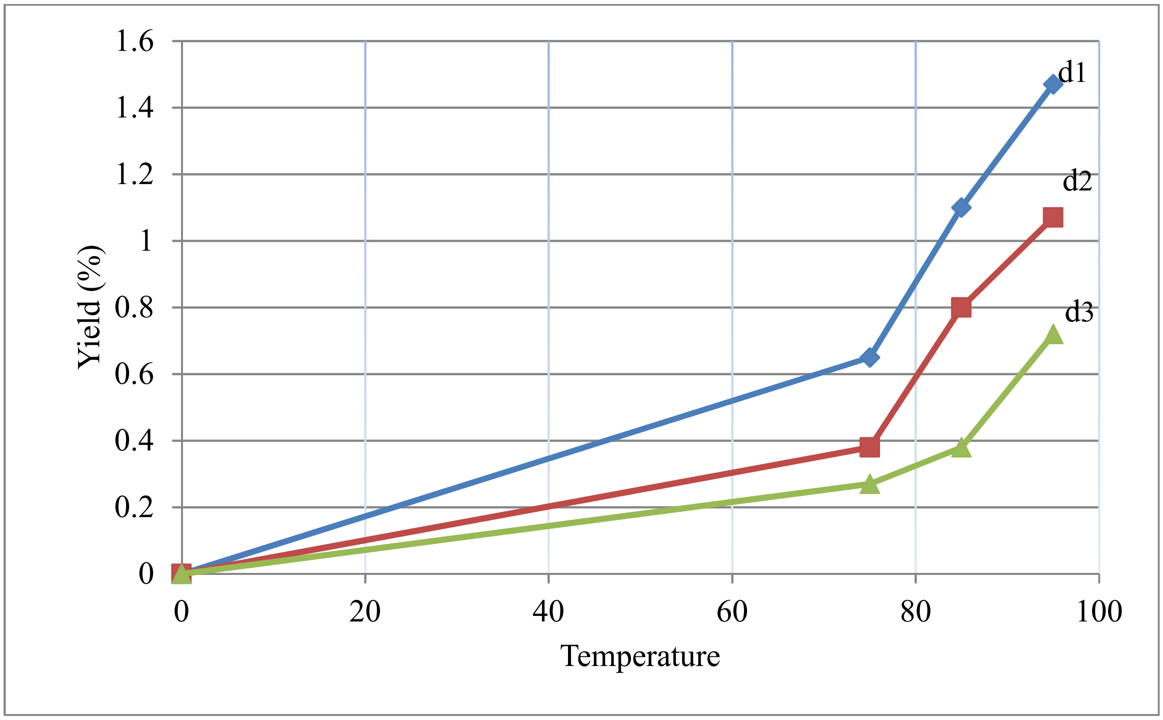
Figure 4.1 Yield of vetiver grass root oil as a function of extraction time and at temperature of (a) 75^0C, (b) 85°C and (c) 95°C and particle size range of d_1, d_2 and d_3 are 0.425-0.75, 0.75-1.075 and 1.075-1.4mm respectively.

4.4.2 Effects of temperature

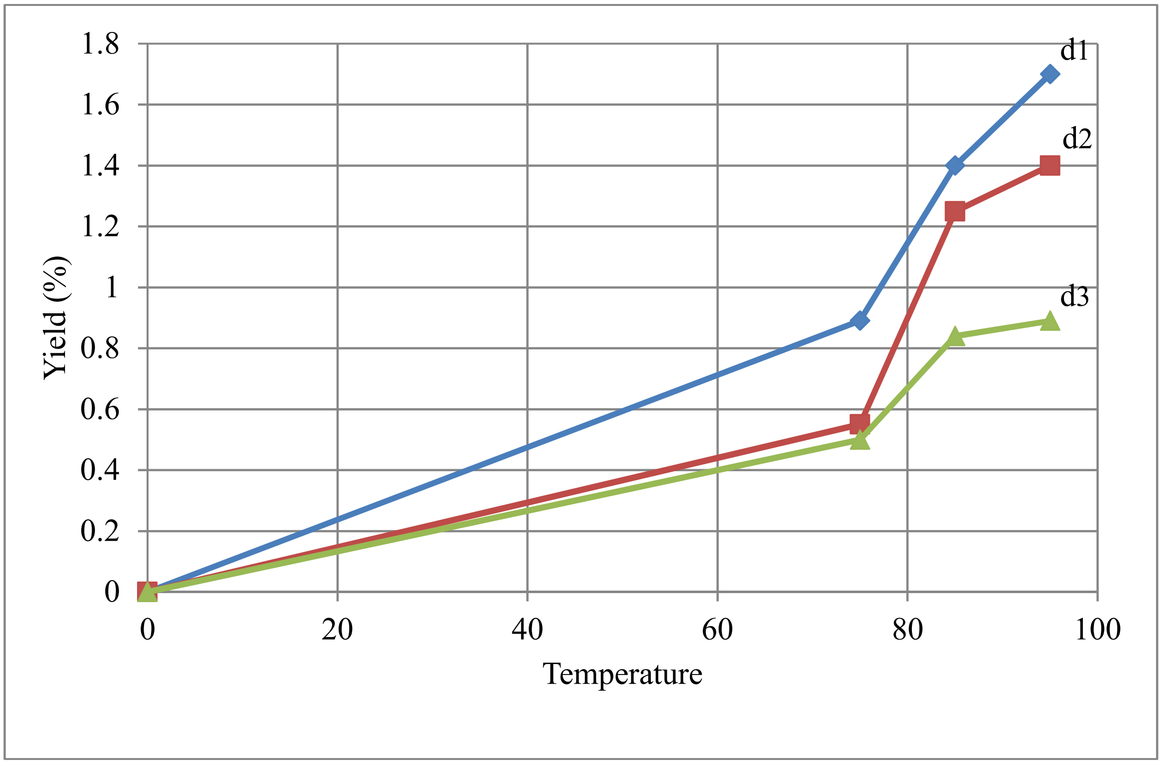
The effect of temperature on the yield of essential oil was obtained from vetiver grass root is shown in Figure 4.2; there is an increase in the average yield from 0.15% to 1.7% when the temperature is rising from 75°C to 95°C for different particle sizes. This observation can be explained from the fact that the heat and the steam generated at 95°C has more rupture effect on the cell structure of the vetiver grass root thus allowing the release of more essential oil at that temperature.



(a)


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Yield (%)
0
20
40
60
80
100
Temperature
d1
d2
d3

(b)


1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Yield (%)
0
20
40
60
80
100
Temperature
d1
d2
d3

(c)

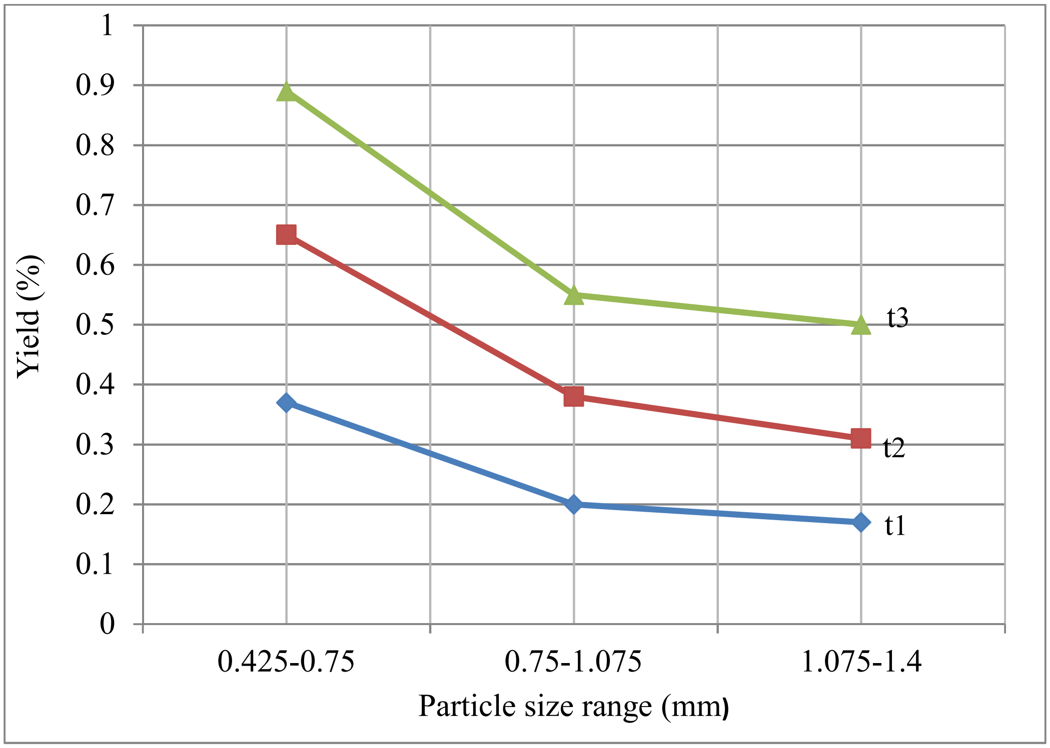
Figure 4.2 Yield of vetiver grass root oil as a function of extraction temperature and at time of (a) 2, (b) 4 and (c) 6hrs and particle size range of d_1, d_2 and d_3 are 0.425-0.75, 0.75-1.075 and 1.075-1.4mm respectively.

The above Figures (4.2) shows that, at (a) the maximum average yield was occurred (0.6%) when by using at time of 2hr, temperature of 95ºC and the particle size range of (0.425-0.07mm) and the minimum average yield was obtained (0.17%) at temperature of 75ºC. At (b) the maximum average yield was obtained (1.47%) at a time of 4hr with the same particle size range and extraction temperature of (a) and the minimum average oil yield was obtained (0.27%) at temperature of 75ºC, time 4hr and particle size range (1.075-1.4mm). At (c) the highest average yield was obtained (1.7%) at particle size range of (0.425-0.75mm) and the minimum average oil yield (0.5%) at particle size range of (1.075-1.4mm) with temperature of 75ºC and 95ºC respectively. Thus for this study, the temperature of 95ºC is the optimal condition and beyond this temperature, the oil will be degraded (Tajjudin M., Rahinan m.2012).

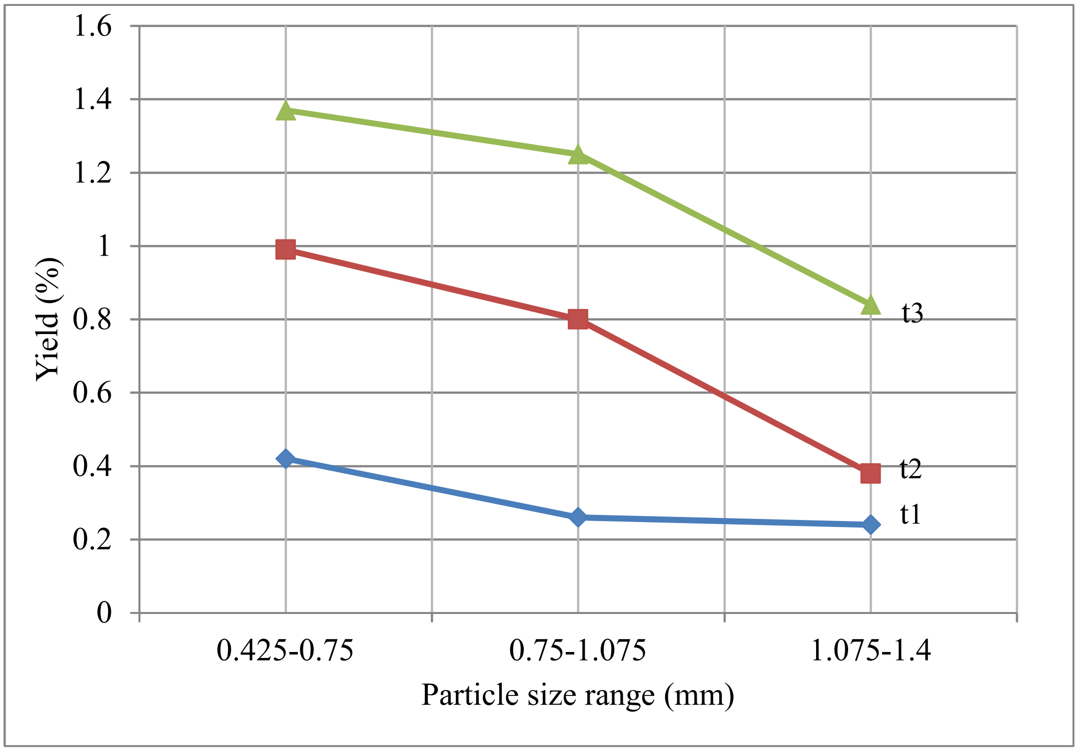
4.4.3 Effect of particle size range

Particle size plays a great role on the yield of vetiver grass root oil. Smaller the size gives high yield while samples with large particle size delivered low yield. (see to Figure 4.3). From Figures 4.3 what we observed from experiments less essential oil were extracted from the larger particles size (>1.4mm) compared to the smaller size of the particles. The reason is that larger particles with smaller contact surface area have more resistant to steam entrance and carried out and less mass transfer occurred.

