CHAPTER I

INTRODUCTION AND LITERATURE REVIEWS

1.1 General Information on Vetiver Grass[1]

Vetiver grass (**Fig 1.1**)[2] is a tall, tufted, perennial, with a straight stem, long narrow leaves and a lacework root system that is abundant, complex, and extensive. It offers an inexpensive yet effective and eco-friendly tool to combat soil erosion. Vetiver is considered to be a rapidly growing variety of grasses. It grows vertically tall and in thick clumps. It has a long fibrous root system which is knitted strongly together like a net in the soil. The roots penetrate vertically 3.0 m deep and horizontally 0.5 m under the ground. It will not affect the root system of other plants. Serving as an underground barrier, the roots hold the soil together, retain water, as well as filter and absorb plant nutrients, and hazardous chemical substances which resultingly help to reduce pollution in the environment. Contour planting of vetiver across the slopes and along the road shoulders help trap silts and filter crop residues, while only letting parts of water to flow through. This effectively reduces soil erosion and prevents surface soil from being washed away. Vetiver is also planted around the base of fruit and perennial trees on the plains, and on dry, deteriorated area in order to preserve rainwater in the soil. Vetiver leaves are also used for mulching to maintain soil moisture. In other cases, vetiver is planted around the reservoirs and ponds, and

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along irrigation canals, and ditches, in order to prevent siltation of these water sources and equally important, to impede toxic chemicals present in the catchment areas from contaminating water sources. However, it is necessary to take proper care of the planted vetiver in order to successfully achieve the above-mentioned benefits.

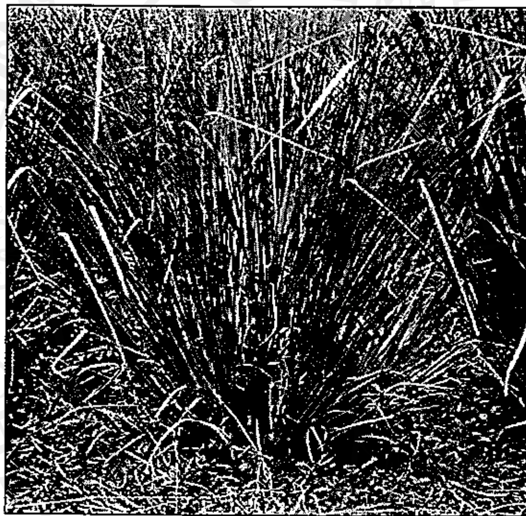

Fig 1.1 Vetiver grass

1.1.1 Morphology of vetiver grass (Fig 1.2)[3]

Culm

Vetiver grass is a prolific tiller growing naturally in clumps with thin, long, and erect leaves. The vetiver clumps may grow densely tufted in a big cluster or scattering over the nearby spaces. The base of the clump is dense which makes it obviously distinctive from other types of grass. With the bases of the leaves laying on top of one another, the culm base looks flat. The genuine culm is a tiny shoot hidden

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in the leaf sheath at the culm base near the soil. The growth of vetiver is determined by tillering which occurs regularly by producing new shoots on the sides, making the clump bigger and bigger. Normally, vetiver grass has a short culm with unclear joints and pedicels. Aerial branching and culm raising slightly above the ground is not commonly found in fertile conditions, but with vetiver tillers grown in bags, in plots with old tillers or in critical conditions.

Leaf

Vetiver leaves will sprout from the bottom of the clump. Each blade is narrow, long and coarse. The edge of the blade is parallel and the apex is acute. Particularly on old leaves, the edge and midrib are spinulose. Normally, the base and the middle of the blade have few spines whereas the apex has numerous spines. All spines are pointing diagonally towards the apex. The ligule at the base of the leaf is observable in a shrinkingly bending form with short silky hair which sometimes cannot be noticed.

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Roots

Vetiver roots are important and the most useful part. Most grass have fibrous roots which spread out from the underground part of the culm and hold the soil in an horizontal pattern. The roots that penetrate vertically into the soil are not deep. In contrast, the root system of vetiver grass does not expand horizontally but

penetrates vertically deep into the soil, whether it be the main roots, secondary roots or fibrous roots.

Inflorescence and Spikelets

Vetiver inflorescence is erect and it appears in the form of a panicle. The panicle and the stalk which is round and long are about 100-150 cm high above the ground. However, for a mature culm, the stalk can be as high as 200 cm. The inflorescence or the panicle alone is about 20-40 cm high and can spread out at a maximum width of 10-15 cm. The sessile spikelet is at the middle, whereas, the pedicelled one is at the tip. Each spikelet is similar in appearance to a spindle. The edge is parallel and oval. The cuneate apex is 1.5-2.5 mm wide and 2.5-3.5 mm long. The surface of the back of the spikelet is rough and consists of minute spines, especially at the edge which can be clearly seen. The lower part of the spikelet is smooth.

Seed and Seedling

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After breeding, the sessile spikelet which is a hermaphrodite flower produces seeds. Each seed is light brown and in spindle shape. The surface is smooth and the apex and base is round. The inner texture is like sticky flour which turns stiff when exposed to strong wind, concentrated sunlight or other critical climatic conditions. Stiffness disables the seed to enlarge, thus impairing its chance to germinate. Since

the seed can germinate only within a limited period of time and some ecotypes which are imported do not seed at all, the vetiver grass cannot spread like a serious weed.

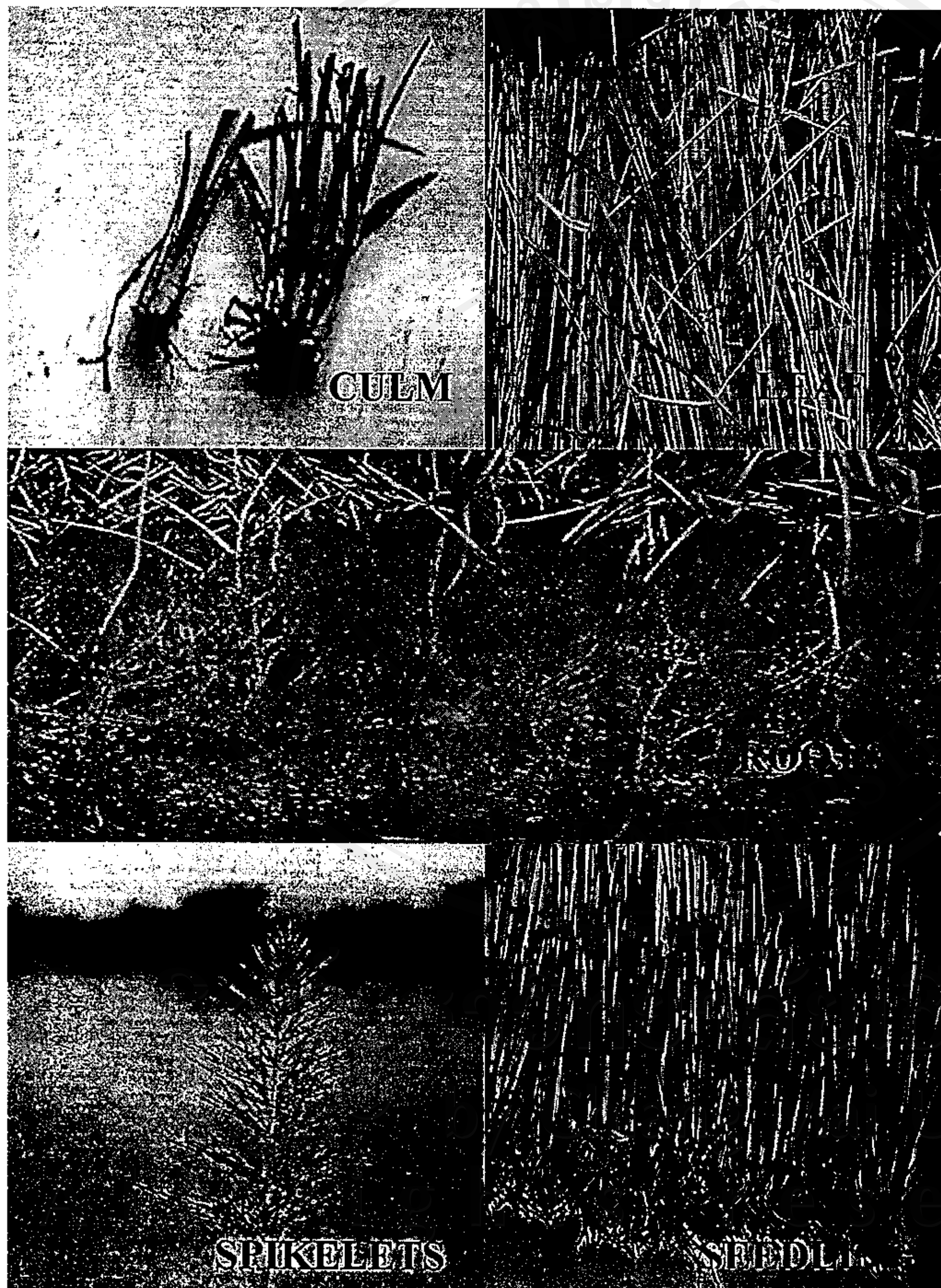

Fig 1.2 Morphology of vetiver grass

1.1.2 Vetiver Ecotypes[3]

According to the results from a systematic plant taxonomy study being conducted on Vetiveria in Thailand, the ecotypes which are commonly found are *Vetiveria zizanioides* and *Vetiveria nemoralis*. Both species naturally grow in a wide range of areas from lowlands to highlands, from the altitude close to mean sea level to as high as 800 m above mean sea level.

