Monographs of *Cymbopogon nardus* (L.) Rendle and Citronella Oil

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ชื่อวิทยานิพนธ์ ข้อกำหนดมาตรฐานสมุนไพรของตะไคร้หอมและน้ำมันตะไคร้หอม
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บทคัดย่อ

ตะไคร้หอม (*Cymbopogon nardus* (L.) Rendle) เป็นพืชในวงศ์ Poaceae หรือ Gramineae ซึ่งในปัจจุบันมีการนำน้ำมันหอมระเหยจากตะไคร้หอมมาใช้ในด้านต่างๆ เช่น สารแต่งกลิ่นในสบู่ ผลิตภัณฑ์ไล่ยุง และเครื่องหอม เป็นต้น งานวิจัยนี้เป็นงานวิจัยชิ้นแรกที่ได้ทำมาตรฐานสมุนไพรตะไคร้หอมและน้ำมันตะไคร้หอม โดยได้เก็บตัวอย่างตะไคร้หอมจากทั่วประเทศไทยทั้งหมด 15 ตัวอย่าง และสุ่มซื้อตัวอย่างน้ำมันตะไคร้หอม 8 ตัวอย่าง เพื่อศึกษามาตรฐานของสมุนไพรดังกล่าวตามวิธีการของ Thai Herbal Pharmacopoeia 2007 โดยมีผลการทดลองดังนี้ ปริมาณเถ้า ปริมาณเถ้าที่ไม่ละลายในกรด ปริมาณน้ำ และ ปริมาณน้ำมันหอมระเหย มีค่าเท่ากับ 6.49±1.11, 2.46±0.13, 2.56±0.98 และ 0.10±0.07 ตามลำดับ และผลการหาปริมาณสารสกัดด้วยตัวทำละลายต่างๆ ได้แก่ เอทานอล น้ำ เฮกเซน และคลอโรฟอร์ม มีปริมาณสารสกัดเท่ากับ 6.26±1.34, 8.04±1.25, 3.22±1.31% และ 7.51±3.33 ตามลำดับ นอกจากนี้ได้ศึกษาหาค่าความหนาแน่นสัมพัทธ์ ค่าดัชนีการหักเห และค่าออปติคัลโรเตชัน ตามวิธีการของ European Pharmacopoeia 1980 โดยให้ผลการทดลองเท่ากับ 0.878-0.902, 1.458-1.484 และ -0.054° - +2.344° ตามลำดับ พร้อมกันนี้ได้นำน้ำมันตะไคร้หอมไปตรวจวิเคราะห์ด้วยเครื่อง GC-MS พบสารที่เป็นองค์ประกอบหลักได้แก่ limonene, citronellal, citronellyl acetate, citral, geranyl acetate, citronellol และ geraniol

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ABSTRACT

Citronella grass (*Cymbopogon nardus* (L.) Rendle) belongs to Poaceae or Gramineae Family and is locally in Thai name as Ta khrai hom. Citronella oil is the essential oil from this plant and it is used for manufacture of soaps, insect repellant product and flavouring agents. Monographs of *C. nardus* and citronella oil have not been reported, so this study will be the first report. Fifteen samples of plant material were collected from each part of Thailand and eight samples of citronella oil were purchased from commercial product. The results showed that fifteen samples have total ash, acid insoluble ash, water content, and volatile oil as 6.49±1.11, 2.46±0.13, 2.56±0.98 and 0.10±0.07, respectively. The results of ethanol-soluble extractive, water-soluble extractive, hexane-soluble extractive and chloroform-soluble extractive content were examined after Thai Herbal Pharmacopoeia 2007, showed 6.26±1.34%, 8.04±1.25%, 3.22±1.31% and 7.51±3.33%, respectively. The relative density, refractive index and optical rotation of eighteen samples of citronella oil were examined after European Pharmacopoeia 1980. showed as 0.878-0.902, 1.458-1.484 and -0.05° - +2.344°, respectively. The results from GC-MS showed the chemical constituents in citronella oil that contained limonene, citronellal, citronellyl acetate, citral, geranyl acetate, citronellol and geraniol.

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LIST OF ABBREVIATIONS AND SYMBOLS

m	mater
mm	millimeter
cm	centimeter
nm	nanometer
g	gram
kg	kilogram
ppm	part per million
β	beta
ρ	ro
α	alpha
γ	gamma
mg	milligram
%	percentage
Hrs	hours
ml	milliliter
etc	et cetera
GC-MS	gas chromatography –mass spectrometry
Fig	figure
°C	degree celcius
m/z	mass-over-charge ratio
w/w	weight by weight
v/w	volume by weight
MIC	minimum inhibitory concentration
MBC	minimum bactericidal concentration
MFC	minimum fungicidal concentration
No	number
CN-PS	*Cymbopogon nardus* from plant sample

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

CN-BS	Citronella oil from the market
SD	standard deviation
TP	Thai Pharmacopoeia
BP	British Pharmacopoeia
EP	European Pharmacopoeia
USP	United States Pharmacopoeia
BHP	British Herbal Pharmacopoeia
THP	Thai Herbal Pharmacopoeia

CHAPTER 1

INTRODUCTION

1.1 Introduction

1.1.1 Background and Rationale

The meaning of “monograph” (mono+graph) is a book or authority describing on something. Thus, “specification of medicinal plants” means the detail of a type of herbal which describe the quality to clarify efficiency and safety for human needs (วีณา วิรัจฉริยากุล, 2534).

In the past, the agreement between dealer and broker was evidently specification. There was the basis in determining quality and characteristics of good herb. It leads to the official agreement as appearing in the Pharmacopoeia such as, Thai Pharmacopoeia (TP), British Pharmacopoeia (BP), The United States Pharmacopoeia (USP), and European Pharmacopoeia (EP). Those were composed by basing on the monograph of the principle. However, making the medicine aside from using the chemical, herbal extracts are also included such as; glycyrrhiza for cough relief. It is observed that in the past 20 years there are advertisements of “herbal” in each Pharmacopoeia. They take place in the supplement such as British Herbal Pharmacopoeia (BHP), Thai Herbal Pharmacopoeia (THP). Most of all, aims to reflect that the use of herbal and it increases, respectively. Moreover, the quality of used herbal is controlled as the same standard (วีณา วิรัจฉริยากุล, 2534).