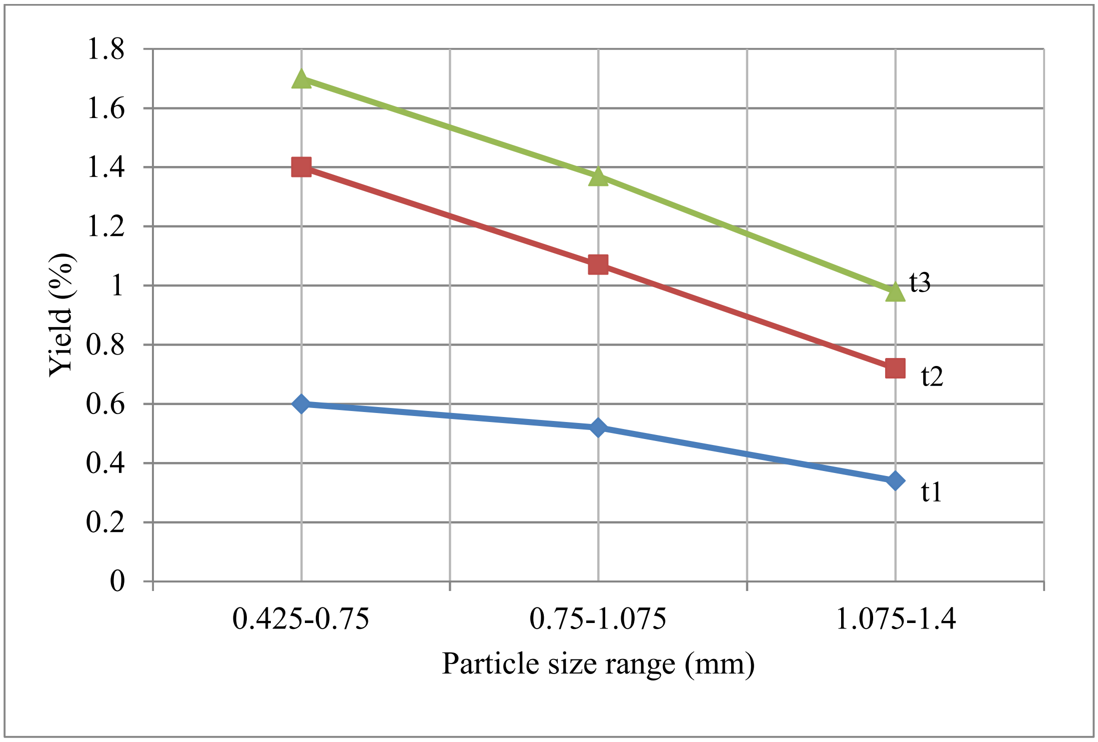
Therefore, less amount of essential oil will be transferred from inside to the surrounding vapor in comparison with the smaller ones. Thus, an increase in particle size would decrease the yield of essential oil. As a rule, particles with mean diameters ranging approximately between 0.25 and 2.0 mm are used (Reverchon and Marco, 2006).


Yield (%)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
t3
t2
t1
0.425-0.75
0.75-1.075
1.075-1.4
Particle size range (mm)

(a)


Yield (%)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
t3
t2
t1
0.425-0.75
0.75-1.075
1.075-1.4
Particle size range (mm)

(b)



(c)

Figure 4.3 Yield of vetiver grass root oil as a function of particle size range and temperature of (a) 75oc, (b) 85oC and (c) 95oC and t1, t2 and t3 are time of extraction 2, 4 and 6hrs respectively.

From the above each Figures (4.3) shows that we can observe the following points: at (a) the maximum average oil yield was obtained (0.89%) at temperature of 75°C, time 6hr and particle size range of (0.425-075mm) and the lowest average yield was obtained (0.17%) at time of 2hr and particle size range (1.075-1.4mm). At (b) the maximum average yield was obtained (1.37%) at temperature of 85°C, time of 6hr and particle size range (0.425-0.75m) and the minimum average yield was obtained (0.24%) at time of 2hr and particle size range (1.075-1.4mm). At (c) the highest average yield was determined (1.7%) at temperature of 95°C time of 6hr and particle size range of (0.425-0.75mm) and minimum average yield was obtained (0.34%) at time of 2hr and particle size range (1.075-1.4mm).

Another observation in agreement with from different literature was that the smallest average particle size of (0.77 mm) produces highest amount of essential oil yield. (Leite Bianca, 2012).

4.5 Analysis of general factorial experiments

4.5.1 Investigating interactions effect between factors on percent yield

4.5.1.1 Interaction effect between time and temperature

In this study, the methods employed in the extraction of the essential oil from a vetiver grass root time and temperature has major significant effects on the final yield of essential oil. As we see from design expert software (general variable) the outputs of interaction effect between temperature and time have a significant interaction for yield obtained by using steam extraction. From this figure 4.4 what we observed that, the yield linearly increases with respect to time and temperature. Maximum yield was obtained (1.7%) at temperature of 95°C and time (6hrs.)

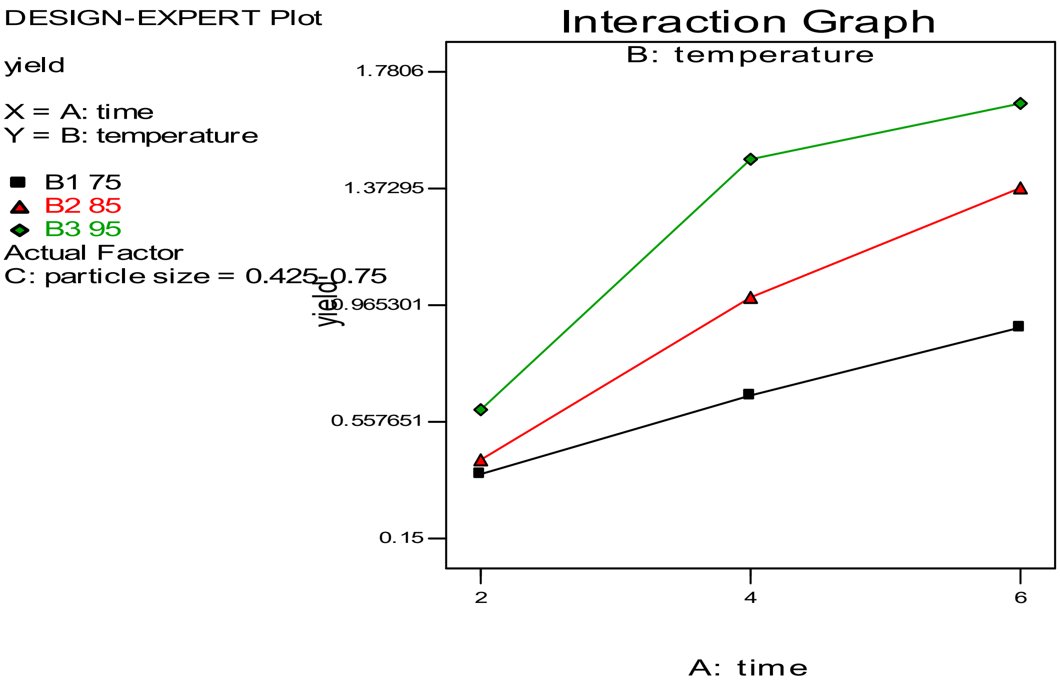


Figure 4.4 Interaction effects of time and temperature

Where, B_1, B_2 and B_3 are codes for temperature 75°c, 85°c and 95°c respectively and particle size range of 0.425-0.75mm and A_1, A_2 and A_3 are codes for extraction time of 2, 4 and 6hrs respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response based on these points.

4.5.1.2 Interaction effect between time and particle size range

In figure 4.5 below shows that the interaction effect of extraction time and particle size range on yield, from this figure what we can observe as, the maximum oil yield was obtained (1.67%) at time of (6hr) and particle size range of C_1 (0.425-.75mm) and the minimum yield was obtained (0.34%) at particle size range of (1.075-1.4mm) and time (2hr). When a particle size decrease, the yield will increases.

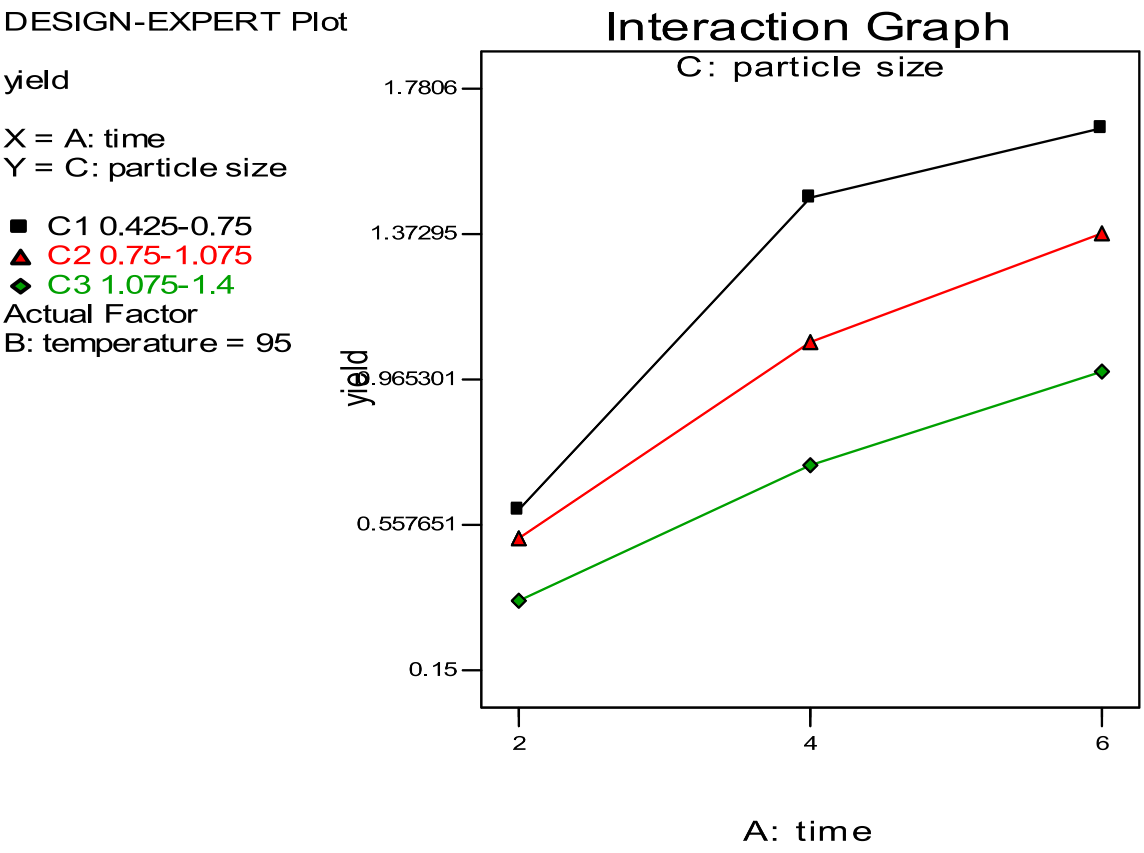


Figure 4.5 interactions between time and temperature on yield

Where, C_1, C_2 and C_3 are codes for particle size ranges 0.425-0.75, 0.75-1.075 1.075-1.4mm respectively and B_1, B_2 and B_3 are codes for extraction time (hrs.) two, four and six respectively.