1.1.2.1 Differences Between *Vetiveria Zizanioides* and *Vetiveria Nemoralis*

- ***Vetiveria zizanioides***

Vetiveria zizanioides is a kind of plant that can suitably and rapidly adapt to the environment. Most imported ecotypes include those from India, Sri Lanka and Indonesia. They are selected ecotypes and planted under conditions which are well taken care of and different from leaving them to grow naturally. For example, their leaves are regularly trimmed to accelerate growth of roots and tillering as well as to prevent budding of inflorescence, which hampers their reproduction and mutation. Therefore, the original characteristics are all well preserved. *Vetiveria zizanioides* which is commonly found in nature grows in various conditions and adapts suitably to those conditions. The grass produces a number of inflorescences and performs cross pollination every year. Cross pollination makes the plant stronger in many ways, especially in terms of genetic components as well as tolerance to diseases and to critical climatic factors of the areas. However, cross pollination can induce mutation, particularly with those in which the roots are extracted for volatile oils. Mutation

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would result in a reduction or fluctuation of the quantity of volatile substances contained in the roots.

- ***Vetiveria nemoralis***

Vetiveria nemoralis or local vetiveria has a limited scope of distribution. It is found only in Southeast Asia covering the countries of Thailand, Laos, Cambodia, Vietnam and Malaysia. Moreover, there are no records of its uses. *Vetiveria nemoralis* is commonly found in dry areas or in soil conditions with good water drainage potential in every region of Thailand, especially in dipterocarp forests. However, there are only a few in the South. This species grows well in areas both with strong and moderate sunlight. The tip of the clump bends over the ground like lemongrass; it is not erect like that of *Vetiveria zizanioides*. In some areas, the grass grows densely in the same manner as a ground cover plant which claws over a vast area, for example in the area of Huai Kha Kang Wildlife Reserve in Uthai Thani Province. *Vetiveria nemoralis* that grows in dipterocarp forests is always affected by forest fires. Although the dry leaves serve as good fuel, the bottom of the clump is very dense, thus preventing the grass from being easily damaged by fires.

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Table 1.1 Comparing the Differences between *Vetiveria Zizanioides* and *Vetiveria Nemoralis*.

Vetiveria zizanioides	*Vetiveria nemoralis*
Origin	**Origin**
- In the central part of the Asia Continent, presumably India - Generally planted for multiplication	- Southeast Asia: Thailand, Laos, Cambodia and Vietnam - Wildly multiplied in natural conditions, and not cultivated for multiplication purpose
General Morphology	**General Morphology**
- Clumpy with long, erect leaves - 150-200 cm high - Capable of rattooning and aerial branching	- Tufted with leaves bending down like lemongrass - 100-150 cm high - Normally incapable of rattooning and aerial branching
Leaf	**Leaf**
- 45-100 cm long and 0.6-1.2 cm broad - Dark green, curved upper surface, white lower surface with a septum, texture clearly seen when held against sunlight	- 35-80 cm long and 0.4-0.8 cm broad - Pale green, upper surface flapped with a triangular ridge, lower surface paler than upper surface, septum not clearly seen when held against sunlight

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- Smooth texture, with wax coating giving soft and waxy appearance	- Coarse texture, with little wax coating, unwaxy appearance
Inflorescence and Spikelet	**Inflorescence and Spikelet**
- 150-250 cm long inflorescence	- 100-150 cm long
- Mostly purple color	- Color varies from creamy white to purple
- Most florets without awn	- Florets with awn
Seed	**Seed**
- Relatively larger	- Relatively smaller
Roots	**Roots**
- Have mild fragrance with volatile oils of 1.4 - 1.6% dry weight	- Have no fragrance
- Can anchor as deep as 100-300 cm	- Shorter roots, can anchor as deep as 80-100 cm
Uses	**Uses**
- Roots are used to extract volatile oils to make perfume, soaps, and other products like handbags, fan, clothes hanging, and also used as herbal medicine and closet insect repellents	- In Thailand, leaves are used for roof thatching, but not popular

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Table 1.2 Samples of 28 Ecotypes in Thailand (According to the List of the Land Development Department).

Vetiveria Zizanioides	*Vetiveria Nemoralis*
1. Kamphaeng Phet 2	1. Udon Thani 1
2. Chiang Rai	2. Udon Thani 2
3. Songkhla 1	3. Nakhon Phanom 1
4. Songkhla 2	4. Nakhon Phanom 2
5. Sonakhla 3	5. Roi Et
6. Surat Thani	6. Chaiyaphum
7. Trang 1	7. Loei
8. Trang 2	8. Saraburi 1
9. Sri Lanka	9. Suraburi 2
10. Chiangmai	10. Huai Kha Khaeng
11. Mae Hong Son	11. Kanchanaburi
	12. Nakhonsawan
	13. Prachuap Khirikhan
	14. Ratchaburi
	15. Chanthaburi
	16. Phisanulok
	17. Kamphaengphet

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1.1.2.2 Other Ecotypes

Collection of vetiver ecotypes is carried out not only by the Land Development Department but also by other agencies such as the Highland Soil Development Office which operates the Highland Agricultural Project and Department of Public Welfare. The hilltribe people, especially the Karen tribe, have long been cultivating and utilizing Mae La Noi and Pang Mapha ecotypes which belong to Vetiveria zizanioides species for making herbal products. These ecotypes have large culms and a tall clump similar to Mae Hong Son ecotype which has been collected from Khun Yuam District that lies at an altitude of about 1,479 m above mean sea level. At the Doi Tung Development Project, vetiver ecotypes were collected from various locations such as Pimai District of Ayutthaya Province and Surat Thani Province. Although this Surat Thani ecotype grown in the project area shares the same name as the ecotype collected from Phra Saeng District of Surat Thani Province by the Department of Land Development, it was imported originally from Indonesia and obtained from the Rubber Research Center in Surat Thani. The study conducted at the Project focused on the use of the vetiver roots in making herbs and extracting volatile oils. For experimentation in highland areas which are under the cool climate condition with cloudy sky in the rainy season and smog in the dry season such as in Pang Tong area, it was found that the indigenous ecotypes of Khun Yuam District and Mae Hong Son Province demonstrated a favorable growth and a resistance to the rotten base and leaf blast diseases.

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1.2 Literature Reviews

Vetiver is more and more interesting because of its roots utilization, for example, it offers an inexpensive effective and eco-friendly tool to prevent soil erosion. The roots hold the soil together and retain water as well as filter and absorb plant nutrients and the hazardous chemical substances which resultingly help reduce pollution in the environment. Due to effective function of vetiver roots which has been extensively interested by most researchers to perform the studies as followed:

In 1957, Triebs *et al.*[4] isolated two primary alcohols, bicyclovetivenol and tricyclovetivenol, which have been determined using classical chemical methods and infra-red spectroscopy. The obtained alcohols are 6-hydroxymethyl-2-methyl-9-isopropenyl-(0:3:5)-bicyclodecane and 2-hydroxymethyl-9-isopropenyl-3:6-endomethylene-(0:3:5)-bicyclo-decane.