Medicinal plants mean natural medicine both dried and fresh without the transformation. Especially, the under ground such as licorice root and aerial part plant such as cinchona bark, sappan wood, senna leaf, bale fruit, and pepper seeds.

To study on herbal plants for using as medication and pharmacy, we have to base on chemical knowledge, botany, pharmacognosy and pharmacology. To verify the herbal plant characteristic we use five human senses; eye, nose, tongue, ear and hand to prove the characteristic of herbal plants as commercial product. That protects the incorrectness in primary using (สมพร ภูติยานันต์, 2542).

Due to in the present world people are more health conscious, especially by applying the natural method of treatment. That brings popularity of herbal product. However, people are still lacking the confidence in its quality and safety since there is no monograph to standardize its quality.

The quality of herbal medicinal plants are base on the following factors (วีณา วิรัจฉริยากุล, 2534):

1. Knowledge and experience of the collector. The incorrect method brings the low quality herbal. Without the knowledge and experience the collector may collect the incorrect part or type.
2. Be careful, herbal preparation should be based on the demand.
3. The ability to control the contamination.
4. The correct method to preserve the herbal plant.
5. The contamination of other substance or rock, soil, and sand due to the carelessness of collector.
6. The adulteration, to add other herbal, or getting rid of the essential extract, and the spoilage of collector.
7. The substitution, purposely assimilation of the collector, for example, to use the Spanish saffron instant of the American saffron.
8. Deterioration, due to the long duration storage or the incorrect method used while storing. For example, the fungus caused by high temperature, and the quality distortion due to the bite of insects.

Essential oils are organic substance produced by plants which can be obtained from roots, stem, bark, leaves, flowers, fruits and seeds. They are volatile and have a special odor which help the plants to protect themselves from insect, bacteria and other microbial attacks. And volatile oils are also useful for plant pollination by inducing insects.

The essential oils have complex constituents. There are more than 50-500 compounds in one type of essential oil. The main compositions are terpenes, including monoterpenes, sesquiterpenes, phenylpropene and terpenes which are composed of oxygen in the form of alcohols, aldehydes, esters and ketones, e.g. geranial and menthol. Limonene, citral, geraniol, menthol and camphor are examples of monoterpenes, while β-bisabolene and β-caryophyllene are sesquiterpenes (Wannisorn *et al*., 2009).

Citronella oil is a type of essential oil. It is used in spa business, aroma therapy, and as insect repellent. However, the essential oil getting from different places might have the difference in chemical compositions, physical characteristics, and physicochemical properties. Therefore, it is important to study and evaluation in order to get more information for the official standard. Those are chemical compositions, pharmacological effects, benefits, and applications. It is for the advantage of people related to essential oil business such as the citronella grower, extract business, importer-exporter, and the specialist who supporting the essential oil manufacturing. For the project "Comparing standard of extracting citronella for sale", we could see that in the past 10 years people were interested in alternative medicine especially, aromatherapy spa. It drives the chance to tourism business and Thai economy. Therefore, various costly essential oil mostly from aboard were imported. In fact, Thailand is one of the countries with full of herbal plants. It includes the plant used in aromatherapy such as jasmine, rose, orchid, and citronella. So, using Thai aroma plants should be developed continually. However, to compete with other country, the quality evaluation is unavoidable. The herbal plant in our research, citronella has the monograph in European Pharmacopoeia (European Pharmacopoeia, 1980), but it does not have monograph in Thailand.

Therefore, it is essential to have official standard in the pharmacopoeia for the purpose of quality controlling and to define the appropriate amount. The wheather have the same time producing same content at same season or not (วีณา วิรัจฉริยากุล, 2534).

The analysis for crude drug quality controlling has been developed continually to physical characteristic, quality, purity, contamination, and active constituent in the monograph. However, the essential oils are getting from different places might have the difference in chemical compositions, physical characteristics, and physicochemical properties.

Since, in Thailand it has not been reported the monograph of *C. nardus* and citronella oil. The monograph of *C. nardus* should be included macroscopic, microscopic, foreign matter, total ash, acid insoluble ash, water content, volatile oil, ethanol-soluble extractive, water-soluble extractive, hexane-soluble extractive and chloroform-soluble extractive content. They were examined after Thai Herbal Pharmacopoeia (2007). And the monograph of citronella oil should be included the relative density, refractive index and optical rotation were examined after European pharmacopoeia, 1980.

1.2 Objectives

The main objectives in this investigation are as follows:

1. To study the monograph of *Cymbopogon nardus* and citronella oil.
2. To standardize of plant materials for commercial product.
3. To compare the quality of citronella oil between the products from the market and house products.

CHAPTER 2

HISTORICAL

2.1 Review of Literatures

2.1.1 Botanical aspects of Citronella grass

Citronella grass has the sciencetific name is *Cymbopogon nardus* (L.) Rendle. It belongs to Poaceae (Gramineae) family. It has been known as Ta khrai hom (Central), Cha khai ma khuut, Ta khrai ma khuut (Norther), Ta-Khrai-Dang (Nakornsrithamara), Sarah Grass, and Citronella Grass (วุฒิ วุฒิธรรมเวช, 2540 และ เพ็ญนภา ทรัพย์เจริญ, 2549).

The genus *Cymbopogon* in Poaceae family is characterized by its species possessing great variability in morphology and chemotypes. The genus is represented by 55–140 taxa, largely confined to old world tropics and sub-tropics. Most species of the genus are aromatic and yield volatile oils of important commercial values (Nath *et al*., 2002).