4.5.1.3 Interaction effect between temperature and particle size range

Figure (4.6) shows that, the interaction effect of particle size range and temperature on yield obtained from general variable expert design. From this figure the maximum yield was obtained (1.67%) at maximum temperature of (95°C), maximum time of 6hr and minimum particle size range c_1 (0.425-0.75mm)

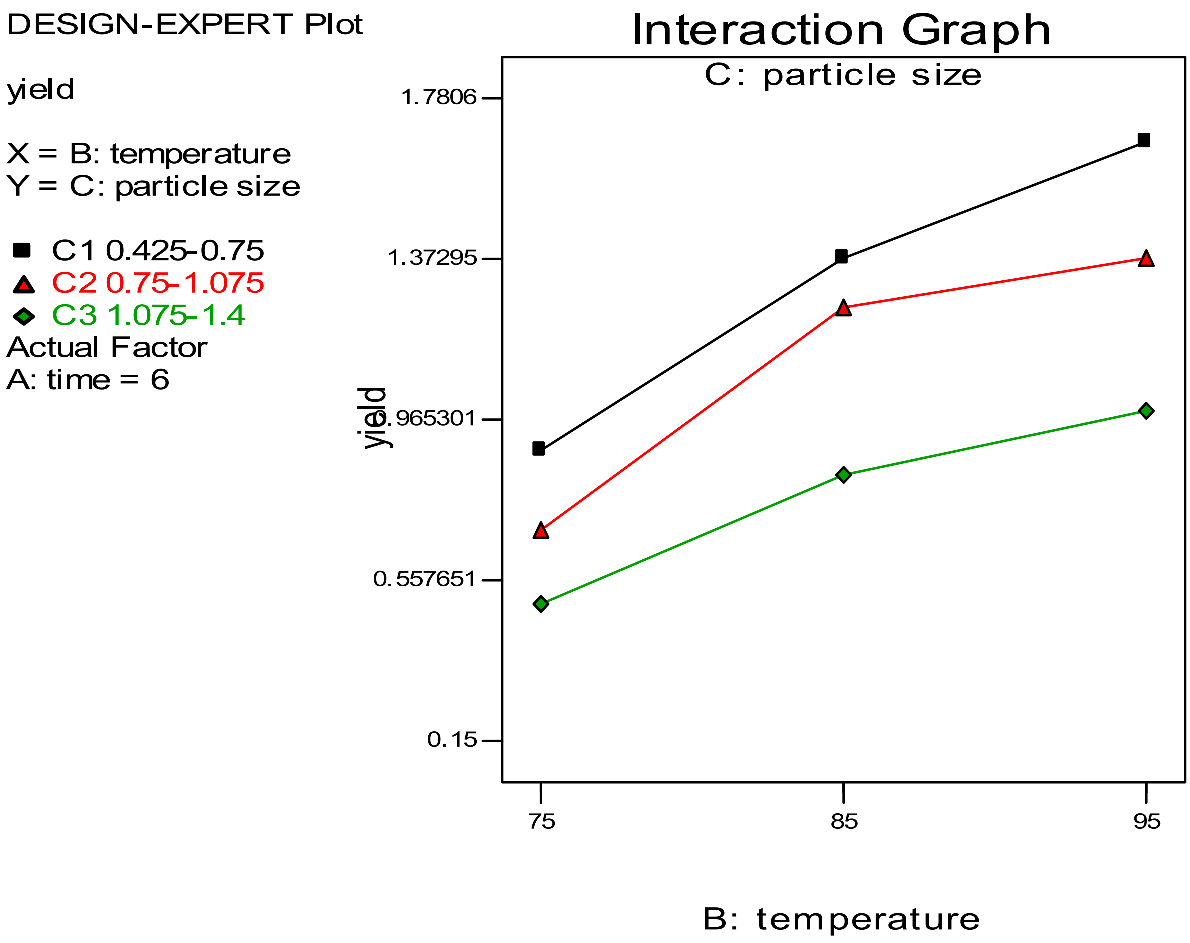


Figure 4.6 Interactions between temperature and particle size range on yield

4.5.2 Optimization of extraction conditions

For this study the optimal condition of steam distillation extraction obtained by the different extraction condition optimization option of the Design Expert software was obtained the temperature of 95ºC, an extraction time of 6 hrs and a particle size range 0.425-0.75mm. Under the optimal condition, the predicted value of oil yield was 1.66%.

The result revealed that the actual experimental value of 1.72% was consistent with the predicted value1.66%. The results confirmed that the general variable method was reliable and accurate enough to predict the oil yield**.**

4.5.3 Regression model equation

The following table shows analysis of variance (ANOVA), obtained from design expert software (general variable) which tell us the major role of different factors on yield. The ANOVA method is the classical method for estimating variance components in designed experiments. It is more accurate than the X-bar and R- chart methods.

Table 4.6 Analysis of variance (ANOVA) for a response of percentage essential oil yield at temperature of 95ºC, time of 6hr and particle size range of (0.425-0.75mm)

Source	Sum of squares	Degree of freedom	Mean square	F value	Prob>F
Model	9.63	26	0.37	31.78	<0.0001
A -Time (hrs.)	4.60	2	2.30	198.	<0.0001
B –Temperature(ºc)	2.40	2	1.20	103.07	<0.0001
C–Particle size (mm)	1.78	2	0.89	76..71	<0.0001
AB	0.43	4	0.11	9.35	<0.0001
AC	0.25	4	0.063	5.4	0.0025
BC	0.11	4	0.027	2.32	0.0827
ABC	0.056	8	6.954E-003	0.60	0.7708
Pure Error	0.31	27	0.012	-	-
Cor Total	9.94	53	-	-	-

The Model F-value of 31.78 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case A-time, B-temperature, C-particle size, AB-interaction of time and temperature, AC-interaction of time and particle size are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant. The P-value of ABC (interaction factors) is 0.7708 > P-value thus, the interactions of temperature, time and particle size ranges are not significant in the model terms.

Table 4.7 Values for reasonable agreements and model adequacy measure

Std.dev	0.11	R-Squared	0.9684
mean	0.72	Adj R-Squared	0.9381
c.v.	14.99	Pred R-Squared	0.8738
press	1.26	Adeq Precision	19.742

Table 4.7 shows that, the Predicted R-squared was obtained (0.8738) are in reasonable agreement with the Adjusted R-squared value of (0.9381). And adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this study the ratio was obtained (19.742) which indicate an adequate signal. This model can be used to navigate the design space.

Table 4.8 Regression coefficients for 95% CI low and 95% CI high

Term	Coefficient Estimate	Degree of freedom	Standard error	95% CI low	95% CI high
Intercept	0.72	1	0.015	0.69	0.75
A[1]	-0.37	1	0.021	-0.41	-0.33
A[2]	0.029	1	0.021	-0.013	0.072
B[1]	-0.26	1	0.021	-0.30	-0.22
B[2]	6.204E-003	1	0.021	-0.036	0.049
C[1]	0.22	1	0.021	0.18	0.26
C[2]	5.926E-003	1	0.021	-0.037	0.048
A[1]B[1]	0.16	1	0.029	0.10	0.22
A[2]B[1]	-0.051	1	0.029	-0.11	9.278E-003
A[1]B[2]	-0.047	1	0.029	-0.11	0.013
A[2]B[2]	-0.036	1	0.029	-0.096	0.025
A[1]C[1]	-0.10	1	0.029	-0.16	-0.041
A[2]C[1]	0.071	1	0.029	0.011	0.13
A[1]C[2]	-0.025	1	0.029	-0.085	0.035
A[2]C[2]	-0.011	1	0.029	-0.071	0.049
B[1]C[1]	-0.040	1	0.029	-0.10	0.020
B[2]C[1]	-0.014	1	0.029	-0.074	0.046
B[1]C[2]	-0.039	1	0.029	-0.099	0.021
B[2]C[2]	0.031	1	0.029	-0.029	0.091
A[1]B[1]C[1]	0.048	1	0.041	-0.037	0.13
A[2]B[1]C[1]	-0.037	1	0.041	-0.12	0.048
A[1]B[2]C[1]	0.013	1	0.041	-0.072	0.098
A[2]B[2]C[1]	-2.963E-003	1	0.041	-0.088	0.082
A[1]B[1]C[2]	0.015	1	0.041	-0.070	0.100
A[2]B[1]C[2]	-7.130E-003	1	0.041	-0.092	0.078
A[1]B[2]C[2]	-0.057	1	0.041	-0.14	0.028

Table 4.7 Continued…

A[2]B[2]C[2]	0.039	1	0.041	-0.055	0.12

From this table (4.7) what we can observe that, the final equation in terms of coded factors was obtained with interaction factors as follows:

Final equations in terms of coded factors are:

Oil yield = 0.72-0.37*A1+0.029*A2-0.26*B1+0.6.204E-0*B2+0.22*C1+5.926E -0*C2+0.16*A1B1-0.051*A2B1-0.047*A1B2-0.036*A2B2-0.10* A1C1+0.071*A2C1-0.025* A1C2-0.011*A2C2-0.04* B1C1-0.014* B2C1-0.39* B1C2+0.031* B2C2+0.048*A1B1C1-0.037*A2B1C1+0.013*A1B2C1-2.963E-00* A2B2C1+0.015*A1B1C2-7.130E-00*A2B1C2-0.057*A1B2C2+0.03*A2B2C2 (4.1)

Growing the individual effect of time, temperature and particle sizes as parameter and their interactions in the entire extraction process was found to be ineffective. Therefore, the final equation in terms of coded factor without the interaction effect is given by a first order regression equation obtained as:

Final equation in terms of coded factors without interaction factors are:

Oil yield = 0.72-0.37*A1+0.029*A2-0.26*B1+0.6.204E-0*B2+0.22*C1+5.926E- 0*C2 (4.2)

Where: A 1 = the difference of time level-1 from the overall average.

A 2 = the difference of time level-2 from the overall average.

B 1 = the difference of temperature level-1 from the overall average.

B 2 = the difference of temperature level-2 from the overall average

C 1 = the difference of particle size level -1 from overall average

C 2 =the difference of particle size level -2 from overall average

Table 4.9 Design summery of essential oil from vetiver grass root

Design summery							
Study type	Factorial	Experiment :54					
Initial design	Full factorial	Blocks	No blocks				
Center point zero (o)							
Design model 3FI							
Response	Name	Unit	Observation	Minimum	Maximum	trans	Model
Y	Yield	%	54	0.15	1.72	none	3FI
Factor	Name	Unit	Type	Low actual	High actual		
A	Time	hrs.	Categorical	2	6	level	3
B	Temperature	^{O}C	Categorical	75	95	level	3
C	Particle size	mm	Categorical	0.425-0.75	1.075-1.4	level	3

Diagnostics case Statistics: The following figure 4.6 indicates that, the relationship between the actual value of the experiment and the value predicted by the model equation developed by the Design Expert Software.