In 1962, the North Indian vetiver variety has yielded a new laevorotatory crytalline sesquiterpene alcohol named as laevojunenol which was dicovered by Shaligram *et al.*[5] It is the optical antipode of the previously isolated dextrorotatory alcohol, junenol. The absolute configuration of these two alcohols which belong to eudesman group of compounds has been determined on the basis of synthesis of dihydrojunenol from santanolide. At the same time, Kalsi *et al.*[6,7] separated two new sesquiterpenes from vetiver oil (*Vetiveria zizanioides* Linn.) known as isobisabolene and khusol ($C_{15}H_{24}O$). Isobisabolene was shown to have the structure on the basis of dehydrogenation, ozonolysis and the formation of bisabolane on hydrogenation. But khusol which was a crystalline primary sesquiterpene alcohol belongs to the antipodal

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group of cadinenic alcohols. A new sesquiterpene hydrocarbon, (-)-γ_2-cadinene, was discovered by Kartha *et al.*[8] It was isolated from Indian vetiver oil on the basis of formation of (+)-cadinene dihydrochloride and other reactions. This report indicated that (-)-γ_2-cadinene belongs to the unusual antipodal group of cadinenes. Besides, khusinol, a new crystalline secondary sesquiterpene alcohol, insulated from North Indian vetiver oil (*Vetiveria zizanioides* Linn.) was shown to have the absolute configuration represented on its conversion to (-)-γ-cadinene under mild conditions. This work was studied by Rao *et al.*[9]

In 1964, Trivedi *et al.*[10] showed that khusitone, a new laevorotatory ketone containing only 14 carbon atoms, was yielded from North Indian vetiver oil (*Vetiveria zizanioides* Linn.). At that time, Kalsi *et al.*[11] also reported a new laevorotatory aldehyde, khusilal ($C_{14}H_{18}O$), which was assigned on the basis of UV, IR and NMR spectra as well as chemical evidences.

In 1966, Seshadri *et al.*[12] insulated khusinoloxide ($C_{15}H_{24}O_2$), a new laevorotary epoxy alcohol, from North Indian vetiver oil (*Vetiveria zizanioides* Linn.). Its structure was assigned on the basis of IR and NMR spectra.

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In 1968, John *et al.*[13] presented zizanoic acid which was identified as a constituent of vetiver oil from Zambia. In the same year, Nigam *et al.*[14] isolated three novel sesquiterpenes including khusinic acid, isokhusinic acid, and khusenol from Angola vetiver oil while some new sesquiterpenic carboxylic acids were reported by Naoki *et al.*[15]. Among these acids, zizanoic acid was reported as the major

constituent. Besides, two minor acids namely isovalencenic acid and epizizanoic acid were also assigned.

In 1969, Fusao *et al.*[16] showed up the structures of additional two minor acids namely cyclopacamphenic acid and epicyclocamphenic acid. It was found that both components consisted of carboxyl groups.

In 1970, MacSweeney *et al.*[17] studied the vetiver sesquiterpenes. It was found that the co-occurrence of the tricyclic vetiver sesquiterpenes, tricyclovetivene, tricyclovetivenol and zizanoic acid with α-vetivone and β-vetivone leaded to postulate a close biogenetic relationship between these widely differing structural types. Akiko *et al.*[18] had also studied on the fraction of sesquiterpene alcohol of the oil and isolated four new alcohols namely vetiselinenol, zizanol, cyclopacamphenol and epicyclopacamphenol. The study was further conducted by Niels *et al.*[19,20] using small-scale dehydrogenation coupled with GLC analysis which showed the composition of the sesquiterpene portions of vetiver oil known as nootkatensis. The study of the oil of vetiver samples of Haiti, Reunion, and North India origin using GLC. The result showed that the vetiver oil from North India origin (khus oil) was distinctively different and it represented a chemical distinct race of *Vetiveria zizanioides* or perhaps a distinct species.

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In 1972, Kaiser *et al.*[21] found (-)-10-epi-γ-eudesmol in the essential oil of *Vetiveria zizanioides.* This compound was assumed to be a precusor of nootkatone-, α- and β-vetivane derivatives occuring in the oil. At that time, new compounds were

also isolated such as β-bisabolol, two new acoradienes, and seven new cedrane derivatives.

In 1973, Hanayama *et al.*[22] isolated two sesquiterpene carboxylic acids, zizanoic and apizizanoic acids, from the essentail oil of vetiver and assigned the structures on the basis of chemical degradation. This work revealed that these sesquiterpenoids also belong to the zizane group.

In 1976, Peter *et al.*[23] studied the structure of three new tricyclic sesquiterpenes from *Eremophila georgei* which were shown to be antipodal to that of the zizane sesquiterpenes of vetiver oil.

In 1982, Takayuki *et al.*[24] identified several rare phenols including 4-vinylphenol, 4-vinyl-2-methoxyphenol and trans-isoeugenol in oil of vetiver. In addition, two new insect-repelling aldehydes; zizanal and epizizanal were isolated from Javanese vetiver oil (*Vetiveria zizanioides*) by Subhash *et al.*[25].

From the alcoholic fraction of North Indian vetiver oil (*Vetiveria zizanioides*), Kalsi *et al.*[26] isolated C_{14} terpenoid namely, norkhusinoloxide. Stereostructure was assigned on the basis of the chemical correlation coupled with its spectral data.

In 1987, a new antipodal sesquiterpene diol (vetidiol) was isolated from North Indian vetiver oil (*Vetiveria zizanioides*) by Kalsi *et al.*[27] The prediction as

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well as the stereostructure of the diol was confirmed by its spectral data and the chemical correlation with khusinol, a known absolute configuration sesquiterpene diol.

In 1988, Kazutoshi *et al.*[28] synthesized (-)-khusimone, the minor essential component of vetiver oil, with insect repellent activity starting from (S)-6,6-dimethyl-5-methoxy-carbonylmethyl-2-cyclohexen-1-one. Lewis acid-catalyzted Diel-Alder reaction was employed to obtain the desired carbon skeleton regio- and stereoselectively. The overall yield of all steps was 6.9%.

In 1991, Sellier *et al.*[29] studied Chinese vetiver oil that showed the 118 characterized compounds. According to their chromatographic (GC) and mass spectral (MS) data, twenty of these compounds were completely indentified.

In 1993, Chen *et al.*[30] isolated acoradiene from vetiver oil which is one kind of spirocyclic sesquiterpenes containing the spiro[4.5]decane nucleus. Moreover, (±)-acoradiene was synthesized via free radical cyclization for the construction of the spiro[4.5]decane nucleus.

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In 1997, Stephanie *et al.*[31] reported the (-)-δ-amorphene as a component of vetiver oil. The enantiomer of this sesquiterpene was prepared by rearrangement of an enantiomeric mixture of germacene D isolated from *Solidago canadensis*.

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In 2001, Zhu *et al.*[32] examined the behavior of Formosan subterranean termites toward one of the components of vetiver grass oil. The results showed that sesquiterpene ketone, nootkatone, which was isolated from vetiver oil having strong repellent and toxicant activity toward Formosan subterranean termites. The lowest effective tested concentration was 10 μg/g substrate.

In 2002, Bowles *et al.*[33] studied the essential oil of vetiver which was blended into an aqueous cream (content of essential oil = 3.5 mL/100g aqueous cream) and 5g was gently massaged five times a day onto the bodies and limbs of 56 aged care facility residents (age range 70-92 years) with moderate to cure dementia.

From previous different studies above, it was found that there were 2 types of vetiver grass which were studied, *Vetiveria zizanioides* and *Vetiveria nemoralis*. From reviewed reports most of researchers emphasized on the structural elucidation and identification of components of vetiver oil which were extracted from vetiver roots. These studies involved the use of chemical techniques such as infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopic (MS) techniques. Most of the identified compounds in vetiver root were in a group of sesquiterpenes (carbon 15 atoms) and their derivatives. Some components were also partialy synthesized. However, there was not any relevant reports concerning the examination of components which play a role in the scent of vetiver roots (*vetiveria zizanioides* Nash). Therefore, this present research was interested in qualitatively study on the aroma compounds in scented vetiver roots using chromatographic and spectroscopic

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techniques to separate and identify volatile components of the scented vetiver extract. By means of sensory evaluation, the aroma component was identified and isolated.

1.3 Introduction of Chromatography[34]

The separation and purification of plant constituents is mainly carried out using one or other, or a combination of chromatographic techniques such as paper chromatography (PC), thin layer chromatography (TLC) and gas liquid chromatography (GLC). The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated.

Chromatography is a separation method based on differences in equilibrium constants for the components of the mixture placed in diphasic system. A chromatographic system is one in which a fluid mobile phase percolates through a stationary phase. The stationary phase is often a bed of nonconsoildated particles, but this is not essential. All that the system required is two phases in relative motion and excellent contact between these phases so that concentrations in the stationary phase are always very near their equilibrium values. The mobile phase is responsible for transporting the sample components through the stationary phase. The velocity of each component, hence its residence time, depends on the both mobile phase velocity and the distribution equilibrium constant for that component.