Cymbopogon represents an important genus of about 120 species and several varieties. *Cymbopogon* species are well known as a source of commercially valuable compounds like geraniol, geranyl acetate, citral (neral and geranial), citronellal, piperitone, eugenol, etc., which are either used as such in perfumery and allied industries, or as starting materials for the synthesis of other products commonly used in perfumery (Cos, 1980).

The plant is a perennial grass; consisting of culms up to 2 m tall, erect, growing in thick tufts, branched rhizome. Leaf-blades are linear, up to 1 m long, 5-20 mm wide, glabrous, aromatic; having ligule (a projection between joining of sheath and blade) ovate, truncate, about 2 mm long, pubes-cent; sheath persistent, basal ones imbricate, curling up when dry. Inflorescence is in a large panicle up to 80 cm long; each spike consists of 4-5 spikelets, rachis and pedicels ciliate, shorter than ½ spikelets, funnel-shaped. Spikelets are in pair, one is sessile the other is pediceled. The sessile spikelet is oblong-lanceolate about 3 mm long consisting of outer glume toothed vein obscure slightly flattered on the dorsal side margins narrowly winged and the upper margins scabrous; the inner glume boat-shaped nearly as long as outer glume 1-3 veins margin ciliate lower lemma oblong membranous ciliate veinless; upper lemma linear with a mucro apex (Farnsworth and Bunyapraphatsara, 1992).

The Citronella is herbaceous plant. Its pseudostem grows to cluster. It has rootstock and looks similar to the lemon grass. However, it has bigger, and longer leaves. The ornamental leaf-like sheath split into several branches. The pseudostems are green and magenta colors (Farnsworth and Bunyapraphatsara, 1992).

Another species is *C. winterianus* Jowitt, it has been known as Old Citronella Grass or Winter Grass. It is believed to have originated from the well-known species. *C. nardus* referred to as Ceylonese (Sri Lankan) commercial citronella.

So, *C. winterianus*, which is closely related to *C. nardus* but the differences are as followed:

***C. winterianus* Jowitt**	***C. nardus* (L.) Rendle**
1. shallow	1. deep root
2. tall culm	2. short culm
3. cross-section of the non-flow-ering branch is a yellow colour	3. cross section of the terminal of non-flow-ering branch is a red color
4. opened panicle	4. contracted panicle
5. lower glume with 3 distinct veins	5. lower glume without distinct vein

2.1.2 Ecology and distribution of *C. nardus* (Farnsworth and Bunyapraphatsara, 1992).

The plants widely spread in well-drained sandy soil require sunlight. They distribute in Southeast Asia.

2.1.3 Propagation of *C. nardus*

The propagation is done by rhizome cutting. Several studies have been carried out on the cultivation of citronella. Nitrogen (120 kg/hector) and phosphorus (60 kg/ hector) are found to be suitable for the cultivation. Nitrogen increases crop yield volatile oil content and its alcohol is terpenes. Ammonium sulfate and neem seeds covered with urea are better than urea. Another study indicated that the good spacing is 50x50 cm and suitable fertilizer is N-P-K (200:75:60 kg/hector). Boron magnesium molybdenum and manganese enhance volatile oil production. Copper at 500 ppm gave the maximum volatile oil yield (200 kg/hector). Both copper and boron showed no effect on the composition of volatile oil. Cultivation trial using red soil showed that nitrogen and phosphorus increased crop yield 51-61%. Besides the effect of fertilizing the studies were extended to insecticides seasonal effect and genetic studies (Farnsworth and Bunyapraphatsara, 1992).

2.1.4 Ethnomedical uses of *C. nardus*

The claimed efficacies in Thai medicinal plants text books are follows.

Stem : treatment of minor aphthous ulcers and cracked lips; as an abortifacient and carminative.

Not specified part used : treatment of chronic gastrointestinal ailments of the children between the ages of 5 and 13 characterized by marked malnutrition usally associated with intestinal parasitium apthous ulcers, minor apthous ulcer, menstrual disorders, stomach discomfort, vomiting and trachoma; as an abortifacient, carminative, fire element tonic and antipyretic.

2.1.5 Chemical constituents of *C. nardus* (Farnsworth and Bunyapraphatsara, 1992):

Leaves : essential oil.

Essential oil : benzoic acid, L-borneol, (-)-borneol, bourbonene, camphene, L-camphene, camphor, β-caryophyllene, Δ^3-carene, carvacrol, carvone, cinnamic aldehyde, citral, citronellal, citronellol, citronellyl acetate, citronellyl butyrate, coumarin, ρ-cymene, elemol, eugenol, eugenol methyl ether, isoeugenol methyl ether, fanesol, geraniol, geraniol acetate, geranyl acetate, geranyl butyrate, geranyl formate, gerianol, hexan-l-ol, D-limonene, L-limonene, limonene oxide, trans-limonene diol, linalool, linalyl acetate, menthol methyl heptenone, methylisoeugenol, myrcene, nerol, nerolidol, ocimene, *cis*-ocimene, *trans*-ocimene, octyl alcohol, patchoulene, phellandrene, D-α-phellandrene, D-β-phellandrene, phenylethyl alcohol, α-pinene, β-pinene, α-pinene, safrole, sabinene, sesquiterpeneoids, γ-terpenene, α-terpenene, α-terpineols, terpenin-4-ol, terpinolene, α-thujene, tricyclene

From the previous reports the main constituents of both from *C. nardus* and *C. citratus* are acyclic terpenes. And the chemical constituents of citronella oil from *C. nardus* are camphene, β-caryophyllene, ρ-cymene, limonene, myrcene, ocimene, ∝-phellandrene, ∝-pinene, β-pinene, ∝-terpinene, terpinolene, ∝-thujiene, borneol, citronellol, elemol, geraniol, linalool, menthane-1-0, nerol, piperitol, η-propyl alcohol, ∝-terpineol, 4-terpineol, citral, citronellal, methyl heptenone, piperitone, citronellyl acetate, geranyl acetate, geranylbutyrate, chavicol, eugenol, methyleugenol, methyl isoeugenol, citronellic acid, caryophyllene oxide (Heiba and Rizk, 1986).