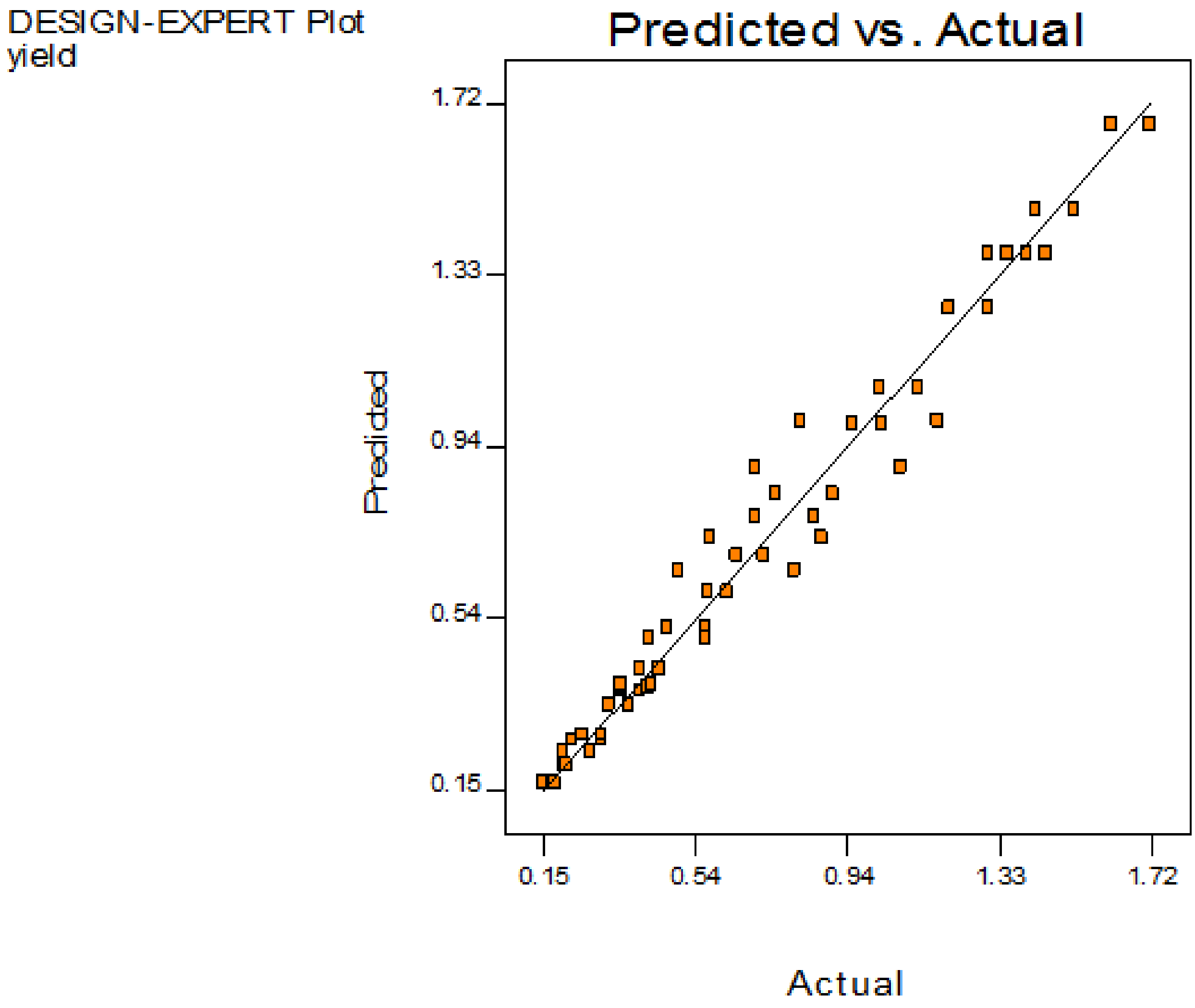


Figure 4.7 Predicted vs. actual value of yield for Steam distillation extraction

The above Figure 4.7 shows that, the closed relationship between the actual value that obtained from steam extraction condition and the predicted value of design expert software (general full factorial).

Table 4.9 Difference between the actual (experimental) value and predicted value.

Std. Order	Actual Value	Predicted Value	Residual	Std. Order	Actual Value	Predicted Value	Residual
1	0.40	0.38	0.025	28	0.85	0.81	0.045
2	0.35	0.38	-0.025	29	1.3	1.25	0.05
3	0.57	0.54	-0.15	30	1.2	1.25	-0.05
4	0.80	0.65	0.15	31	0.55	0.49	0.065
5	1.08	0.89	0.19	32	0.42	0.49	-0.065
6	0.70	0.89	-0.19	33	0.78	0.95	-0.17
7	0.45	0.43	0.025	34	1.12	0.95	0.17
8	0.4	0.43	-0.025	35	1.45	1.38	0.075
9	0.81	0.99	-0.18	36	1.3	1.38	-0.075
10	1.17	0.99	0.18	37	0.15	0.16	-0.075
11	1.35	1.38	-0.025	38	0.15	0.16	-0.01
12	1.40	1.38	0.025	39	0.25	0.28	-0.025
13	0.63	0.60	0.022	40	0.3	0.28	0.025
14	0.57	0.60	-0.025	41	0.42	0.5	-0.075
15	1.43	1.48	-0.05	42	0.57	0.5	0075
16	1.52	1.48	0.05	43	0.2	0.24	-0.035
17	1.62	1.67	-0.05	44	0.27	0.24	0.035
18	1.72	1.67	0.05	45	0.42	0.38	0.04
19	0.2	0.21	-5.000E-00	46	0.34	0.38	-0.04
20	0.21	0.21	5.000E-003	47	0.9	0.83	0.075
21	0.35	0.39	-0.035	48	0.75	0.83	-0.075
22	0.42	0.39	0.035	49	0.37	0.34	0.035
23	0.65	0.69	-0.035	50	0.3	0.34	-0.035
24	0.72	0.69	0.035	51	0.58	0.72	-0.14
25	0.25	0.28	-0.025	52	0.86	0.72	0.14
26	0.3	0.28	0.025	53	1.02	0.99	0.035
27	0.76	0.81	-0.045	54	0.95	0.99	-0.035

From this Table (4.10) what we can see, the actual value of essential oil yield that determined by using steam extraction has a slightly difference at the end of a process (residual value) with that of predicted value from design expert software (general full factorial).

4.6 Chemical composition analysis of essential oil

4.6.1 Gas chromatography-mass spectrometry analysis

The GC-MS library available in the laboratory at the Addis Ababa University college Natural Science Chemistry Department is the NIST14 L Mass Hunter GC-MS (1) 5977 MS firm. The essential oil of vetiver contained that maximum percentage of area was obtained ((3S,3aR,6R,8aS)-7,7-dimethyl-8methyleneoctahydro 1H-3a,6-methanoazulen-3-yl)methanolulen-3-y1methanol).

Table (4.10) below shows that, mostly of the essential oil contained sesquiterpene alcohol and hydrocarbon derivatives that eluted at different retention times depending on the boiling point of the eluted component. The GC chromatogram obtained revealed a high concentration of oil indicated by presence of one large peak which eluted at 16.780 minute with peak areas of percentage 23.37% (See to Figure 4.7). The mass spectrographs of the identified constituents are given in Table (4.10) below.

Table 4.10 Chemical composition of vetiver grass root essential oil.

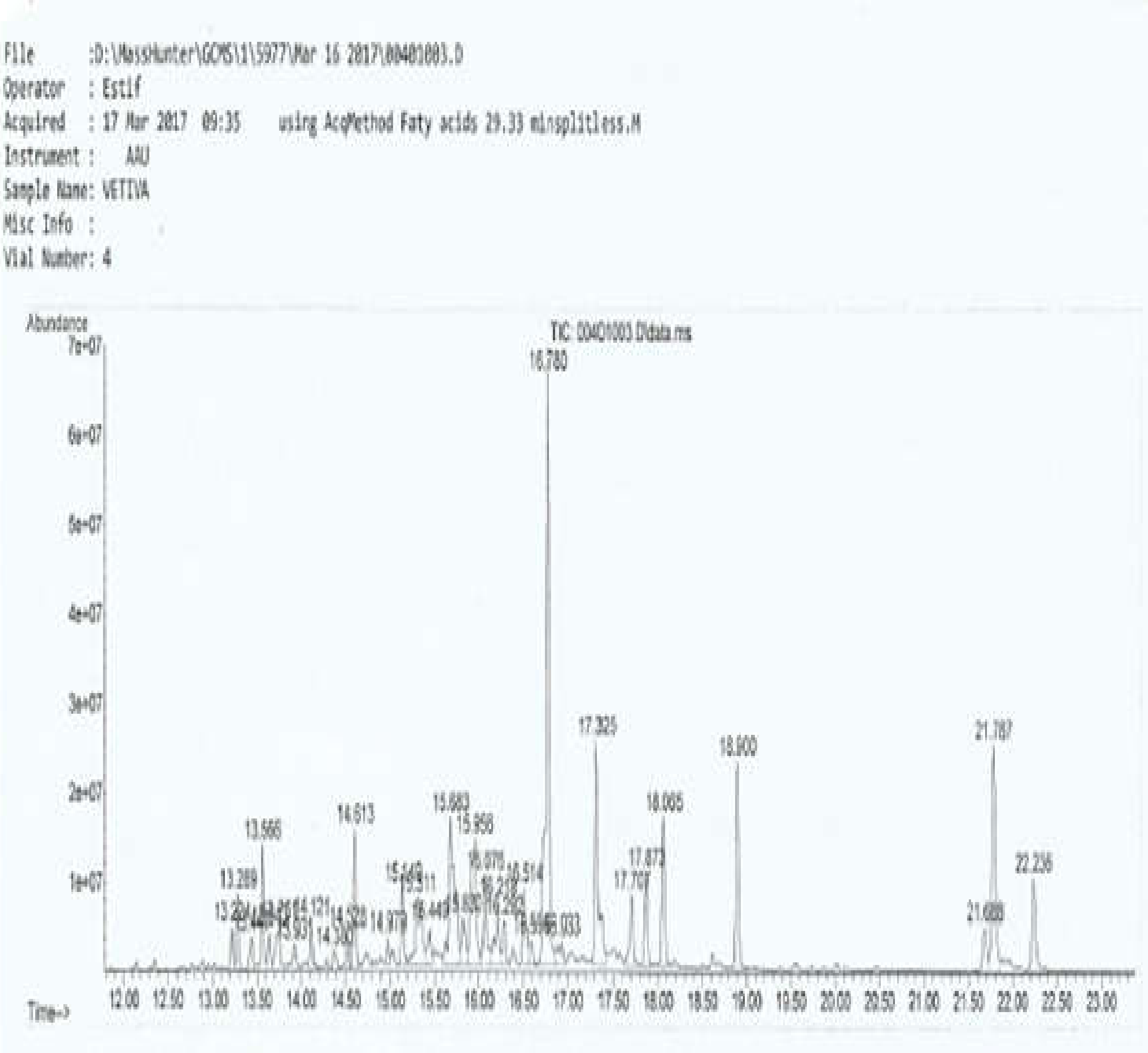
Structural assignment	Library Match Quality (%)	Retention time (min)	Area (%)
(3S, 3aS, 6R, 8aS)-3, 8, 8-Trimethyl	99	13.244	1.24
(8R, 8As) -8 8a-dimethyl	98	13.441	1.11
(1R, 4aS, 8aS) -1-Isopropyl -4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphtathalene	99	13.566	3.15
γ – muurolene	98	13.751	2.34

Table 4.10 Continued

Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-1-(1-methylethyl)-	98	13.751	2.34
Epizonarene	91	14.121	1.53
α – calacorene	91	14.380	0.9
β – vatirenene	95	14.528	1.33
2-((4aS, 8R, 8aR) -4a, 8-dimethyl-3,4,4a, 5, 6,7,8,8a octahydronaphthalene-2-y1)propan -2-o1.	98	14.613	4.22
α – ylangene	94	14.979	1.97
(3aR,6R,8aR)-7,7-dimethyl-8-methyl enexahydro-1H-3a,6-methanaozulen-3(2H) -one	98	15.149	2.95
Isoledene	95	15.311	5.89
Cyclohexen ,6-ethenyl -6- methyl -1-	90	15.449	1.6
(3S, 3aS, 5R, 6S, 7aS) -3, 6, 7, 7-Tetrame thyloctahydro-3a,6-ethanoinden-5-o	99	16.076	4.13
Decahydro-4a-methyl-1	94	16.218	3.21
Methyl tetradecanoate	99	16.292	1.8
(R) -2- ((4aS,8aR) -4a-methyl -8-methylene-1,4,4a,5,6,7,8,8a-octahydronaphthalen-2-y1)	99	16.514	3.88
((3S,3aR,6R,8aS)-7,7-dimethyl-8 methyleneoctahydro-1H-3a, 6-methanoazulen-3-yl) methanolulen-3-y1) methanol.	99	16.780	23.37
(5R, 10R)- 6, 10-Dimethyl-2-(propan-2-ylidene) spirol [4,5] dec-6-en-8-one	98	17.707	3.24
(z) -2-((8R, 8aS) -8, 8a –Dimethl-3,4,6,7,8,8a-hexahydronaphthalen-2(1H)-ylidene) propanal	99	17.83	3.12
2(3H) – Naphthalenon, 4, 4a,5,6,7,8-hexahydro -4,4a,-dimethylin-6-(1-methylethylidene)-, (4R-cis).	99	18.065	5.38

Table 4.10 continued

Hexadecanoic acid, methyl ester	99	18.900	7.2
9,12 octadecadienoic acid , methyl ester	99	21.688	1.91
11-octadecenoic acid, methyl ester	99	21.787	0.99
Methyl stearate	99	22.236	4.38



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Operator  : Estif
Acquired  : 17 Mar 2017  09:35     using AcqMethod Faty acids 29.33 minsplitless.M
Instrument :    AAU
Sample Name: VETIVA
Misc Info  :
Vial Number: 4
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Figure 4.8 Gas chromatography-mass spectrometry result profile

In this present study, there are twenty-six volatiles components were identified in the vetiver grass root essential oil obtained by using gas chromatography mass spectrometry which has been qualified the minimum quality of (90%).

Among these components, ((3S, 3aR, 6R, 8aS)-7, 7-dimethyl-8 methyleneoctahydro-1H-3a, 6-methanoazulen-3-yl) methanol was obtained in peak retention time (16.780min) and high percentage of area occupied (23.37%) which has been observed from Figure (4.8) and Table (4.11) respectively.