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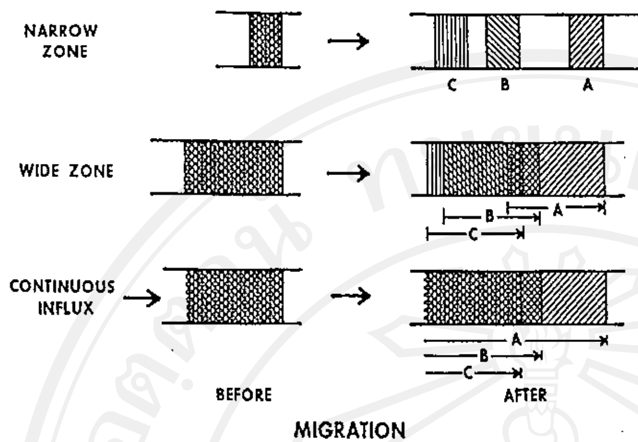

Fig.1.3 Differential migration of three components of a mixture. When the sample zone is narrow (top), its components are completely separated, but there is overlap when it is wide (middle). Continuous influx of sample (bottom) can yield only a fraction of the fastest migrant (A) in pure form.

Fig. 1.3 is a schematic representation of the spatial separation of a sample mixture of 3 components (A, B, and C), initially confined to a rectangular zone, by migration to the right. If the migration distance is large compared to the width of the original sample zone (top), the fastest-moving component (A) will be completely separated from the slower-moving component (B), which also separates from the slowest component (C). However, if the initial sample zone is wide in comparison with the migration distance (middle), only a portion of the fastest and slowest components (A and C), respectively, will be separated from the mixtures, and B will not be isolated at all. Similarly, if a larger amount of sample mixtures allowed to flow into the system continuously from the left (bottom), only a small portion of A can be

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obtained in pure form, and neither B nor C can be isolated. This illustrates three types of differential migration methods. For isolation purposes, migration from narrow zones (top of **Fig. 1.3**) is the method of choice, but for analytical purposes, migration from wide zones (middle of **Fig. 1.3**; frontal analysis) or even from a continuous stream (bottom of **Fig. 1.3**; break-through analysis) may be acceptable.

In this experiment, chromatographic techniques including thin layer chromatography (TLC), classical liquid chromatography (CC) and gas chromatography (GC) were used for study the aroma components in scented vetiver roots. These techniques are explained as followed.

1.3.1 THIN LAYER CHROMATOGRAPHY[35]

In thin layer chromatography (TLC) the separation process and the mechanism of retention is exactly analogous to those involved in LC except that the stationary phase is in the form of a laminate sheet, whereas in LC it is in the form of a cylindrical packed bed. Furthermore, in TLC the flow of mobile phase is driven by surface tension forces and not by fluid pressure. As in LC, the vast majority of TLC separations are carried out employing silica, or silica based material as the stationary phase. However, there are a significant number of alternative materials that have been employed for special separations but few of them have achieved common use. Silica gel is one alternative for use as stationary phases that will be briefly discussed.

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- **Silica Gel**

Silica gel is prepared by the precipitation of a silicate solution with acid, or by hydrolysis of silicon derivatives. The surface area and diameter of the silica gel particles depend on the method of precipitation. Variations in pH during precipitation can produce silica gels with surface areas ranging from 200-800 m^2/g. It has been shown that the silica gel provides three types of surface hydroxyl group: bound, reactive and free. Relative reactivity and adsorption follow the order bound > free > reactive. Thus, control of the distribution of surface function can have a significant effect on the chromatographic properties of silica. Special methods have been developed for preparing spherical particles with specific pore characteristics. Thermal treatment can also effect the pore size. Many of the silicas currently in use have a pore size of 6 nm.

A number of distributors and manufactures supply precoated TLC plates, but not all carry a complete selection of sorbent/backing-plate combinations in the four major sizes (5x20, 10x20, 20x20, 20x40 cm). Some commercial 20x20 cm plates have prescored lines on the back at 5, 10, and 15 cm so they can easily be broken into smaller plates. There is little literature available comparing plate-layer characteristics. To the benefit of the method, only two or three major manufactures produce each sorbent type, so diversity with respect to a given sorbent is kept to a minimum. However, different manufactures of the plates themselves employ different coating techniques, which in turn has an effect on the characteristics of sorbent that has been coated on the plates. The recently introduced hydrophilic modified precoated plates have broadened the spectrum of selectivity available for TLC. These plates are

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referred to as "HPTLC-NH_2 precoated plates," in which γ-aminopropyl groups are bonded to the surface of silica gel skeleton, and "HPTLC-CN precoated plates," with γ-cyanopropyl moieties as the functional groups. In term of polarity the two hydrophilic modifications can be classified as follows: Normal SiO_2 > NH_2 > CN > RP. Since the surface NH_2 and CN groups are of medium polarity, no wetting problems are encountered with the corresponding plates even at the maximum level of modification.

- **Stationary Phase Selection**

The stationary phase selection procedure is represented in **Table 1.3**. If the solutes are ionic in nature, or can be made so by buffering the solution to a particular pH, then an ion exchange media might be chosen for the stationary phase. It is seen that there are three to chose from. Alternatively the substances present in the mixture may have different polarities, in which case a polar stationary phase might be appropriate and it can be seen that there are basically three to chose from.

Table 1.3 Stationary Phase Selection.

Dominant Interactions that Control Retention		
IONIC	POLAR	DISPERSIVE
Ion Exchange Resin Ion Exchange Cellulose Ion Exchange Bonded Phase	Silica Gel Alumina Other Inorganic adsorbents Polymer Beads	Polymer Beads Reversed Phase

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However, this choice is much oversimplified. If the solutes are strongly polar (e.g. a group of peptides or perhaps proteins), despite being strongly polar, as a result of their complexity and the abundance of polar groups present in each molecule, they all might exhibit approximately the same polarity. As a result they would not be necessarily separated on a polar stationary phase. In fact they may differ to a far greater extent in their dispersive characteristics and therefore might be better separated on a dispersive stationary phase. For simple polar molecules however, a polar stationary phase would be a logical choice. Finally, if the solutes differ largely in their dispersive interactive capability, then a dispersive stationary phase will probably be appropriate. The general type of stationary phase can thus be estimated but there remains a choice to be made within the groups of ionic, polar and dispersive stationary phases themselves.

1.3.2 Classical Liquid Chromatography[36,37]

The techniques of liquid chromatography which are now considered to be classical played an important role in separation of natural products. The extensive use of liquid column chromatography in the past was evidently the result not only of the extension of the applicability of separation, analytical and preparative methods but also of the simplicity and cheapness of chromatographic equipment.

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- **Columns**

In classical liquid chromatography, glass tubes (polyacrylate tubes are also commercially available) are almost always used in the preparation of columns. Their dimensions are dictated primarily by the amount of the sample: laboratory columns

usually have a diameter of 2-70 mm and a length of 15-150 cm. The amount of sorbent is necessary given by its bulk density. Various materials differ to such an extent in their bulk densities and swelling properties that no universal rule for the weight of sorbent to be used for a given tube volume can be given. The ratio of the column diameter to its length cannot be recommended generally. As the homogeneity of the bed is of prime importance for efficiency, a rather wide range of ratios of diameter to length may be tolerated. The efficiency of the separation and the complexity of the separated mixture should be taken into account in order to have a sufficiently, but not excessively, long column: the elution of a too strongly sorbed component could prolong the experiment too much and the separation effect would deteriorate. Most workers choose a ratio of diameter to length between 1:10 and 1:100.

- **Column preparation**

The preparation of the columns is a fundamental operation. All theoretical consideration are valid only for a correctly packed column; only on a perfectly packed column can a good separation be achieved. In this respect no procedure, however perfect, can replace experimental skill. The technique itself may differ for various types of sorbents and for high efficiency liquid chromatography. The main requirement is that the column should be packed regularly so that it does not contain either air bubbles or channels through which an excessively large part of the mobile phase could flow ineffectively. The column should be fastened in a vertical position and the bottom of the column carefully scaled with a porous layer of wool or a similar material. The column is filled to one third of its height with the mobile phase, and a

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thick suspension of suitably adjusted, degassed sorbent in the mobile phase is poured into it. It is recommended that the sorbent suspension should be poured into the column immediately in order to prevent stratification, i.e., classification directly in the column according to a particle size. A certain time is necessary before the material becomes settled and a compact bed is formed (for a column 1m high, approximately 5 hrs. are required). The column is then stabilized by washing it with the mobile phase. Filling the column with the sorbent poured into the solvent is limited to liquid-solid or liquid-liquid chromatography. When the column is packed in a wet state, the tube is first filled with a larger amount of a mobile phase (up to 70-90% of the column length) and the outflow is regulated with the stopcock. The prepared classified sorbent is poured into the tube through a funnel, which should not touch the level of the mobile phase.

- **Sample application**

The sample should be applied to the top of the column as evenly as possible, in as concentrated a solution of eluting solvent as possible, avoiding disturbance of the column packing. The top of the column can be protected with a thin layer of sand, glass wool, filter paper or ballotini beads. When all of the sample has been adsorbed, the void can be filled with the solvent and the chromatogram developed. The supply of solvent can be replenished as required.