The steam-distilled volatile oil obtained from the partially dried grass (citronella grass, *C. nardus*) was analysed by capillary GC and GC-MS. The partially dried grass contained 35 components, of which 29 constituents, comprising 92.7% of the total volatiles, were completely identified, while six constituents (7.3%) of the total volatiles, were partially identified. The oil contained 16 monoterpenes (73.3%), five hydrocarbons (8.9%), two aldehydes (30.0%), eight alcohols (40.4%) and one phenolic hydrocarbon (0.5%). Citronellal (29.7%) was the major component identified, followed by geraniol (24.2), γ-terpineol (9.2%), *cis*-sabinene hydrate (3.8%), β-myrcene (2.9%), borneol (2.5%), nerol (1.5%). Nine sesquiterpenes (11.5%), including six hydrocarbons (5.0%) and three alcohols (6.5%), were also identified. *(E)*-nerolidol (4.8%) was the predominant sesquiterpene, followed by caryophyllene (2.2%) and germacren-4-ol (1.5%). Four non-terpenic constituents, comprising 1.4% of the sample, were detected, with the major aliphatic constituent being 3,3,5-trimethyl-1,5-heptadiene (0.7%). Six components, comprising 7.3% of the oil, were partially identified, with half of them being monoterpenes. These components included bis (2-ethyl hexyl)-1,2-benzene dicarboxylic ester (2.0%) and benzene dicarboxylic acid derivative (1.7%) (Vijender and Mohd, 2003).

The light yellow essential oil *C. citratus* and *C. nardus* were obtained in yields of 1.6% and 1.3%, respectively. The chemical compounds were identified by GC-MS in the *C. citratus* oil sample, which represented 99.8% of the detected compounds that included geraniol (45.2%), neral (32.4%) and myrcene (10.6%) as major components. This oil consisted of two monoterpene hydrocarbon (10.6%) and nine oxygenated monoterpenes (86.4%). This sample appeared similar to previous reports on oil chemotypes from Togo but it differed from that previously described in Cuba with composes of geraniol (52.3%), cis-pinocaveol (20.2%), neral (9.8%) and 1, 2-epoxide (3.6%). This oil consisted of seven oxygenated monoterpenes (79.0%), five sesquiterpene hydrocarbon (12.3%) and four oxygenated sesquiterpenes (2.6%). This composition was similar to previous reports on *C. nardus* oils from Togo but differed from those described in Thailand with geraniol (35.7%), *trans*-citral (22.7%), *cis*-citral (14.2%) and geranyl acetate (9.7%) as major components, in India by Mahalwal and Ali with citronellal (29.7%), geraniol (24.2%), γ-terpineol (9.7%) and *cis*-sabinene hydrate (3.8%). The sample of *C. citratus* essential oil was notably richer in citral (neral and geraniol upper than 77%) than that of *C. nardus* (with only a total of 1.1% in citral) (Koba *et al*., 2009).

The chemical comparison of essential oils of *C. nardus* from leaves of Benin and Congo were subjected to hydrodistillation to obtain essential oil. The chemical analysis was carried out by using GC-MS for identification of components of the two essential oils. These observations could be explained by some qualitative and/or quantitative differences observed between the constituents of the two essential oils. The more important constituents are monoterpenes (more than 91% in essential oil of Congo and 86% in those of Benin) represented by citronellal and geraniol (respectively 41.3 and 23% in essential oil of Benin versus 37.5 and

29.4% in those of Congo). These monoterpenes are associated with their acetates (4.4 and 1.7 in essence of Benin versus 1.5 and 1.8% for sample of Congo). Oxygen containing compounds were also abundant and are adjoining 90% of total essences. The propotions of aldehydes are high (40% in average). Some sesquiterpenes are present in these preparation, represented by α-farnesene (1.5%) and elemol (4.8%) in *C. nardus* specie of Benin and by β-elemene (1.5%), trans-β-caryophyllene (2.5%) and δ-cadinene for Congolese essential oil. Hydrocarbons (mycrene and limonene exclusively) represents only 3% (Abena *et al*., 2007)

2.1.6 Chemical constituents of *C. winterianus* (Thai medicinal plants, 1992):

Essential oil: (-)-borneol, bonene, β-caryophyllene, citronellal, citronellol, citronellyl acetate, citronellyl butyrate, elemol, eugenol methyl ether, isoeugenol methyl ether, farnesol, geraniol, geranyl formate, limonene, linalool, linalyl, methylheptenone, nerol, terpinen-4-ol.

2.1.7 Monograph of *C. winterianus*

Moreover, there is the monograph of *C. winterianus* in European Pharmacopoeia. It showed the relative density, reflective index, and optical rotation are 0.8881-0.895, 1.463-1.475 and -4 - +1.5, respectively. Citronellal was used to be the positive standard in citronella oil. It was identified by using thin layer chromatography (TLC) technique and proved by anisaldehyde spraying reagent. The result showed the violet occurs at the position of citronellal which is the extract in citronella oil. From the element analysis by using Gas chromatography-Mass spectrometer (GC-MS) found the element as follows; limonene (1-5%), citronellal (30-45%), citronellyl acetate (2-4%), neral (2%), geranial (2%), geranyl acetate (3-8%), citronellol (9-15%), and geraniol (20-25%) (European pharmacopoeia, 1980).

2.1.8 Pharmacological activities and clinical trials of *C. nardus* and *C. winterianus* : (Farnsworth and Bunyapraphatsara, 1992)

The green soft leaves of citronella grass are softer than normal lemongrass. The stems and leaves are very smelly and full of essential oil. Therefore, people don't use it for cooking. Its yellow flowers are on the top, each bouquet is dried (เพ็ญนภา ทรัพย์เจริญ, 2549 และ วุฒิ วุฒิธรรมเวช, 2540).