4.6.2 Determination of the functional groups present using FT-IR

The FT-IR spectra of vetiver grass root essential oil contain bands in the range of 3405 - 3120 cm^{-1} and these correspond to N-H and in cases where the oil contains solvent molecules, O-H. Range of standard broad band centered between 3450 - 3350 cm-^{1} corresponds to O-H stretching of hydroxyl groups (alcohols, phenols and carboxylic acids) (Szymczycha-madeja A, 2013).

Bands are also observed between 1647.11 and 1542 cm^{-1} and these are due to C-N and C-C vibration of aromatic structures respectively. The absorption at 1655.74 cm^{-1} was principally associated with absorbed water, since the hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures which can easily be hydrated.

The band at 1386 and 1168 cm^{-1} attribute to C–H deformation and C–O–C vibration in hemicelluloses, respectively. The prominent band at 1034 cm^{-1} was attributed to the C–O, C–C stretching or C–OH bending in terpenes. The sharp band at 892 cm^{-1} corresponding to the C-1 group frequency or ring frequency, was characteristic of vetivone. (refer to figure 4.8).

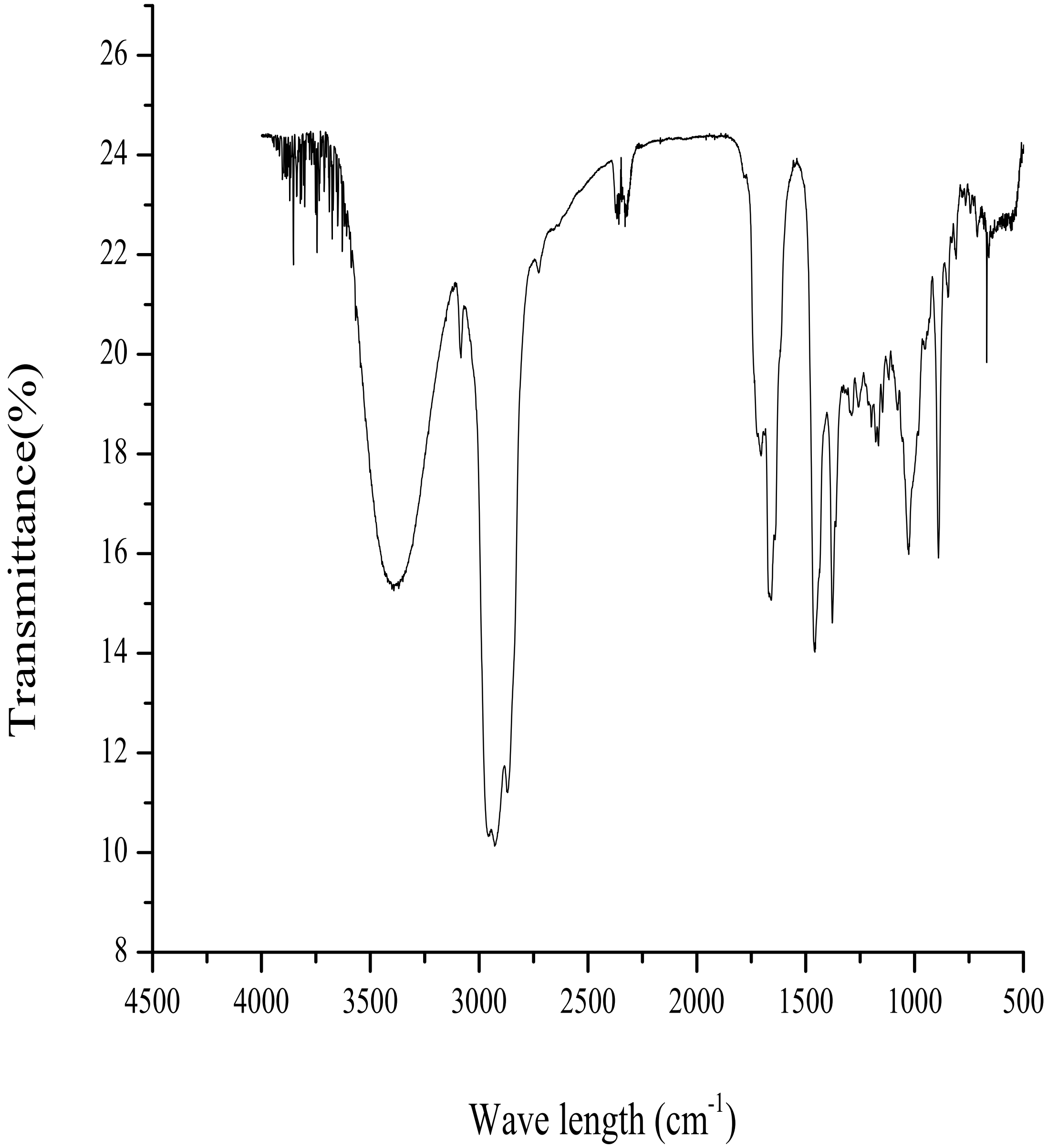

Figure 4.9 IR spectrum of vetiver grass root essential oil

5. Conclusion and Recommendations

5.1 Conclusions

For this study, the vetiver grass roots were collected from Bako (Anno agro industry farm plc) around 230km far from Addis Ababa. The study also was intended to study the influence of different factors (extraction time, temperature and Particle sizes) on the quality and quantity of extraction of essential oil from dried vetiver grass root. Variability of these operating conditions is the pre-dominant factors for the yield of the essential oil.

However, in this study steam distillation extraction method was used due to no thermal decomposition of oil constituents and most widely accepted process for large-scale oil production, superior to the other hydro distillation processes and it was recommended by so many literatures. The experimental apparatus for steam distillation extraction modification setup were done in leather industry development institute (LIDI) laboratory Addis Ababa, Ethiopia.

The yields obtained from the vetiver grass roots extracted using the laboratory scale steam distillation setup were given average yield of maximum approximately (1.72%) at 6hr,95^{o}C and at particle size of 0.425-0.75mm and minimum obtained was(0.15%) at 2hr,75^{o}C and 1.075-1.4mm of particle size. It is clearly seen design expert software and excel graph, that decreasing particle size range of raw material and increasing of extraction time and temperature of vetiver grass roots would increase the yield percentage. The maximum yield determined the optimum value of time (6hrs), 40g of dried raw material, temperature of (95^{o}C) and particle size range of (0.425-0.75mm), 1.72% was recorded. Some physical and chemical properties of the vetiver grass roots essential oil was determined and obtained comparable results with that of literature values. In these present study the extracted essential oil contains a large percentage of ((3S, 3aR, 6R, 8aS)-7, 7-dimethyl-8 methyleneoctahydro-1H-3a, 6-methanoazulen-3-yl) methanol (23.37%) as indicated by the strong intense vibration frequency in the FT-IR and in the GC-MS spectrograph by using the steam extraction technique.

5.2 Recommendations

Based on the present study finding, the following recommendations are forwarded.

In this study the effect of roots age and growth conditions of vetiver grass on were not studied. Therefore, further study should be done in order to extract much better essential oil yield. Because, studies showed that the younger roots give higher vetiver oil yields (NEDFi, 2005). Pretreatment of the raw vetiver grass is important for the quality yield and to select the best cultivated raw vetiver that will give better essential oil content of the product (leite bianca 2012).

It is important to know how much raw consumption is needed during extraction of essential oil, this is because if there is long storage time of raw vetiver grass root its quality will reduced.

I also recommend that it is important to control the temperature of the dryer or set the appropriate set of drying temperature to prevent over drying that in turn will burnt and denatured the raw material.

Optimization of the steam distillation extraction equipment is also recommended for more convenient operation and to increase the vetiver oil yield obtainable from the equipment.

In order to improve quality and increase shelf life of the essential oil, the freshly distilled essential oil need to be dehydrated to remove water either by anhydrous sodium sulphate or natural evaporation by air drying.

My final recommendation, further study should be conducted on the quality of the vetiver grass roots essential oil obtained at each operating conditions using GC-MS.

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Appendix

Appendix.A Physical characterization of vetiver grass root.

Figure A.1 Result of raw vetiver grass root characterization

በአዲስ አበባ ዩኒቨርስቲ
ተፈጥሮ ሳይንስ ኮሌጅ
የምግብ ሳይንስና ኒውትሪሽን ማዕከል

ADDIS ABABA UNIVERSITY
College of Natural Sciences
Center for Food Science and Nutrition

Date: January 17, 2017
Ref: FDSN/187/09/17

To: - Dereje Emishaw

Please find below the result for the sample submitted to our center.

No	Sample Type	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)
1	Vetiver grass root	5.80±0.00	2.81±0.09	1.50±0.00	1.6±0.00

Approval
Quality control

Name	Signature	Date
W/t Woinshet Abera		17/01/2017

Regards,

Kaleab Baye (PhD)
Chair, Center for food science and nutrition

Center for Food Science & Nutrition

Tel. –01-1-655-19-27 P.O. Box - 1176 Fax - +251-111-23-94-69 Email – foodsc@sc.aau.edu.et
Website:http://www.sc.aau.edu.et/foodscience

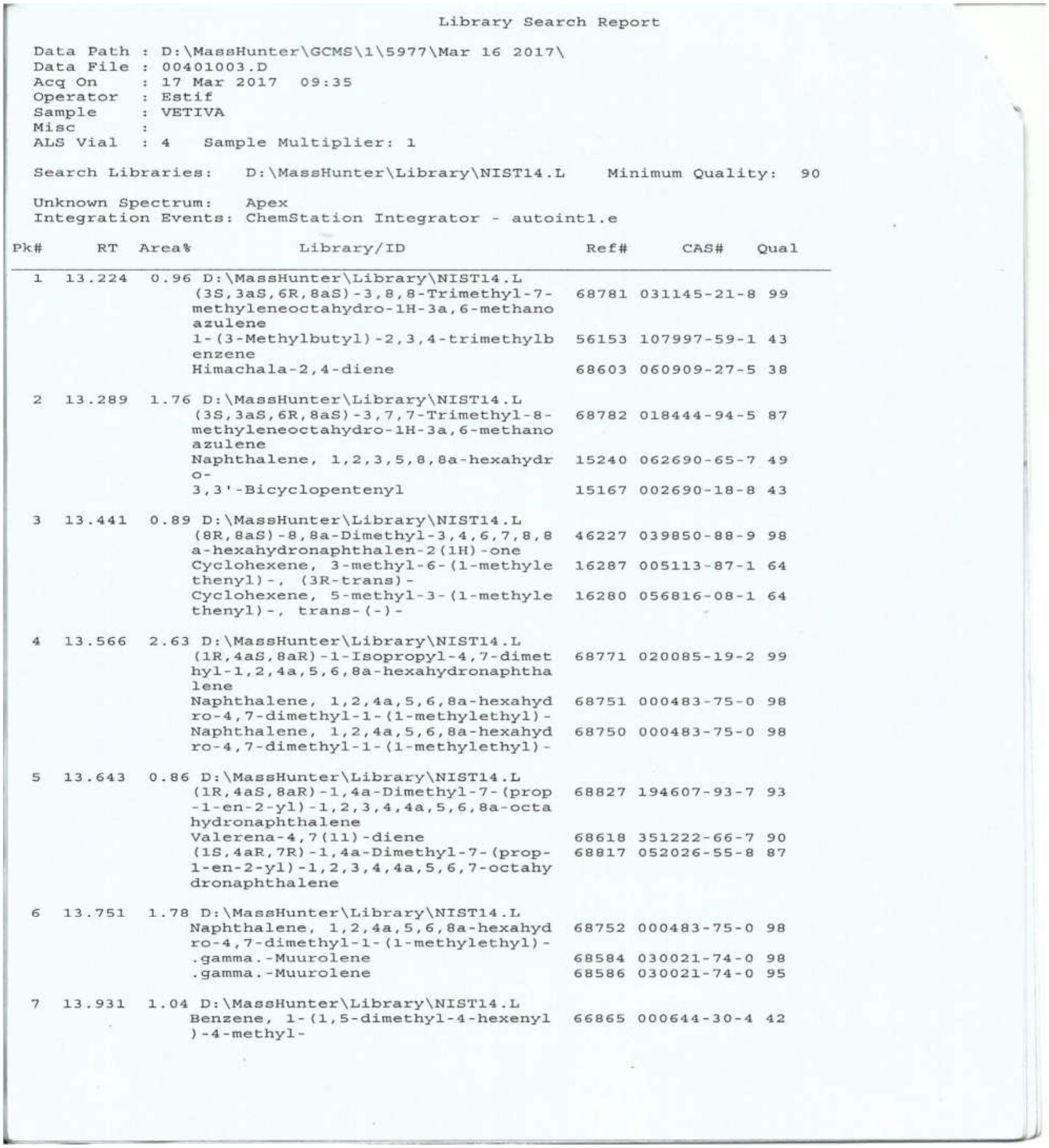
Appendix.B. Tabulated yields of vetiver oils from vetiver grass found in literature

Table B.1 Yields and extraction times for the extraction of vetiver oil from Brazilian vetiver roots using different extraction methods (Martinez et al, 2004)

Method	Temperature (°c)	Time (min)	Pressure (bar)	Yield (%)
Hydro distillation	100	720	-	0.31+/-0.01
Solvent extraction	70	300	-	1.91+/-0.19
Supercritical carbon dioxide extraction;	50	100	190	1.38

Appendix.C. Gas chromatography mass-spectrometry result.