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- **Elution procedures**

There are three principal elution procedures: isocratic, fractional and gradient elution.

Isocratic. By isocratic is meant the operation of the chromatographic column, by allowing a solvent mixture of unvarying composition to run through the column until separation is complete.

Fractional elution. If only one solvent is used, ready elution of only some of the components of the original mixture from the column may result. To remove those which are more firmly held, a stronger eluting agent will be required. Sometimes it may be necessary to use several different solvents of gradually increasing strength for the successive desorption of different components. This is known as stepwise elution.

Gradient elution. The technique of gradient elution involves the use of a continuously changing eluting medium. The effect of this gradient is to elute successively the more strongly adsorbed substances and at the same time to reduce tailing. This means that the chromatographic bands will tend to be more concentrated and thus occupy less of the column. This desirable effect may be ascribed to the 'straightening' of the isotherms by the concentration gradient.

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1.3.3 Gas Chromatography[38]

Gas chromatography (GC) is one of the most important and definitely the most economic of all separation methods. Its applicability ranges from the analysis of permanent gases and natural gas to heavy petroleum products, oilgosaccharides, lipids, etc. Moreover, as far as chromatographic efficiency and GC system selectivity is concerned, no other separation technique can complete with gas chromatography. The dictum "if the separation problem can be solved by gas chromatography, no other technique has to be tried out" is now generally accepted. A schematic drawing of a modern gas chromatographic system is shown in **Fig 1.4**.

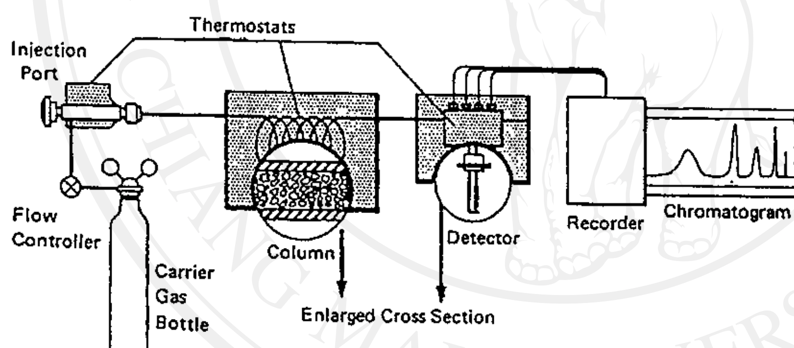

Fig. 1.4 Schematic of gas chromatograph.

Gas chromatography naturally devices itself into two distinct classes: (1) analytical – in which the purpose is to obtain qualitative information and (2) preparative – in which the goal is the collection and recovery of purified fractions for further identification, chemical synthesis, reference materials, or production of chemicals. The instrument of gas chromatograph will be briefly discussed.

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1.3.3.1 Sample inlet systems

Ideally a sample should be introduced onto the few plate of the column. However, capillary columns have a very low gas volume, in the order of 10 μL per plate, which mean that equally small liquid samples are needed otherwise the column would be saturated and column efficiency decreased. Specially designed glass-lined split/splitless injectors are used where the proportion of sample introduced on to the column can be varied from splitless mode where all the sample goes onto the column to split mode and where a predetermined ratio, e.g. 1:200 is set so that 1/200 of the gaseous volume enters the column, the rest being vented through the gas control valves. Splitter and splitless injector are the most popular to use as injector in GC technique which will be briefly discussed.

- **Splitter Injector**

Injector of samples into open tubular columns is a somewhat more demanding process than injection onto packed columns. The sample capacity of open tubular columns is much lower than that of packed columns. This requires much smaller injection volume, since sample volumes will rarely be larger than 2 μL, and usually closer to 0.5 μL. To handle this situation, the splitter was developed. This injector consists of a heated liner (like the flash vaporizer) where the sample is introduced by syringe and vaporized. A small plug of glass wool or chromatographic support is located downstream from this area in the split liner, to facilitate mixing of the vaporized sample with the carrier. The flow of the mixture is then split into two streams, one entering the capillary column and the other vented through a back pressure regulator. This method of decreasing the column flow is done without

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affecting the column pressure, and ensures that column overload does not occur. Sample transfer to the column is fast, resulting in little band broadening due to the injector volume. The schematic diagram of a splitter injection is shown in **Fig. 1.5**.

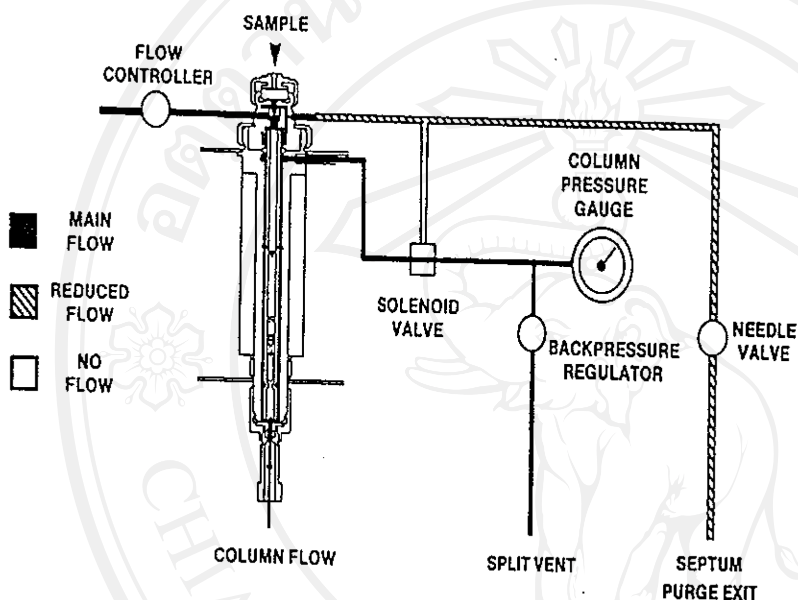

Fig. 1.5 Schematic diagram of a splitter injection for capillary columns.

- **Splitless Injector**

An alternative injector for open tubular columns is the splitless injector which is similar enough in external construction to the splitter that a single injection port can be modified to provide both split and splitless modes. As with the splitless, small (0.5 to 1 μL) volumes of liquid are injected, making it attractive for trace analysis. This injector uses a straight glass or quartz liner in place of the split liner, and a solenoid valve diverts the purge flow during injection so all carrier and sample present in the liner is deposited into the column. The injection process is therefore much slower

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(the rapid vaporization step required for the other injectors is not needed) than that used in split mode, and is usually done at lower temperatures. This makes the splitless injector attractive for the injection of thermally labile samples, those samples with components which elute near the tail of the solvent front, and samples that are very dilute. The lower temperature is used to refocus the sample volume, which is then deposited as a whole into the column. An alternative to this thermal refocusing is to make use of the so-called "solvent effect" in which the solvent plug is used as a secondary stationary phase to retard and reform the solute components at the head of the column. The schematic diagram of a splitless injection is shown in **Fig. 1.6.**

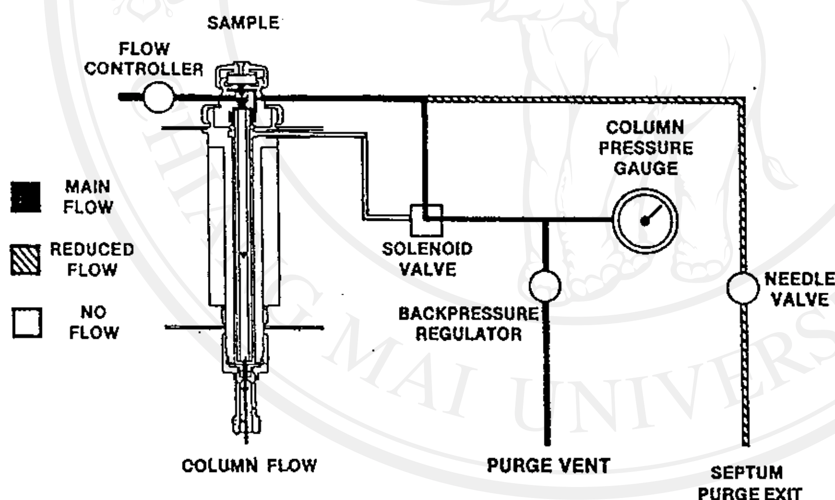

Fig. 1.6 Schematic diagram of a splitless injection for capillary columns.