The citronella grass presents a lot of properties. Its rhizome is used for women's medicinal such as; uterus compressing, diureticalness, and driving leucorrhea. Leaves give essential oil that can be used as ingredient in soap, and mosquito spray. The stem has hot and bitter taste that is good composing for medicine healing of mouth hemorrhoids, blood driving (in women), uterus compressing, abortion, intestine gas releasing, and making insecticide. Moreover, the crashed stem is good for mosquito protection (พเยาว์ เหมือนวงษ์ญาติ, 2530 และ สันติสุข โสภณสิริ, 2537).

Due to the citronella shows interesting benefits from Thai folk wisdom. Hence, there are several experimental researches on its biological actions. For example, citronella oil which can be used as insect repellent (Cos,1980). Testing with the malaria mosquito found that the cream containing essential oil 1.25%, 2.5%, and 5% can protect the mosquito for 2 hrs. Besides, the intensity at 10% can protect the mosquito for 4 hours (กิตติพันธ์ ตันตระรุ่งโรจน์, 2543). The cream containing 5% galangal oil, 2.50% citronella oil, and 0.5% vanillin has ability to protect mosquito more than 6 hrs (กิตติพันธ์ ตันตระรุ่งโรจน์, 2543). Testing with the culex, found that the cream containing essential oil has higher efficiency than the cream without essential oil. Using citronella oil to test with the carrier of malaria, dengue fever, and elephantiasis found that it can protect mosquito for 8-10 hrs (Tyagi *et al*., 1998). The intensity that can protect the common house mosquito at EC_{50} and EC_{95} are 0.031% and 5.259%, respectively. The 1% intensity of essential oil can protect the mosquito for 75.19%.

The citronella oil was reported for insect repellant activity by using the preparation which contains essential oil from *C. nardus* was patented for treatment of fleas in dog. The mixture of 90% ethanol with citronella extract, olive oil and essential oil with civet scent, which was tested with the common house mosquito and the female culex found that, it had long time active to protect mosquito (114-126 minutes). Furthermore, it had ability to control and defeat the mosquito larva (เนาวรัตน์ ศุขะพันธ์, 2536).

Citronella oil at 10% of intensity is good for hunting a carid larva for 8 hrs (Thorsell *et al*., 2006). Besides, it can chase away the rice pest without any effect to the rice quality (Paranagama *et al*., 2003). The citronella also has the ability to chase away the moth (Sinchaisri *et al*., 1988) and other insects (Sugiura *et al*., 2002).

The active ingredients in citronella oil extract for mosquito repellant are camphor (Masui and Kochi, 1974 ; Schearer, 1984), cineol (Scriven, 1984; Verma, 1981; Hwang *et al*., 1985), eugenol (Chogo, 1981; Tunon *et al*., 1994; Marcus and Lichtenstein, 1979], Linalool (Bower *et al*., 1993), citronella and citral (Vartak and Sharma, 1993).

An attempt to develop a cream preparation of citronella oil from *C. nardus* for use as a mosquito repellent was carried out at the Faculty of Pharmacy, Mahidol University. It was found out a cream containing 17.0% of oil showed mosquito repellent activity for 3 hrs (Farnsworth and Bunyapraphatsara, 1992). There is clinical study by applying the cream containing 14% citronella oil and the cream without citronella oil on 40 of volunteers. The result showed that it is effective in 13 from 20. While another volunteers without applying the cream are unprotected from mosquito. Thailand Institute of Scientific and Technological Research has an experiment on the 14% intensity citronella oil and found that it can protect the mosquito for 2 hrs. It is close to the effect of 20% dimethyl phthatate mixed with 5% diethyl toluene amide (ศศิธร วสุวัต, 2533).

There is the research of citronella oil efficiency by gram smoking for 72 hrs. The result shows that it can protect the pest under 50% without the side effect to its grown (Ketoh *et al*., 2005). However, it effects to the parasite of the gram pest (Ketoh *et al*., 2002). Citronella extract with the neem seed extract and galangal 200 ml altogether in 20 liters of water has effect to defeat the embryo of aphis and bean pod borer. However, it is not cover to the bean fly (อรัญ งามผ่องใส, 2546). The 100 ppm of citronella is not so effective to the cabbage pest but it can kill the moina to 90% in 20.70 hrs (กนก อุไรสกุล, 2002). Besides, 10% of ethanol extract (dried citronella 200g / ethanol 4 liters) is good for reducing the jumping fleas, kale pest. However, it tends to reduce the kale weight. The shampoo containing citronella extract can kill the fleas in pet (วสุ วิฑูรย์สฤษฎ์ศิลป์ และ ศุทธิชัย พจนานุภาพ, 2545).

It has the report of essential oil from the leaves of *C. winterianus* which relieved the symptom of grand mal, testing with the male swiss of mice 2-3 months old mouse with the weight of 25-30 g. The animal were randomly housed in appropriate cages at 22±1 °C on a 12 h light / dark cycle (lights on 06.00-18.00) with free access to food (Purina) and water. The behavioural screening of the mice was performed following parameters and animal were observed at 0.5, 1, and 2 h after administration of essential oil (EO) (100, 200 and 400 mg/kg). Then used to assess the anticonvulsant effect of the EO. Mice were kept individually in transparent mice cages (25 cm x 15 cm x 15 cm) for 30 min to acclimatize to their new environment before the commencement of the experiment. Induced in mice with pentylenetetrazole (PTZ) (60 mg/kg) or picrotoxin (PIC) (8 mg/kg) and the animal were observed for convulsion for a period of 15 or 20 min and repeated following the pretreatment of animals with either EO and diazepam (DZP) (3 mg / kg) or control vehicle prior to the administration of any the convulsant agents used. The citronella oil contain 3 significant substances, geraniol (40.06%), citronellal (27.44%), and citronellol (10.45%), testing with the 100, 200, and 400 mg / kg of essential oil. It can be confirmed that the extract from *C. winterianus,* citronella has higher efficiency to cure the disorder, comparing to cure with PTZ and PIC from the experiment of citronella and its effect to nervous system inhibitory (Quintans *et al*., 2008).