Figure C.1 GC-MS analysis results for the vetiver oil obtained by steam distillation at 6 hrs. 95oc and 0.425-0.75mm**.**



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  Operator  : Estif
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  Misc      :
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Pk#      RT  Area%            Library/ID                     Ref#      CAS#    Qual
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                  methyleneoctahydro-1H-3a,6-methano
                  azulene
                  1-(3-Methylbutyl)-2,3,4-trimethylb   56153 107997-59-1 43
                  enzene
                  Himachala-2,4-diene                  68603 060909-27-5 38

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                  methyleneoctahydro-1H-3a,6-methano
                  azulene
                  Naphthalene, 1,2,3,5,8,8a-hexahydr   15240 062690-65-7 49
                  o-
                  3,3'-Bicyclopentenyl                 15167 002690-18-8 43

  3  13.441  0.89 D:\MassHunter\Library\NIST14.L
                  (8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8   46227 039850-88-9 98
                  a-hexahydronaphthalen-2(1H)-one
                  Cyclohexene, 3-methyl-6-(1-methyle   16287 005113-87-1 64
                  thenyl)-, (3R-trans)-
                  Cyclohexene, 5-methyl-3-(1-methyle   16280 056816-08-1 64
                  thenyl)-, trans-(-)-

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                  (1R,4aS,8aR)-1-Isopropyl-4,7-dimet   68771 020085-19-2 99
                  hyl-1,2,4a,5,6,8a-hexahydronaphtha
                  lene
                  Naphthalene, 1,2,4a,5,6,8a-hexahyd   68751 000483-75-0 98
                  ro-4,7-dimethyl-1-(1-methylethyl)-
                  Naphthalene, 1,2,4a,5,6,8a-hexahyd   68750 000483-75-0 98
                  ro-4,7-dimethyl-1-(1-methylethyl)-

  5  13.643  0.86 D:\MassHunter\Library\NIST14.L
                  (1R,4aS,8aR)-1,4a-Dimethyl-7-(prop   68827 194607-93-7 93
                  -1-en-2-yl)-1,2,3,4,4a,5,6,8a-octa
                  hydronaphthalene
                  Valerena-4,7(11)-diene               68618 351222-66-7 90
                  (1S,4aR,7R)-1,4a-Dimethyl-7-(prop-   68817 052026-55-8 87
                  1-en-2-yl)-1,2,3,4,4a,5,6,7-octahy
                  dronaphthalene

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                  Naphthalene, 1,2,4a,5,6,8a-hexahyd   68752 000483-75-0 98
                  ro-4,7-dimethyl-1-(1-methylethyl)-
                  .gamma.-Muurolene                    68584 030021-74-0 98
                  .gamma.-Muurolene                    68586 030021-74-0 95

  7  13.931  1.04 D:\MassHunter\Library\NIST14.L
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                  )-4-methyl-
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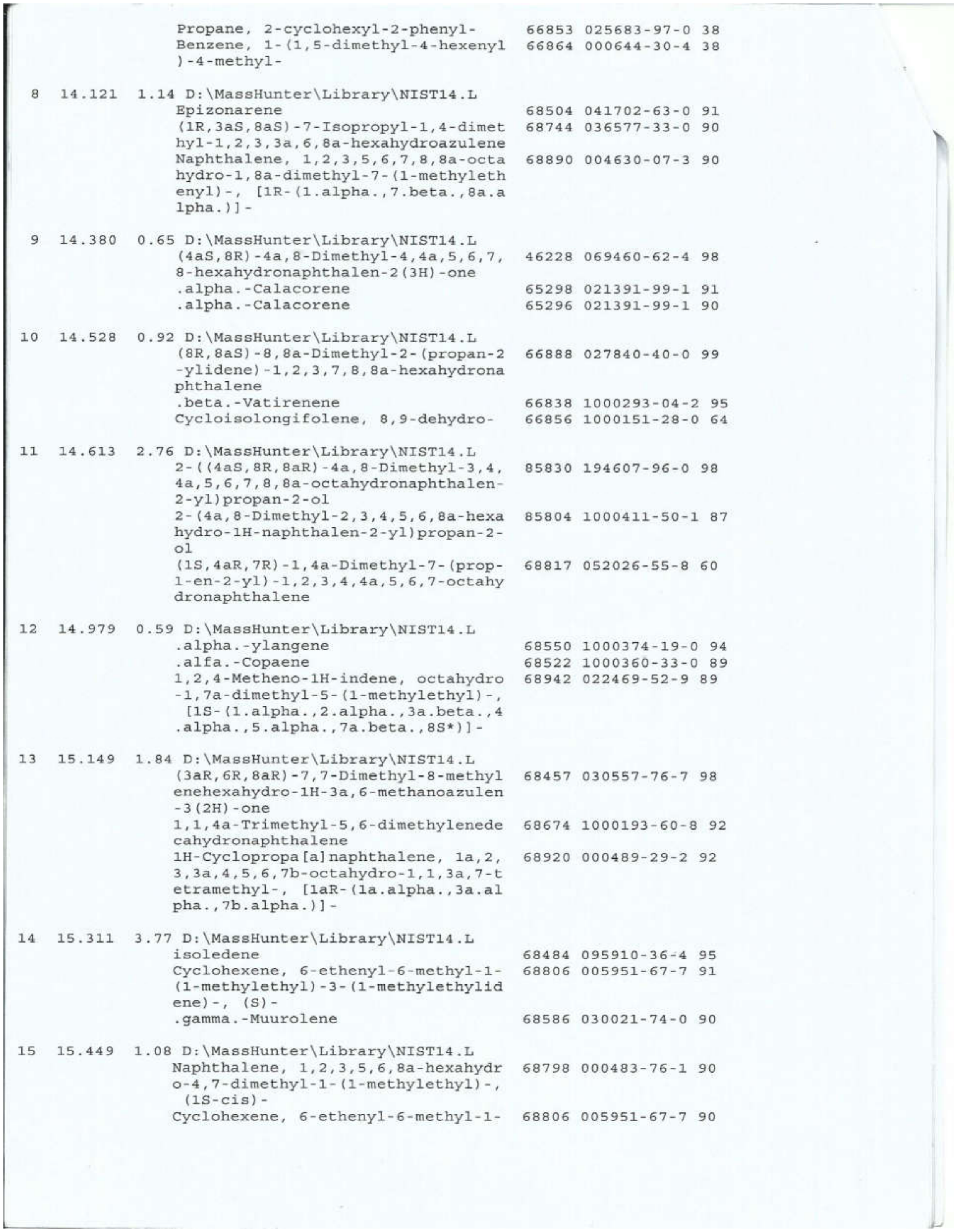

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                   )-4-methyl-

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                   (1R,3aS,8aS)-7-Isopropyl-1,4-dimet   68744 036577-33-0 90
                   hyl-1,2,3,3a,6,8a-hexahydroazulene
                   Naphthalene, 1,2,3,5,6,7,8,8a-octa   68890 004630-07-3 90
                   hydro-1,8a-dimethyl-7-(1-methyleth
                   enyl)-, [1R-(1.alpha.,7.beta.,8a.a
                   lpha.)]-

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                   (4aS,8R)-4a,8-Dimethyl-4,4a,5,6,7,   46228 069460-62-4 98
                   8-hexahydronaphthalen-2(3H)-one
                   .alpha.-Calacorene                   65298 021391-99-1 91
                   .alpha.-Calacorene                   65296 021391-99-1 90

 10  14.528  0.92 D:\MassHunter\Library\NIST14.L
                   (8R,8aS)-8,8a-Dimethyl-2-(propan-2   66888 027840-40-0 99
                   -ylidene)-1,2,3,7,8,8a-hexahydrona
                   phthalene
                   .beta.-Vatirenene                    66838 1000293-04-2 95
                   Cycloisolongifolene, 8,9-dehydro-    66856 1000151-28-0 64

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                   2-((4aS,8R,8aR)-4a,8-Dimethyl-3,4,   85830 194607-96-0 98
                   4a,5,6,7,8,8a-octahydronaphthalen-
                   2-yl)propan-2-ol
                   2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexa   85804 1000411-50-1 87
                   hydro-1H-naphthalen-2-yl)propan-2-
                   ol
                   (1S,4aR,7R)-1,4a-Dimethyl-7-(prop-   68817 052026-55-8 60
                   1-en-2-yl)-1,2,3,4,4a,5,6,7-octahy
                   dronaphthalene

 12  14.979  0.59 D:\MassHunter\Library\NIST14.L
                   .alpha.-ylangene                     68550 1000374-19-0 94
                   .alfa.-Copaene                       68522 1000360-33-0 89
                   1,2,4-Metheno-1H-indene, octahydro   68942 022469-52-9 89
                   -1,7a-dimethyl-5-(1-methylethyl)-,
                    [1S-(1.alpha.,2.alpha.,3a.beta.,4
                   .alpha.,5.alpha.,7a.beta.,8S*)]-

 13  15.149  1.84 D:\MassHunter\Library\NIST14.L
                   (3aR,6R,8aR)-7,7-Dimethyl-8-methyl   68457 030557-76-7 98
                   enehexahydro-1H-3a,6-methanoazulen
                   -3(2H)-one
                   1,1,4a-Trimethyl-5,6-dimethylenede   68674 1000193-60-8 92
                   cahydronaphthalene
                   1H-Cyclopropa[a]naphthalene, 1a,2,   68920 000489-29-2 92
                   3,3a,4,5,6,7b-octahydro-1,1,3a,7-t
                   etramethyl-, [1aR-(1a.alpha.,3a.al
                   pha.,7b.alpha.)]-

 14  15.311  3.77 D:\MassHunter\Library\NIST14.L
                   isoledene                            68484 095910-36-4 95
                   Cyclohexene, 6-ethenyl-6-methyl-1-   68806 005951-67-7 91
                   (1-methylethyl)-3-(1-methylethylid
                   ene)-, (S)-
                   .gamma.-Muurolene                    68586 030021-74-0 90