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1.3.3.2 Column oven

The column is perhaps the most important feature of a GC system. It contains the stationary phase and thus effects the separation of components in a mixture. The column may be made of glass or metal and typically 2-6 mm i.d. and 1-

3 m in length when packed with the stationary phase material or 0.2-0.7 mm i.d. and 10-100 m long if in the form of a capillary column. Columns are formed into a coil of between 4 and 8 inch (10-20 cm) in diameter and specially designed end fittings are used to connect the columns to the injector and detector with minimum dead volumes. Aluminium or copper tubing can be used for the column, however, an active oxide film may form on the inner surface, which can initiate catalytic reactions in some sensitive components and therefore generally avoided. Stainless –steel or glass tubing is therefore used for the most columns. Capillary or open tubular columns consist of long narrow tubing coated on the inner surface with a 0.2-5.0 μm film of stationary phase. Columns are usually stainless steel or glass for packed columns and silica for capillary columns.

1.3.3.3 Temperature-Programmed Analysis

In temperature-programmed analysis, a controlled change of the column temperature occurs as a function of time. The initial temperature, heating rate, and terminal temperature must be adjusted to the particular separation problem. The initial temperature is chosen so that the low boiling compounds are optimally separated. Selection of the program rate depends on the nature of the solutes and the complexity of the sample. The final temperature is adjusted to give reasonable total analysis times. Temperature programming has been applied since the beginning of gas chromatography (1960), but only since the end of the 1980s have the possibilities been fully exploited. This might be, in part, because of the time-consuming nature of temperature optimization by trial and error, but above all because of ignorance of the importance of the temperature programming rate to selectivity. This is especially so

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for polar columns which is much more pronounced than on nonpolar columns. Today, software is available to optimize a temperature program for a particular separation on the basic of only two experimental programmed runs.

1.3.3.4 Detectors

The purpose of a detector is to monitor the carrier gas as it emerges from the column and generate a signal in response to variations in its composition due to eluted components. The following detectors will be discussed: thermal conductivity detector (TCD) and flame ionization detector (FID).

- **Thermal Conductivity Detector**

The first detection device we will consider is the thermal conductivity detector (TCD) shown in **Fig. 1.7**, which is based on very old measurement technology. This detector measures the change in a bulk transport property of the gas stream, and is therefore universal in response. There are two basic TCD varieties available, hot wire based and thermistor based detectors.

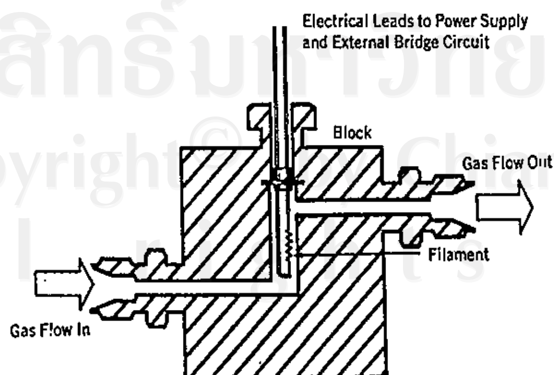

Fig. 1.7 Schematic diagram of the essentials of a thermal conductivity detector.

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The most commonly used TCD is the hot wire version or katharometer along with the relevant Wheatstone bridge circuit. The carrier gas is passed over short sections of coiled wire made from gold or rubidium sheated tungsten. Two cell compartments contain wires bathed by the sample stream, while an additional two compartments accept the flow from a reference column. The wires in these four compartments from the legs of a Wheatstone bridge circuit, which the chromatographic integrator or recorder in place of the electrometer. The wire is electronically heated to between 300 and 450 °C, but the continuous stream of carrier serves to remove much of the heat from the cell. High thermal conductivity gases such as hydrogen and helium are especially efficient heat removers.

The other type of TCD is one based on thermistor elements instead of hot wires. The thermistors are semiconductors in which the resistance decreases sharply with increasing temperature. This type of TCD has a somewhat higher sensitivity, but at a cost of operating temperature range. The thermistors will fail at temperatures much above 150°C, thus the detector cannot be used with high boiling point solutes. The thermistor TCD is, however, very useful for work on gaseous samples. The thermistor elements are much less prone to oxidative damage than the hot wires, but they tend to be sensitive to reducing agents. This usually precludes the use of hydrogen as the carrier gas. Since the thermistors are small, it is often possible to build detectors of much lower internal volume than the hot wire based cells. This allows the use of thermistor detectors with lager bore capillary columns.

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- **Flame Ionization Detector**

The flame ionization detector (FID) is as popular for routine analysis as the TCD and its schematic diagram is shown in **Fig. 1.8**. In fact, it is rare to find a gas chromatograph which is not equipped with both of these detectors. It is relatively simple in construction, and is easy to operate and maintain. The FID may be considered universal in response for organic compounds. A hydrogen-air flame is maintained above the tube which delivers carrier and sample from the column. The flame itself, in the absence of an organic sample species, contains few ions and has a high electrical resistance. When a sample containing carbon-hydrogen bond leaves the column and enters the flame it is burned, and a cascade of ions is produced. This increases the electrical conductivity of the flame by many orders of magnitude. A collector electrode above the flame is maintained at a potential of 180 to 300 V above that of the sample delivery tube. This system of electrodes, in combination with a sensitive electrometer, measures the flame conductivity and produces the response signal.

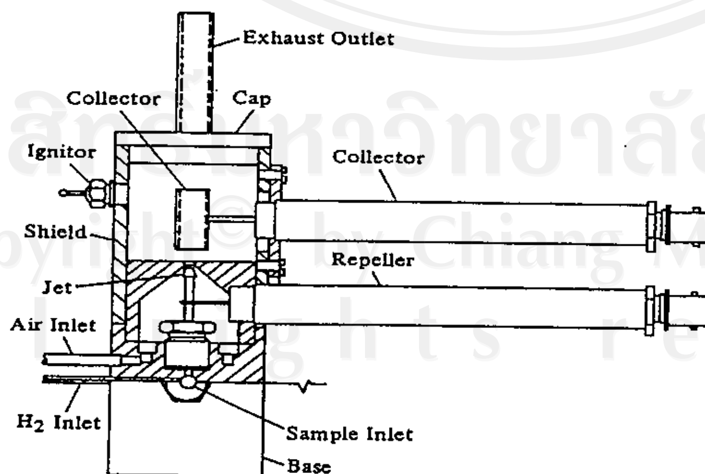

Fig. 1.8 A schematic diagram of a flame ionization detector.

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The FID is one of the most sensitive and fast responding detectors available. It can easily sense the presence of 10^{-11} g of hydrocarbon, and the response will be linear over a wide concentration range. The high sensitivity makes the FID very suitable for use with capillary columns, where very small amounts of material are analyzed. The device has high baseline stability and shows little or no sensitivity to changes in the carrier gas flow rate. It can therefore be used in temperature programmed applications without the viscosity-induced disruptions which plague the TCD. The dead volume of the detector is very small, since the sample is released directly into the flame.

1.4 Gas Chromatography-Mass Spectrometry[39]

Gas Chromatography-Mass Spectrometry (GC-MS) is a combination of two powerful analytical tools which are gas chromatography for the highly efficient gas-phase separation of components in complex mixtures, and mass spectrometry (MS) for the confirmation of identity of these components as well as for the identification of unknowns. Due to a part of gas chromatography that was partial described in previous sections therefore, in this section the details of mass spectrometric technique would be briefly explained.

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The principle of MS is the production of gas-phase ions that are subsequently separated according to their mass-to-charge (m/z) ratio and detected. The resulting mass spectrum is a plot of the relative abundance of the generated ions as a function of the m/z ratio. Extreme selectivity can be obtained, which is of utmost importance in quantitative trace analysis.

1.4.1 Instrumental Aspects of GC-MS

In GC-MS systems, sample introduction is performed from the open capillary chromatographic column, either directly or via an open split coupling. The ionization of the analytes is generally performed by either electron ionization or chemical ionization. After the production of ions, they are separated according to their m/z ratio in the mass analyzer. Although linear quardrupole analyzers are most widely applied, other analyzer types, i.e., magnetic sector and quardrupole ion trap are applied as well. The detection of ions is mostly performed by means of an electron multiplier.

In GC-MS systems, analyte ionization, mass analysis, and ion detection take place in a high-vacuum system. In benchtop GC-MS systems, the vacuum system consists of one pumped chamber, evaluated by means of a small turbomolecular pump, backed by a mechanical pump. Large systems as well as modern research-grade mass spectrometers generally contain two differentially pumped vacuum chambers, separated by means of a baffle containing a slit, i.e., the ion source housing and the analyzer region.

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Efficient means to collect and handle the enormous amounts of data that are generated in the operation of a mass spectrometer, especially in on-line combination with chromatography, are of utmost importance. Highly advanced computer programs are currently available for use in handing, interpretation, and reporting the data.

1.4.2 Analyte Ionization

Various ionization techniques are available for organic mass spectrometry. In this section the most important techniques in relation to GC-MS are following discussed; electron ionization (EI) and chemical ionization (CI).