The test of toxicity shows that in the rate 1:1 it kills the half of all guinea pigs (Dhar *et al*., 1973).

Pharmacological activities and clinical trials of the whole plants of *C. nardus* were reported that an alcohol-water (1:1) extract did not exhibit cytotoxic activity against 9KB. And the LD_{50} of an alcohol-water (1:1) extract in the mouse is 1.0 mg/kg. Besides that the citronella powder solution in the water can cure bone pain and joint pain (ตะไคร้หอมผงสำเร็จรูป (a), 2546).

The herbal insect repellent incense made from citronella powder has the preparation process as follows; 2 portions of sawdust, 1 portion of camphor, the dried chopped citronella, 1 button of citronella oil, paste, and incense stick made from dried bamboo (กรมวิทยาศาสตร์บริการ, 2553).

Furthermore, there is the aroma mosquito sand made from citronella. It protects the mosquito bite that leads to many diseases like Chikungunya, Dengue Fever, and other disease caused by the mosquito (ตะไคร้หอมผงสำเร็จรูป (b), 2553).

The fresh whole plant material of *C. citratus* yielded an essential oil on hydro-distillation which was analyzed by GC-MS and also evaluated for antifungal activity against five species of the genus *Aspergillus*, namely *A. Flavus*, *A. parasiticus*, *A. niger* and *A. famigatus*. The oil was dominated by monoterpene hydrocarbons which accounted for 94.25% of the total oil and characterized by a high percentage of geraniol (39.53%), neral (33.31%), and myrecene (11.41%). The antifungal activity tests showed that the oil was active against all the five *Aspergillus* species. The extent of inhibition of fungal growth was dependent on the concentration of the oil. The activity of the oil against the mycotoxigenic fungi had minimum inhibitory concentration (MIC) value ranging from 15 to 118 mg/ml. These results show that the essential oil of *C. citratus* has antifungal activities against fungi that are the producers of poisonous mycotoxins found in foods. This oil can be used in food preservation systems to inhibit the growth of moulds and retard subsequent mycotoxin production (Josphat *et al*., 2011).

For *C. winterianus*, the essential oil showed anti fungal activity against *Microsporum gypseum*, *Trichophyton eguinum* and *T. rubrum*. The antifungal assay using the vapor-agar contact method showed that the crude essential oil markedly suppressed the growth of several species of *Aspergillus*, *Penicillium* at a dose of 250 mg/L in air.The most active compounds among the 16 examined volatiles, consisting of 6 major constituents of the essential oil and 10 other related monoterpenes were citronellal and linalool. Citronellal and linalool completely inhibited the growth of all tested fungal strains at a dose of 14 to 56 mg/L. The α- and β-pinene showed an inhibitory activity against some fungi, whereas the other 8 volatile compounds lacked this properties. Additionally, the composition of essential oils is affected by many factors, including the cultivation conditions of the plants and isolation techniques (Kazuhiko *et al*., 2003).

The chemical composition and antimicrobial activity of *C. winterianus* Jowitt (Poaceae) and *Carum carvi* L. (Apiaceae) essential oils were investigated against 19 fungal and 7 bacteria species. Among the tested species were food contaminants, spoilage fungi, as well as plant or fungi and animal pathogens. In order to determine minimal inhibitory concentrations

(MICs), minimal fungicidal concentrations (MFCs) and minimal bactericidal concentrations (MBCs), microdilution tests were used. Citronellal and trans-geraniol were dominant in *C. winterianus* oil wheras carvone and limonene were the main components in *c. carvi* oil. The essential oil of *C. cavi* possessed stronger antifungal and antibacterial potential more than citronella oil (Simic *et al*., 2008).

Chemical composition and antimicrobial activity of exotic plants essential oils to potentially control *Paenibacillus larvae*, the causal agent American foulbrood disease (AFB) were determined. AFB represents one of the main plagues that effect the colonies of honeybees *Apis mellifera* L. with high negative impact on beekeepers worldwild. Essential oils tested were niaouli and tea tree from Myrtaceae, and citronella grass (*C. nardus*) and palmarosa (*C. martini*) from Gramineae. The components of essential oils were identified by SPME-GC/MS analysis. The antimicrobial activity of the oils against *P.larvae* was determined by the broth microdilution method. *In vitro* assay of *M. viridiflora* and *C. nardus* oils showed the inhibition of the bacterial strains at the lowest concentrations tested, with minimal inhibitory concentration (MIC) mean value about 320 mg/l for broth oils, respectively. This property could be attributed to the kind and percentage of the components of the oils. Terpenene-4-ol (29.09%), α-pinene (21.63%) and limonene (17.4%) were predominant in *M. viridiflora*, while limonene (24.74%), citronelal (24.61%) and geraniol (15.79%) were the bulk of *C. nardus*. The use of these essential oils contributes to the screening of alternative natural compounds to control AFB in the apiaries; toxicological risks and other undersirable effects would be avoided as resistance factor developed by the indiscriminate use of antibiotics (Fuselli *et al*., 2010).

The plant diversity with confirmed activities in the central nervous system is dominated by higher plants, mainly by dicotylidols. In this review 30 species belonging to 13 families and 23 genera have been reported to possess anti-seizure activity. It is accepted that a refined assessment of the chemical composition of tested essential oils/ constituents should be performed using GC/MS to perform a quantitative analysis, which would provides additional information of their contents and consequently, confirmation of their therapeutic effects. The species and respective essential oils that showed the Poaceae family. The *C. winterianus* used the leaves are anticonvulsant activity was observed in pentylenetetrazole, pilocarpine, and strychnine tests. Anticonvulsant effect was blocked by flumazenil in pentylenetetrazole model and the essential oil showed presence of geraniol, citronellal, and citronellol as the main compounds (Silva, 2010). The *C. citratus* used the leaves are Anticonvulsant activity was observed in pentylenetetrazole, pilocarpine, strychnine, and maximal electroshock tests (Blanco, 2009). From many reports of citronella and citronella oil, it has been proved that it is one of the herbs that should be developed. In Thailand, we are still lacking of the information in herbal standard which is the essential information. Without the standard, products from those herbals are low quality. Therefore, this research will be the information to specify the quality of citronella used in the herbal product manufacturing.