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                   Naphthalene, 1,2,3,5,6,8a-hexahydr   68798 000483-76-1 90
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                    (1S-cis)-
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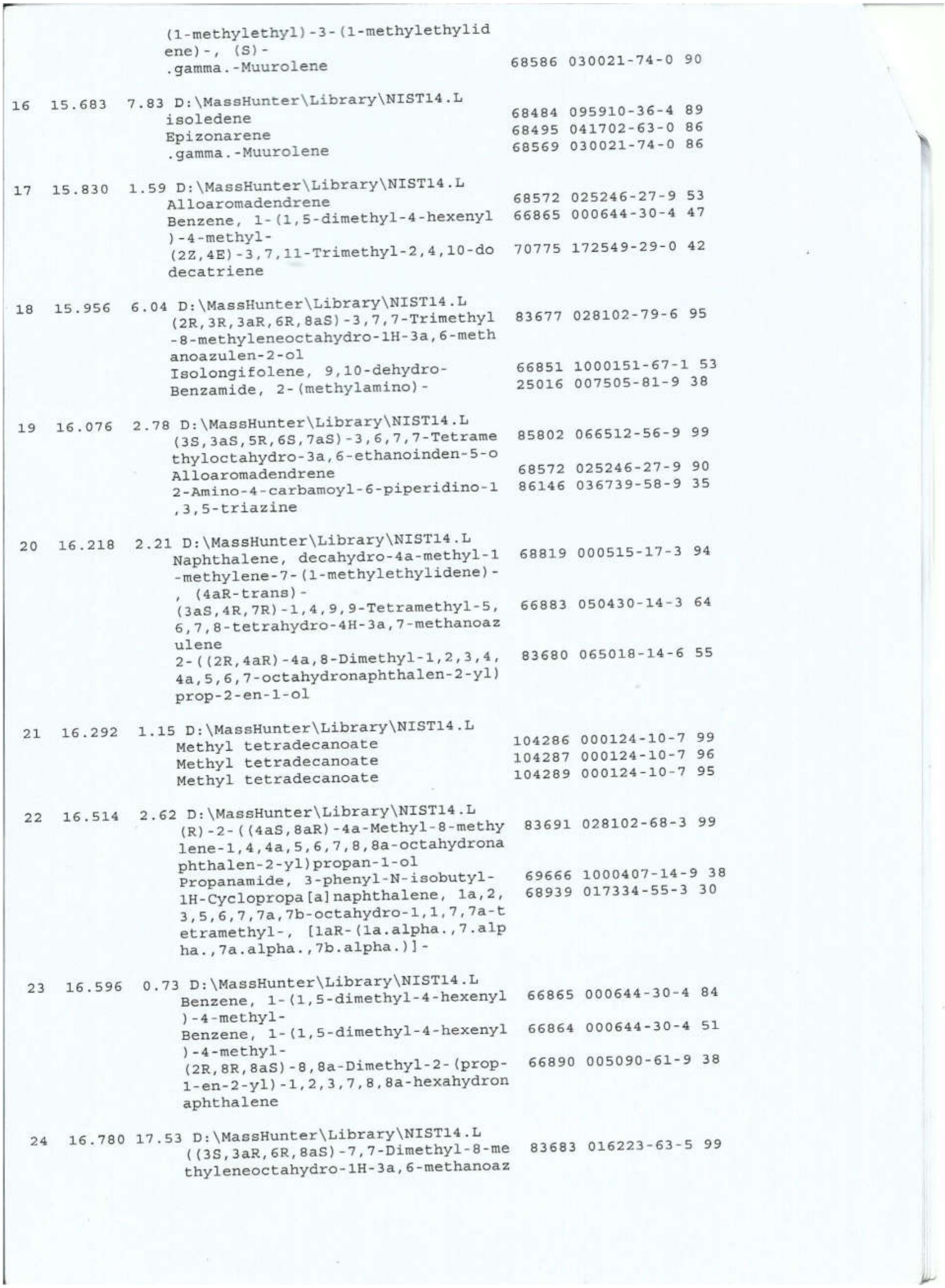

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                       (2Z,4E)-3,7,11-Trimethyl-2,4,10-do   70775 172549-29-0 42
                       decatriene

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                       -8-methyleneoctahydro-1H-3a,6-meth
                       anoazulen-2-ol
                       Isolongifolene, 9,10-dehydro-        66851 1000151-67-1 53
                       Benzamide, 2-(methylamino)-          25016 007505-81-9 38

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                       thyloctahydro-3a,6-ethanoinden-5-o
                       Alloaromadendrene                    68572 025246-27-9 90
                       2-Amino-4-carbamoyl-6-piperidino-1   86146 036739-58-9 35
                       ,3,5-triazine

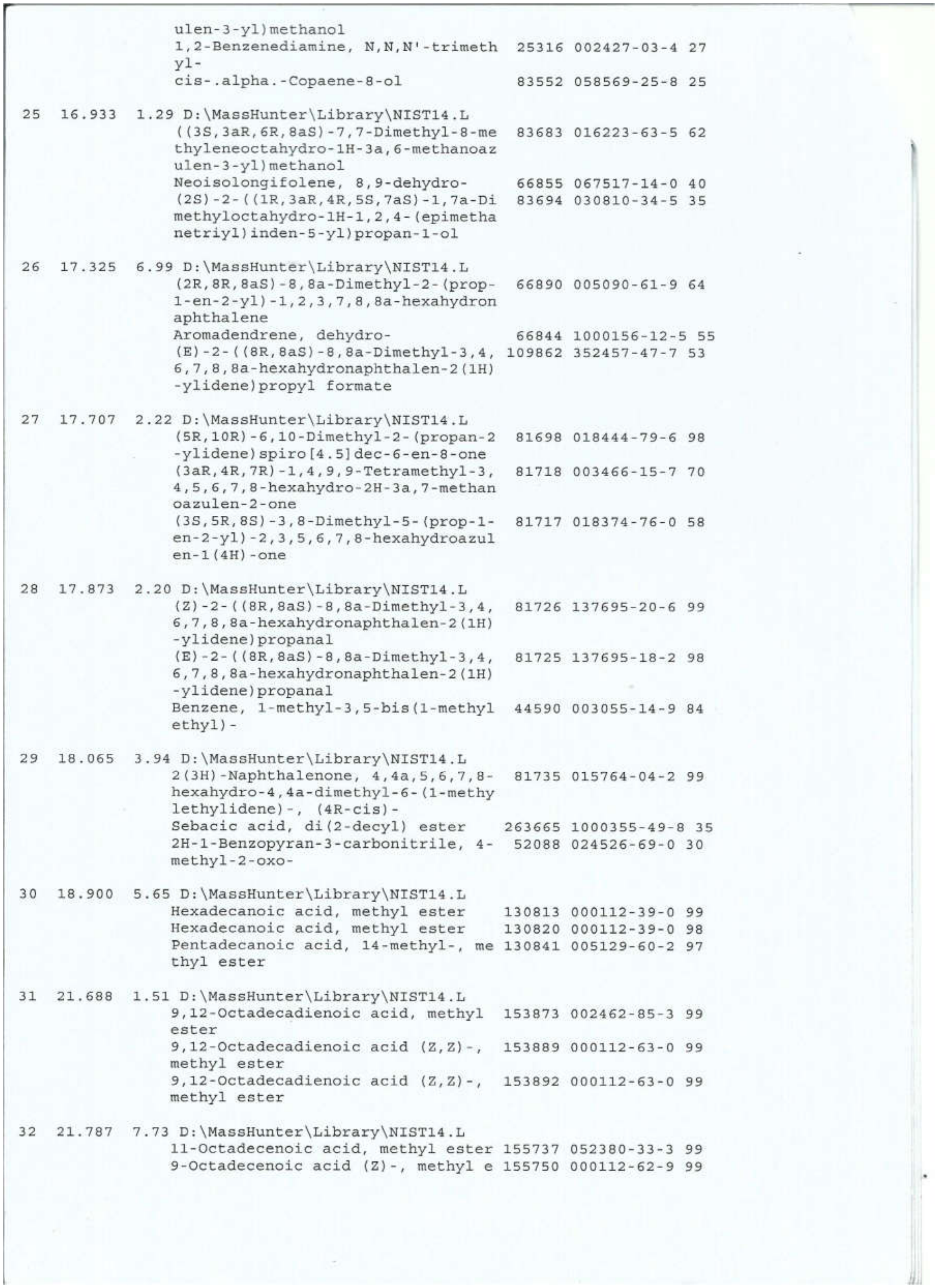
20  16.218   2.21 D:\MassHunter\Library\NIST14.L
                       Naphthalene, decahydro-4a-methyl-1   68819 000515-17-3 94
                       -methylene-7-(1-methylethylidene)-
                       , (4aR-trans)-
                       (3aS,4R,7R)-1,4,9,9-Tetramethyl-5,   66883 050430-14-3 64
                       6,7,8-tetrahydro-4H-3a,7-methanoaz
                       ulene
                       2-((2R,4aR)-4a,8-Dimethyl-1,2,3,4,   83680 065018-14-6 55
                       4a,5,6,7-octahydronaphthalen-2-yl)
                       prop-2-en-1-ol

21  16.292   1.15 D:\MassHunter\Library\NIST14.L
                       Methyl tetradecanoate               104286 000124-10-7 99
                       Methyl tetradecanoate               104287 000124-10-7 96
                       Methyl tetradecanoate               104289 000124-10-7 95

22  16.514   2.62 D:\MassHunter\Library\NIST14.L
                       (R)-2-((4aS,8aR)-4a-Methyl-8-methy   83691 028102-68-3 99
                       lene-1,4,4a,5,6,7,8,8a-octahydrona
                       phthalen-2-yl)propan-1-ol
                       Propanamide, 3-phenyl-N-isobutyl-    69666 1000407-14-9 38
                       1H-Cyclopropa[a]naphthalene, 1a,2,   68939 017334-55-3 30
                       3,5,6,7,7a,7b-octahydro-1,1,7,7a-t
                       etramethyl-, [1aR-(1a.alpha.,7.alp
                       ha.,7a.alpha.,7b.alpha.)]-

23  16.596   0.73 D:\MassHunter\Library\NIST14.L
                       Benzene, 1-(1,5-dimethyl-4-hexenyl   66865 000644-30-4 84
                       )-4-methyl-
                       Benzene, 1-(1,5-dimethyl-4-hexenyl   66864 000644-30-4 51
                       )-4-methyl-
                       (2R,8R,8aS)-8,8a-Dimethyl-2-(prop-   66890 005090-61-9 38
                       1-en-2-yl)-1,2,3,7,8,8a-hexahydron
                       aphthalene

24  16.780  17.53 D:\MassHunter\Library\NIST14.L
                       ((3S,3aR,6R,8aS)-7,7-Dimethyl-8-me   83683 016223-63-5 99
                       thyleneoctahydro-1H-3a,6-methanoaz
```



```
                ulen-3-yl)methanol
                1,2-Benzenediamine, N,N,N'-trimeth  25316 002427-03-4 27
                yl-
                cis-.alpha.-Copaene-8-ol            83552 058569-25-8 25

25  16.933  1.29 D:\MassHunter\Library\NIST14.L
                ((3S,3aR,6R,8aS)-7,7-Dimethyl-8-me  83683 016223-63-5 62
                thyleneoctahydro-1H-3a,6-methanoaz
                ulen-3-yl)methanol
                Neoisolongifolene, 8,9-dehydro-     66855 067517-14-0 40
                (2S)-2-((1R,3aR,4R,5S,7aS)-1,7a-Di  83694 030810-34-5 35
                methyloctahydro-1H-1,2,4-(epimetha
                netriyl)inden-5-yl)propan-1-ol

26  17.325  6.99 D:\MassHunter\Library\NIST14.L
                (2R,8R,8aS)-8,8a-Dimethyl-2-(prop-  66890 005090-61-9 64
                1-en-2-yl)-1,2,3,7,8,8a-hexahydron
                aphthalene
                Aromadendrene, dehydro-             66844 1000156-12-5 55
                (E)-2-((8R,8aS)-8,8a-Dimethyl-3,4, 109862 352457-47-7 53
                6,7,8,8a-hexahydronaphthalen-2(1H)
                -ylidene)propyl formate

27  17.707  2.22 D:\MassHunter\Library\NIST14.L
                (5R,10R)-6,10-Dimethyl-2-(propan-2  81698 018444-79-6 98
                -ylidene)spiro[4.5]dec-6-en-8-one
                (3aR,4R,7R)-1,4,9,9-Tetramethyl-3,  81718 003466-15-7 70
                4,5,6,7,8-hexahydro-2H-3a,7-methan
                oazulen-2-one
                (3S,5R,8S)-3,8-Dimethyl-5-(prop-1-  81717 018374-76-0 58
                en-2-yl)-2,3,5,6,7,8-hexahydroazul
                en-1(4H)-one

28  17.873  2.20 D:\MassHunter\Library\NIST14.L
                (Z)-2-((8R,8aS)-8,8a-Dimethyl-3,4,  81726 137695-20-6 99
                6,7,8,8a-hexahydronaphthalen-2(1H)
                -ylidene)propanal
                (E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,  81725 137695-18-2 98
                6,7,8,8a-hexahydronaphthalen-2(1H)
                -ylidene)propanal
                Benzene, 1-methyl-3,5-bis(1-methyl  44590 003055-14-9 84
                ethyl)-

29  18.065  3.94 D:\MassHunter\Library\NIST14.L
                2(3H)-Naphthalenone, 4,4a,5,6,7,8-  81735 015764-04-2 99
                hexahydro-4,4a-dimethyl-6-(1-methy
                lethylidene)-, (4R-cis)-
                Sebacic acid, di(2-decyl) ester    263665 1000355-49-8 35
                2H-1-Benzopyran-3-carbonitrile, 4-  52088 024526-69-0 30
                methyl-2-oxo-

30  18.900  5.65 D:\MassHunter\Library\NIST14.L
                Hexadecanoic acid, methyl ester    130813 000112-39-0 99
                Hexadecanoic acid, methyl ester    130820 000112-39-0 98
                Pentadecanoic acid, 14-methyl-, me 130841 005129-60-2 97
                thyl ester