- **Electron Ionization**

The oldest and most frequently applied ionization technique is electron ionization (EI). In EI, the analyte vapor is subjected to bombardment by energetic electrons (typically 70 eV). Most electrons are elastically scattered, others cause electron excitation of the analyte molecules upon interaction, while a few excitations cause the complete removal of an electron from the molecule. The last type of interaction generates a radical cation, generally donated as $M^{+\bullet}$, and two electrons:

$$M + e^- \rightarrow M^{+\bullet} + 2e^-$$

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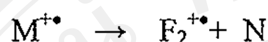
The $M^{+\bullet}$ ion is called the molecular ion. Its m/z ratio corresponds to the molecular mass M_r of the analyte. The ions generated in EI are characterized by a distibution of internal energies, centered around 2 to 6 eV. Typical fragmentation

reactions of a molecule M upon electron ionization result in the formation of an ionized fragment accompanied by the loss of either a radical $R^{\bullet}$ or a neutral N:

$$M^{+\bullet} \rightarrow F_1^{+} + R^{\bullet}$$



$$M^{+\bullet} \rightarrow F_2^{+\bullet} + N$$

$$F_2^{+\bullet} \rightarrow F_3^{+} + R^{\bullet}$$

- **Chemical ionization**

Chemical ionization (CI) is based on gas-phase chemical reactions and can be considered as a versatile ionization technique. Chemical ionization is primarily based on ion-molecule reactions between reagent gas ions and the analyte molecules, e.g., proton transfer (addition), charge exchange, electrophilic addition, and anion abstraction in positive-ion CI (PCI) and proton transfer (abstraction) in negative-ion CI (NCI).

In the most GC-MS applications, CI is performed at ion source pressures between 1 and 100 Pa. Reagent gases are produced by bombardment of the reagent gas, e.g., methane, isobutane or ammonia, by energetic electrons (100 to 400 eV), i.e., by EI, followed by a series of ion-molecule reactions. Some frequently used reagent gases are shown **in Table 1.4**. Upon EI of ammonia in CI source, protonated ammonia NH_4^+ and $(NH_3)NH_4^+$ are the most abundant ions formed. They can react with an analyte molecule M in a proton-transfer reaction, resulting in a product ion with low internal energy. The m/z of this protonated molecule corresponds to $M_r + 1$ and can thus be used to determine the molecular mass of the analyte.

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Table 1.4 Proton Affinity (PA) of Some Frequently Used Reagent Gases for CI.

Reagent gas	PA (kJ/mol)
H_2	422
CH_4	536
H_2O	723
i-C_4H_{10}	823
NH_3	857
Pyridine	921

1.4.3 Mass Analysis

Ions can be separated on the basis of their mass-to-charge ratios using electric or magnetic fields arranged so as to spread them in time or space. Among the many distinct types of mass analyzers we consider two: sector magnetic fields and quadrupole mass filters.

- **Sector Magnetic Instruments**

In a single-focusing sector instrument, the ions mass m and z elementary charges and a particular kinetic energy introduced into a magnetic field B. The kinetic energy of the ions is determined by the voltage V (typically 5 to 8 kV) with which the ions are accelerated toward the source exit slit. When the magnetic force is counterbalanced by the centrifugal force, ions are transmitted to the detector. This leads to the following equation:

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$$m / z = \frac{B^2 r^2 e}{2V}$$

where e is the elementary charge and r is the radius of curvature of the path through the magnetic field.

Upon ionization, ions with a distribution of ion kinetic energies are generated. The resolution of the mass analysis by a sector instrument can be applied by means of an electrostatic analyzer (ESR). In principle, the ESR can be applied in various geometries, e.g., the Nier-Johnson geometry (EB) and the reversed geometry (BE) (**Fig. 1.9**). In most cases, the instrument is designed in such a way that velocity focusing takes place so that ions with one particular m/z value but different kinetic energies are deflected toward one focal point. Instruments with both a magnetic and an electrostatic sector, usually called double-focusing instruments, are capable of high-resolution mass determination with ion transmittance.

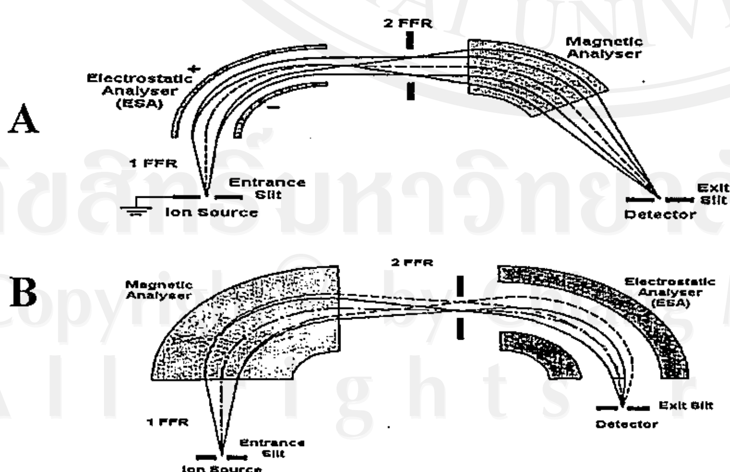

Fig. 1.9 Geometries of double-focusing sector instrument: Nier-Johnson (EB) geometry (A) and reversed geometry (B).

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- **Quadrupole Instruments**

In the quadrupole mass filter (**Fig 1.10**), ions drift through an assembly of four parallel rods. DC voltages of opposite sign are applied to opposite rods. An oscillating radiofrequency (rf) field also applied to opposite rods, successively reinforces and then overwhelms the DC field. An ion moved through the array experiences a force only in the plane normal to the rod length. Provided it has an appropriate m/z ratio, the ion's oscillations will be stable, and it will drift drown the rod assembly and reach the detector. Mass scanning is achieved by sweeping the DC and rf voltages, keeping their ratio and the oscillator frequency constant. Typical operating parameters include rf voltages, V, of several thousand volts, frequencies in the 10^6 Hz range, and DC voltages of several hundred volts.

The ease with which electric fields (as opposed to magnetic fields) can be controlled, and the linear mass scale, are advantages of this instrument. After calibration, ions of any mass-to-charge ratio can be electrically selected. Computer control and data collections are simple, and the instruments are very reliable. The resolution can be adjusted electrically, and operation is possible at unit resolution or in a wide bandpass mode for transmission of a group of ions. High resolution is not available and the mass range is limited. However, the small size, fast scan speed, and relative mechanical simplicity of the device (although the ion motion itself is complex) have made this a very widely used mass analyzer.

A typical time required to record a mass spectrum (m/z 20 to 800) is 1 second. Depending on the experiment, hundreds of scans are made and the data

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captured in separate files. If the data are taken while the sample is being introduced from a chromatograph, the result is a series of mass spectra recorded as the components elute.

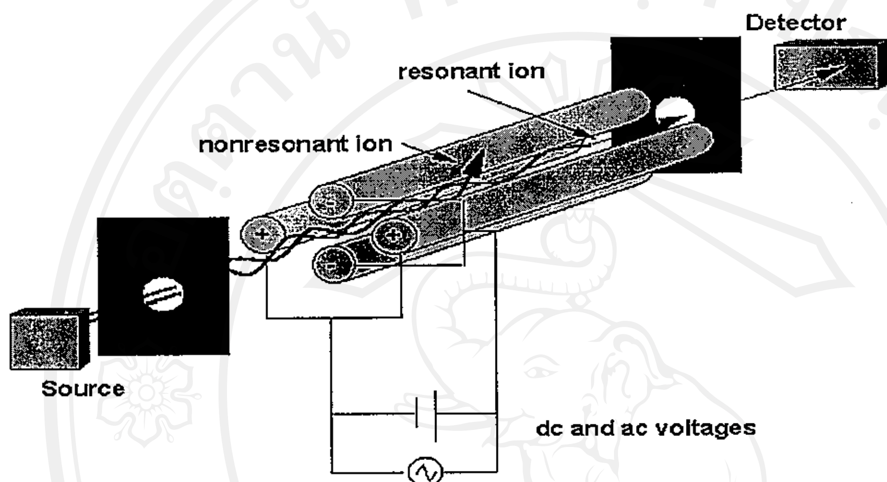

Fig. 1.10 Schematic diagram of linear quadrupole instrument

1.4.4 Gas Chromatography-Mass Spectrometry Interfacing

For use in combination with packed-column GC, a variety of interfaces for GC-MS were developed. The aim of these devices was to achieve analyte enrichment, i.e., a better ratio between analyte and carrier gas.