However, in Thailand it has not been reported of the monographs of *C. nardus* and citronella oil. So, this study will be the first report of the monographs of *C. nardus* and citronella oil. This report will be useful for standardization and quality control of the plant materials for commercial production.

CHAPTER 3

MATERIALS AND METHODS

3.1 Material

3.1.1 Plant materials

The *Cymbopogon nardus* (L.) Rendle (Figure 3-1) in this study was collected from several parts of Thailand as showed in Table 1. A voucher specimen of this plant was deposited in herbarium collection of the Department of Medical Science, Ministry of Public Health, Thailand. The samples were dried at 50 °C for 48 hours in hot air oven and were reduced to coarse powder using a grinder. Dried plant powder was kept in well-closed container and protected from light at 4 °C until examination.

Figure 3-1 *Cymbopogon nardus* (L.) Rendle

Table 3-1 Source of plant materials(*C. nardus*) from each part of Thailand.

No.	Sample code	Source (province)	Part of Thailand	Date of collection	Note
1	CN-PS-0101	Bangklam district, Songkhla	South	21-01-2008	With flower
2	CN-PS-0201	Nathawi district, Songkhla	South	29-01-2008	Without flower
3	CN-PS-0301	Khonburi hospital, Nakhon Ratchasima	Northeast	25-04-2008	Without flower
4	CN-PS-0401	Authong hospital, Suphan Buri	Central	2-05-2008	Without flower
5	CN-PS-0501	Phaya-Mengrai hospital, Chiangrai	North	7-05-2008	Without flower
6	CN-PS-0601	Khaokor district, Phetchabun	North	11-05-2008	Without flower
7	CN-PS-0701	Khaokhitchakut hospital, Chanthaburi	East	19-05-2008	Without flower
8	CN-PS-0801	Mueng district, Krabi	South	27-05-2008	Without flower
9	CN-PS-0901	Nayong district, Trang	South	20-06-2008	Without flower
10	CN-PS-1001	Pabon hospital, Phattalung	South	25-06-2008	Without flower
11	CN-PS-1101	Lampang Conservation, Lampang	North	8-07-2008	Without flower

Table 3-1 Source of plant materials (*C. nardus*) from each part of Thailand (continued).

No.	Sample code	Source (province)	Part of Thailand	Date of collection	Note
12	CN-PS-1201	Nop-Pitam district, Nakhonsithammarat	South	14-07-2008	Without flower
13	CN-PS-1301	Ongkharat district, Nakhonnayok	Central	24-07-2008	Without flower
14	CN-PS-1401	Mueng district, Satul	South	4-08-2008	Without flower
15	CN-PS-1501	Mueang district, Ubon Ratchathani	Northeast	17-08-2008	Without flower

3.1.2 Citronella oils

Table 3-2 Source of citronella oil from the purchase products (No.1-8) and steam distillation (No. 9-18).

No.	Sample code	Source and details	Product
1	CN-BS-0101	Citronella oil Thai-China Flavours & Fragrances Industry Co., Ltd. Code No. 40012 N.W. 1.00kg. G.W. 1.11 kg. Mfd. 16-11-2007 Exp. 16-11-2008	5011532/1911
2	CN-BS-0201	Citronella oil Srichand Lenitedted Dispensary Co., Ltd. (Product from Spain) 450 cc. Mfg. 27-8-2005 Exp. 31-5-2010	295

Table 3-2 Source of citronella oil from the purchase products (No.1-8) and steam distillation (No. 9-18) (continued).

No.	Sample code	Source and details	Product
3	CN-BS-0202	Citronella oil Srichand Lenited Dispensary Co., Ltd. (Product from Spain) 450 cc. Mfg. 27-11-2003 Exp. 27-5 2006	156
4	CN-BS-0301	Essential oil The royal project foundation. (10 cc) Kasetsart University Mfg.- Exp.-	-
5	CN-BS-0302	Essential oil The royal project foundation. (10 cc) Kasetsart University Mfg.- Exp.-	-
6	CN-BS-0401	Citronella oil From the market , Hat yai, Songkhla. (100 cc) Mfg.- Exp.-	-
7	CN-BS-0501	Citronella oil (Kasima Back to Nature) Ayuttaya Soi 10, Suan Lum Night Bazaar, Lumpini, Bangkok. 100 cc. Mfg.- Exp.-	-

Table 3-2 Source of citronella oil from the purchase products (No.1-8) and steam distillation (No. 9-18) (continued).

No.	Sample code	Source and details	Product
8	CN-BS-0601	Citronella oil Union Chemical Co., Ltd. (100 cc) Mfg.- Exp.-	-
9	CN-PS-0101	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (2 cc) From Bangklam district, Songkhla Extracted Date: 21-1-2008	01
10	CN-PS-0201	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (1 cc) From Nathawi district, Songkhla Extracted Date: 29-1-2008	01
11	CN-PS-0301	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (2 cc) From Khonburi hospital, Khonburi district, Songkhla Extracted Date: 25-4-2008	01
12	CN-PS-0401	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (5 cc) From Authong hospital, Authong district, Suphan Buri Extracted Date: 2-5-2008	01

Table 3-2 Source of citronella oil from the purchase products (No.1-8) and steam distillation (No. 9-18) (continued).