31  21.688  1.51 D:\MassHunter\Library\NIST14.L
                9,12-Octadecadienoic acid, methyl  153873 002462-85-3 99
                ester
                9,12-Octadecadienoic acid (Z,Z)-,  153889 000112-63-0 99
                methyl ester
                9,12-Octadecadienoic acid (Z,Z)-,  153892 000112-63-0 99
                methyl ester

32  21.787  7.73 D:\MassHunter\Library\NIST14.L
                11-Octadecenoic acid, methyl ester 155737 052380-33-3 99
                9-Octadecenoic acid (Z)-, methyl e 155750 000112-62-9 99
```

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                 ster
                 7-Octadecenoic acid, methyl ester  155720 057396-98-2 99

 33  22.236  3.35 D:\MassHunter\Library\NIST14.L
                 Methyl stearate                    157879 000112-61-8 99
                 Methyl stearate                    157884 000112-61-8 99
                 Methyl stearate                    157882 000112-61-8 98


Essential oil.M Thu Mar 23 17:59:24 2017
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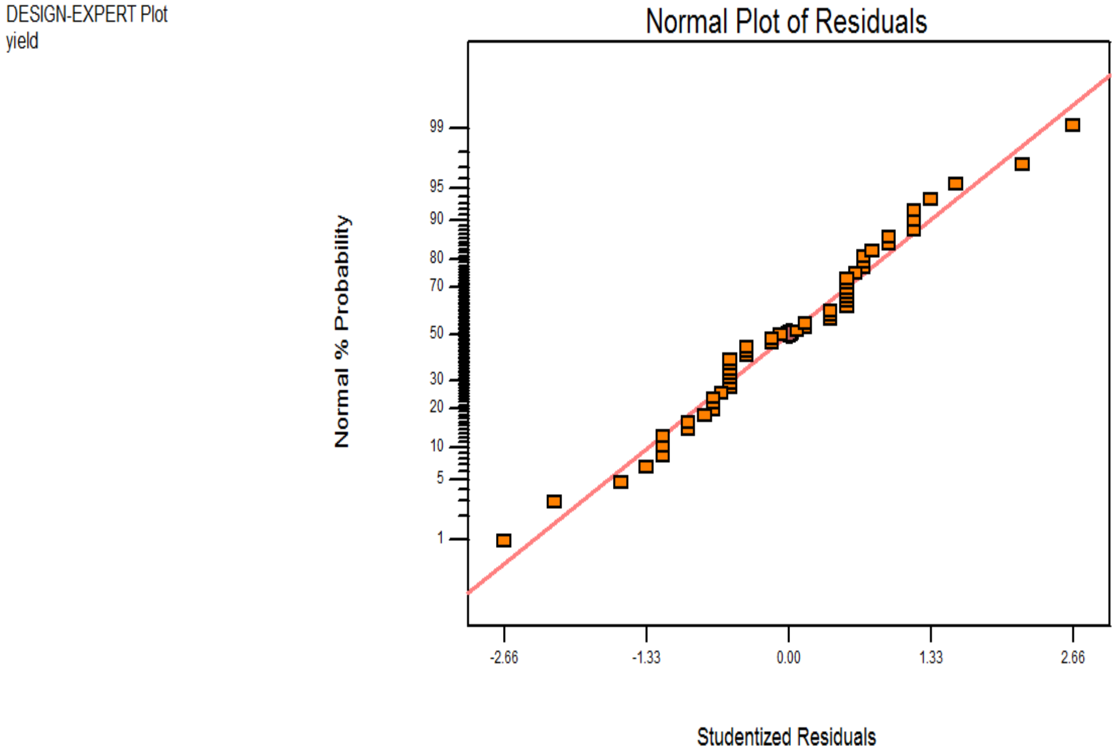
Appendix.D Steam distillation extracted data analysis by using design expert soft ware

Table D.1 Analysis of experimental design (actual) data of essential oil from vetiver grass root at different extraction conditions.

Std.	Run	Block	Factor 1 Time (hr)	Factor2 Temperature (ºC)	Factor 3 Particle size (mm)	Response Yield (%)
1	13	Block 1	2	75	0.425-0.75	0.4
2	2	Block 1	2	75	0.425-0.75	0.35
3	20	Block 1	4	75	0.425-0.75	0.5
4	19	Block 1	4	75	0.425-0.75	0.8
5	16	Block 1	6	75	0.425-0.75	1.075
6	47	Block 1	6	75	0.425-0.75	0.7
7	1	Block 1	2	85	0.425-0.75	0.45
8	51	Block 1	2	85	0.425-0.75	0.4
9	5	Block 1	4	85	0.425-0.75	0.815
10	11	Block 1	4	85	0.425-0.75	1.17
11	52	Block 1	6	85	0.425-0.75	1.35
12	38	Block 1	6	85	0.425-0.75	1.4
13	10	Block 1	2	95	0.425-0.75	0.625
14	40	Block 1	2	95	0.425-0.75	0.575
15	35	Block 1	4	95	0.425-0.75	1.425
16	8	Block 1	4	95	0.425-0.75	1.525
17	22	Block 1	6	95	0.425-0.75	1.62
18	53	Block 1	6	95	0.425-0.75	1.72
19	50	Block 1	2	75	0.75-1.075	0.2
20	54	Block 1	2	75	0.75-1.075	0.21
21	3	Block 1	4	75	0.75-1.075	0.35
22	44	Block 1	4	75	0.75-1.075	0.42
23	39	Block 1	6	75	0.75-1.075	0.65
24	49	Block 1	6	75	0.75-1.075	0.72

25	32	Block 1	2	85	0.75-1.075	0.225
26	42	Block 1	2	85	0.75-1.075	0.3
27	46	Block 1	4	85	0.75-1.075	0.7
28	33	Block 1	4	85	0.75-1.075	0.85
29	29	Block 1	6	85	0.75-1.075	1.3
30	18	Block 1	6	85	0.75-1.075	1.2
31	28	Block 1	2	95	0.75-1.075	0.57
32	36	Block 1	2	95	0.75-1.075	0.47
33	31	Block 1	4	95	0.75-1.075	1.02
34	12	Block 1	4	95	0.75-1.075	1.12
35	4	Block 1	6	95	0.75-1.075	1.45
36	7	Block 1	6	95	0.75-1.075	1.3
37	9	Block 1	2	75	1.075-1.4	0.15
38	15	Block 1	2	75	1.075-1.4	0.18
39	23	Block 1	4	75	1.075-1.4	0.25
40	25	Block 1	4	75	1.075-1.4	0.3
41	43	Block 1	6	75	1.075-1.4	0.425
42	17	Block 1	6	75	1.075-1.4	0.57
43	24	Block 1	2	85	1.075-1.4	0.2
44	21	Block 1	2	85	1.075-1.4	0.27
45	48	Block 1	4	85	1.075-1.4	0.43
46	45	Block 1	4	85	1.075-1.4	0.35
47	34	Block 1	6	85	1.075-1.4	0.9
48	41	Block 1	6	85	1.075-1.4	0.75
49	6	Block 1	2	95	1.075-1.4	0.37
50	30	Block 1	2	95	1.075-1.4	0.32
51	37	Block 1	4	95	1.075-1.4	0.58
52	26	Block 1	4	95	1.075-1.4	0.87
53	27	Block 1	6	95	1.075-1.4	1.025
54	14	Block 1	6	95	1.075-1.4	0.95

Normal plot vs residuals



Appendix.E Graph of Normal plots vs residual

Table E.3 Factors contributions on yield of essential oil from general variable software design.

Factors	Contribution (%)
A	46.28
B	24.09
C	17.93
AB	4.37
AC	2.53
BC	1.08
ABC	0.56

Appendix.F Laboratory equipment and samples photos

Figure F. 1 Vetiver grass at Bako, Anno agro industry farm plc. 2017.

Figure F. 2 Root processing from the ground by digging at Anno agro industry farm plc. (2017).

Figure F. 3 During milling process using Attrition mill.

Figure F.4 vibrating shaker (Retsch) during sieves analysis of particle size.

Figure F. 4 Steam distillation setup photo at LIDI laboratory.

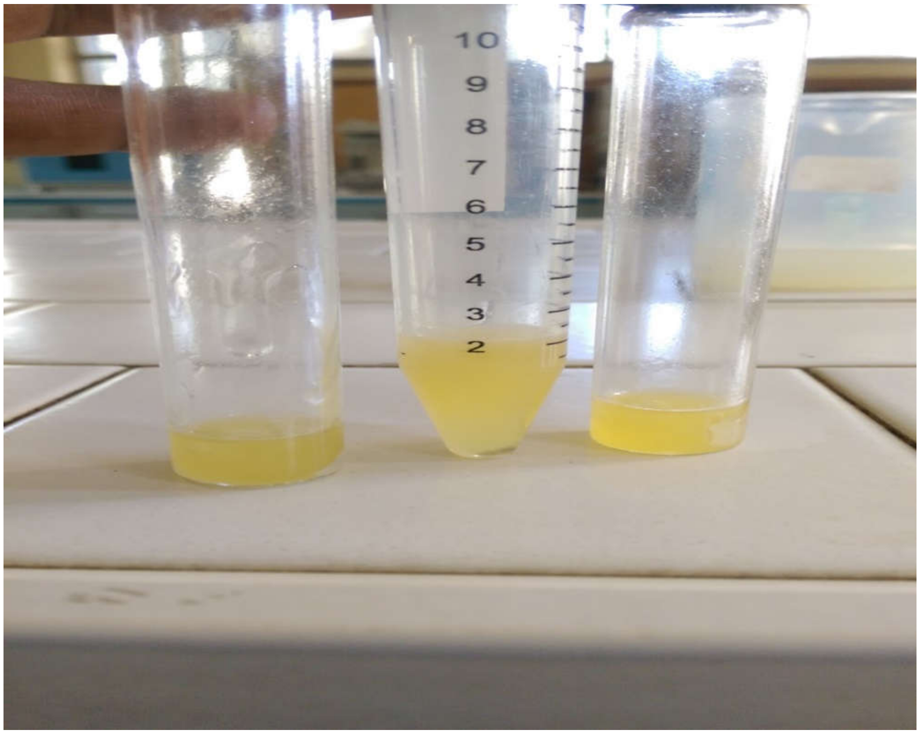


Figure F. 5 Sample of extracted essential oil from vetiver grass roots.

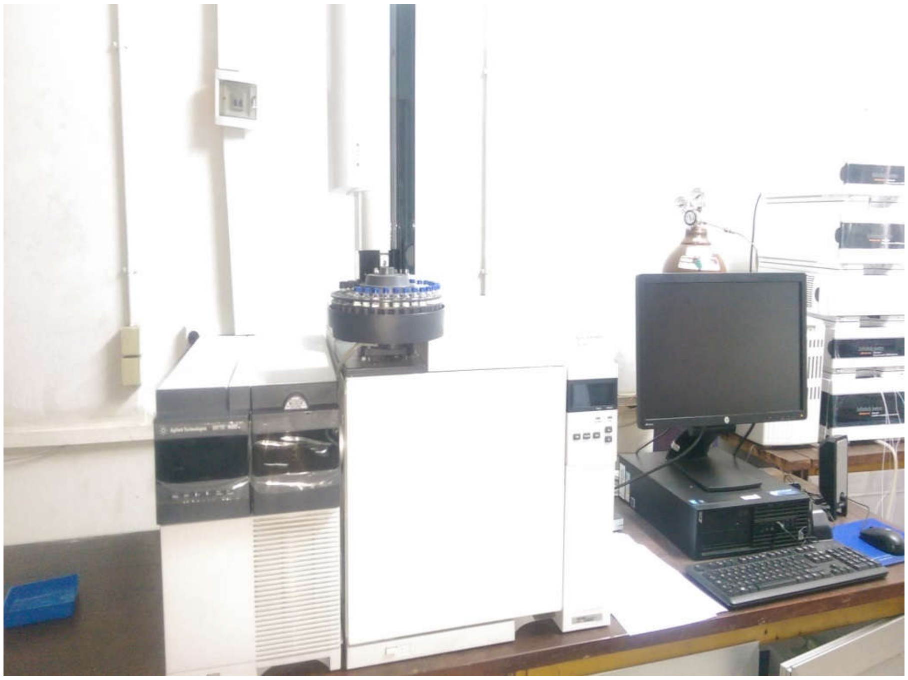


Figure F. 6 Gas chromatography-mass spectrometry.