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With the introduction of capillary columns for GC and GC-MS, an analyte enrichment interface is no longer required as the optimum flow rate of such a column is readily amenable to the vacuum system of a benchtop GC-MS system. At present, two types of GC-MS coupling are applied, i.e., the direct coupling and the open split interface. In the direct coupling, the column effluent of the GC column is directly

introduced into the ion source of the mass spectrometer. While this approach is very simply, it has some disadvantages that are avoided by the use of an open split coupling (**Fig. 1.11**). In a direct-coupled GC-MS, the column outlet is at high vacuum, resulting in changes in the chromatogram similar to those obtained from a GC-FID. As the complete output of the GC column is introduced into the source, the risk of source detuning and contamination is gramming, and sample contaminants. While in direct coupling, the vacuum system must be switched off for changing the GC column; this is not required with the open split coupling.

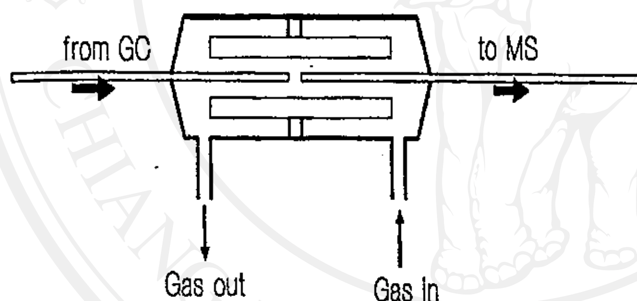

Fig. 1.11 Schematic diagram of the open split coupling for GC-MS.

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1.5 Solid Phase Microextraction and Applications[40]

Solid Phase Microextraction (SPME) utilizes a short, thin, solid rod of fused silica (typically one cm long and 0.11 mm outer diameter), coated with an absorbent polymer. The fiber is the same type of chemically inert fused silica used to make capillary GC columns and it is very stable even at high temperatures. The coated fused silica (SPME fiber) is attached to a metal rod, and both are protected by a metal sheath that covers the fiber when it is not in use. For convenience, this assembly is placed in a fiber holder and, together, the system resembles a modified syringe (**Fig. 1.12**).

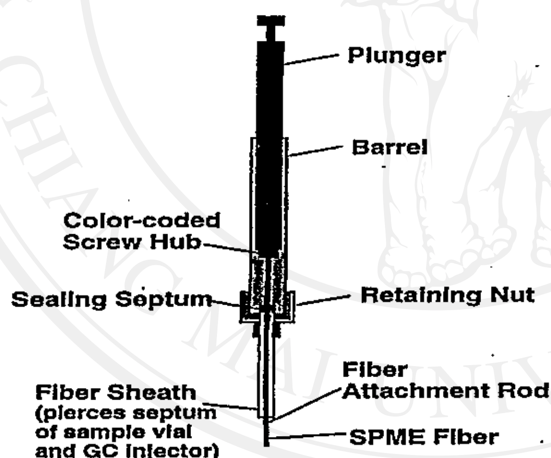

Fig. 1.12 SPME fiber with holder.

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The SPME extraction technique consists of two processes: (1) analytes partition between the sample and the fiber coating, and (2) the concentrated analytes desorb from the coated fiber to an analytical instrument. To perform the extraction, an aqueous sample containing organic analytes or a solid sample containing volatile

organic analytes is placed in the vial, then closed with a cap and the septum. To sample, the SPME protective sheath pierces the septum then the plunger is lowered to either immerse the fiber directly into the aqueous sample or expose it to the sample headspace (**Fig. 1.13**). The target analytes are subsequently extracted from the sample matrix into the fiber coating. After a pre-determined absorption time, the fiber is withdrawn back into the protective sheath, then the sheath is pulled out of the sampling vial. The sheath is immediately inserted in the GC or HPLC injector and the plunger is again lowered to expose the fiber. This time, the fiber is exposed to a high temperature in the injector liner (GC) where the concentrated analytes are thermally desorbed and, consequently refocused onto the GC column.

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Fig. 1.13 SPME headspace sampling.

Different types of sorbents will extract different groups of analytes; therefore, many different fiber coating have been developed. Similar to selecting an analytical GC column where "like dissolves like" a fiber is chosen based on its selectivity for certain target analytes and their volatility ranges. Nonpolar coatings (e.g., poly(dimethylsiloxane)) retain hydrocarbons very well. In contrast, polar fiber coating (e.g., polyacrylate and carbowax) extract polar compounds such as phenols and carboxylic acid very effectively. The affinity of the fiber coating for target analytes is crucial in SPME sampling because both the matrix and fiber coating are competing for analytes. For example, a polar coating chosen to extract polar compounds from water must have a stronger affinity for the analytes than water in order for them to be extracted.

1.5.1 DESORPTION

Once the analyte extraction is complete, the coated fiber containing the analytes is ready to be desorbed into a GC or HPLC injector for analysis. The extraction step establishes the more critical experiment parameters (speed, sensitivity, accuracy, and precision). Desorption, which is closely related to the efficiency of the chromatographic separation and the precision of quantitation, has a substantial influence on the quality of data obtained. For GC analysis, the desorption process involves inserting the fiber into a hot GC injector. As the temperature increases, the coating/gas partition coefficients decrease and the fiber coating's ability to retain analytes quickly diminishes. Typically, a desorption time of two minutes is adequate to release all analytes from the fiber coating. On the other hand, desorption using solvents and subsequent HPLC analysis is prefered for nonvolatile and thermally

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labile analytes. In this case, the SPME fiber is inserted into a desorption chamber which replaces the injection loop on the injector valve. When the valve is in the "load" position, the fiber is introduced into the desorption chamber under ambient pressure. The mobile phase or a stronger organic solvent is then used to release the analytes from the fiber. Additionally, a heater can be installed in the device to expedite the desorption process. Desorption volumes are typically the same as the injection loop volume.

1.5.2 Preparation of GC

The assumption here is that chromatographic conditions have been optimized for the analytes, i.e., an appropriate column, temperature program, and detector have been selected. Sample introduction with SPME does not preclude any of the normal GC techniques. Cryogenics in the column oven are advantageous when the sample contains analytes with a wide range of volatility (for example, vinyl chloride and PAH's). Any detectors including a mass spectrometer can be used successfully. Moreover, various techniques used for confirmation, such as splitting the sample to two columns or introducing an effluent splitter at the column, will be unaffected by SPME introduction. Only the injector conditions should be optimized for SPME.

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1.5.3 Injector Temperature

SPME fibers are generally desorbed under hot, isothermal conditions, although, temperature-programmable injectors are popular for minimizing decomposition of labile compounds and eliminating discrimination based on volatility. Rapid desorption from the fiber is necessary for sharp peaks without

sample carryover. Injector temperature is normally 10-20 °C below the temperature limit of the fiber and/or the GC column (usually 200 to 280 °C).

1.5.4 Selection of the Fiber, Sampling Vial, and Vial Septa

Supelco, Inc., Bellefonte, PA, 16823, manufactures the SPME fibers and holders, and they are continually improving fiber technology. For example, five phases are commercially available for GC injection. The first phase introduced suitable for non-polar compounds and is available in three film thicknesses, with the thinner films recommended for samples with less-volatile analytes. More recently, phases for polar and very volatile compounds were introduced. **Table 1.5** summarizes the fibers that are commercially available.

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Table 1.5 Commerially Available SPME Fibers for GC and GC-MS.

Phase	Applications
Polydimethylsiloxane **Three film thicknesses are available:** • **7 μm** • **30 μm** • **100 μm**	Non-polar phase (for many semipolar compounds: aromatics, esters, many pesticides) 100 μm used for relatively volatile compounds; the thinner phases are for non-polar and semipolar compounds of low volatility.
85-μm Polyacrylate	Polar compounds such as phenols, esters.
65-μm Carbowax/divinylbenzene	More polar than polyacrylate, for alcohols.
75-μm PDMS/divinylbenzene	Moderately polar, for amines.
65-μm Carboxen/PDMS	Highly volatile compounds including vinyl chloride, sulfur gases.

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For samples with a mixture of different classes of analytes, two or three fibers should be compared and considered for a phase giving the best overall performance. Sensitivity is not the only issue, other considerations are a relative lack

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of affinity for interfering compounds and the ability of the fiber to be desorbed easily so that no sample carryover occurs.

1.6 The Scope and Purposes of This Research

The research was conducted in order to study the aroma components that play a role in *Vetiveria zizanioides* Nash (scented vetiver) roots.

A solid-phase microextraction followed by capillary gas chromatography-mass spectrometry was utilized for the investigation of the volatile components involving in aroma character of the root. Then, separation and isolation of the components of interest from the extracted oil of the root were performed using column liquid chromatographic technique followed by thin layer chromatography and preparative gas chromatography. The purity of isolated component was confirmed by GC-MS and its aroma character was justified by a simple method of sensory evaluation.

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