No.	Sample code	Source and details	Product
13	CN-PS-0501	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (4 cc) From Phaya-Mengrai hospital, Chang rai Extracted Date: 7-5-2008	01
14	CN-PS-0601	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (1.7 cc) From Khaokor district, Phetchabun Extracted Date: 11-5-2008	01
15	CN-PS-0701	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory. (7 cc) Prince of Songkla University From Khaokitchakut hospital, Chanthaburi Extracted Date: 19-5-2008	01
16	CN-PS-0901	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory. (14 cc) Prince of Songkla University From Nayong district, Trang Extracted Date: 20-6-2008	01

Table 3-2 Source of citronella oil from the purchase products (No.1-8) and steam distillation (No. 9-18) (continued).

No.	Sample code	Source and details	Product
17	CN-PS-1001	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (21 cc) From Pabon district, Phattalung Extracted Date: 25-6-2008	01
18	CN-PS-1101	Citronella oil Pharmacognosy and Pharmaceutical Botany laboratory Prince of Songkla University. (14 cc) From Lampang Extracted Date: 8-7-2008	01

3.2 Chemicals and reagents

Table 3-3 Chemicals

Chemical	Source
- Ethanol	- Commercial grade (P.S. Science distributor, Thailand)
- Water	- Distilled (Prince of Songkla University)
- Chloroform	- Commercial grade (P.S. Science distributor, Thailand)
- Hexane	- Commercial grade (P.S. Science distributor, Thailand)
- Ethyl acetate	- Commercial grade (P.S. Science distributor, Thailand)
- Hydrochloric acid	- AR grade (Lab scan analytical Science, Thailand)
- Acetic acid, glacial	- AR grade (Lab scan analytical Science, Thailand)

Table3-3 Chemicals (continued)

Chemical	**Source**
- Sulfuric acid	- AR grade (Baker analysis, Thailand)
- Sodium sulfate anhydrous	- Laboratory reagent grade (Fisher Science, UK.)
- Petroleum ether	- AR grade (OReC, New Zealand)
- Toluene	- AR grade (Baker analysis, U.S.A.)
- Limonene	- AR grade (Sigma-aldrich, U.S.A.)
- Citronellal	- AR grade (Fluka, Switzerland)
- Citronellol	- AR grade (Sigma-Aldrich, U.S.A.)
- Geraniol	- AR grade (Sigma-Aldrich, U.S.A.)
- TLC-plate silica gel GF_{254}	- Merck, Germany
-Anisaldehyde	-AR grade (Fluka, Switzerland)
-Limonene	-AR grade (Sigma-Aldrich, USA)
-Citronellal	-AR grade (Fluka, Switzerland)
-Citronellol	-AR grade (Sigma-Aldrich, USA)
-Citronellyl acetate	-AR grade (Sigma-Aldrich, USA)
-Geranyl acetate	-AR grade (Sigma-Aldrich, USA)
-Geraniol	-AR grade (Sigma-Aldrich, USA)
-Citral	-AR grade (Sigma-Aldrich, USA)

Table 3-4 Reagents

Anisaldehyde spray reagent	Anisaldehyde (5 ml) was mixed with 10 ml of acetic acid and 5 ml of HCl (37%), and then adjusted the volume to 100 ml with distilled water.

Table 3-4 Reagents (continued)

Liebermann-Burchard test	5 ml acetic anhydride and 5 ml concentrated sulphuric acid are added carefully to 50 ml absolute ethanol, while cooling in ice. The reagent must be freshly prepared
Kede reagent	5 ml freshly prepared 3% ethanolic 3,5-dinitrobenzoic acid is mixed with 5 ml 2M NaOH.
Vanillin-sulfuric acid reagent	1% ethanolic vanillin (solution I) 10% ethanolic sulfuric acid (solution II)

3.3 Instruments

Table3-5 General instruments

Instrument	**Model**	**Company**
Hot plate	SLR	Schott Gerate, Germany
Hot air oven	DIN 12880-KI	Memmert, Germany
Muffle furnace	85P	Barkmey Division, U.S.A.
Rotary Evaporator	Laborota 4002	Heidolpht, Germany
Sartorius Moiture Analyzer	MA 100	Sciencetific promotion Co., Ltd, Germany
UV-visible spectrophotometer	Genesis 5	Viber lourment, France
Water bath	WB 14	Memmert, Germany
Refractometer	A88001	Bellinghamt Stanley limited, England
Optical rotation	A065260638	Analytical Lab Science, Japan
Gas Chromatography	5890	Hewlett Packard, U.S.A.
Mass Spectrometry	5972	Hewlett Packard, U.S.A.

3.4 Methods

3.4.1 Monograph of *C. nardus.*

3.4.1.1 Identification of medicinal plants (Thai Herbal Pharmacopoeia, 2007)

Determined the herbal plant to check the shape, size, color, test and use the an organoleptic method. Identify with the sample from the fresh material, dry material and powdered drug should be the macroscopic and microscopic study.

3.4.1.2 Foreign matter (Thai Herbal Pharmacopoeia, 2007)

Foreign matter is material consisting of any or all of the following:

1) part of the organ or organs from which the drug is derived, other than the parts named in the definition and description or for which a limit is prescribed in the monograph.
2) any organs, other than those named in the definition and description. The amount of foreign matter is not more than the percentage prescribed in the monograph. Vegetable drugs are as free as possible from moulds, insects and other animal.

Weigh 100 to 500 g of the substance being examined or the quantity specified in the monograph and spread it in a thin layer. Detect the foreign matter by inspecting with the unaided eye or with the use of 6 x lens. Separate, weigh and calculate the percentage present.

3.4.1.3 Water content (Thai Herbal Pharmacopoeia, 2007)

Introduce 200 ml of toluene and about 2 ml of water into dry flask. Distil for about 2 hours, allow to cool to room temperature and read the water volume to accuracy of 0.05 ml. Place in the flask a quantity of the substance, weighed to the nearest centigram, expected to give about 2 to 3 ml of water. If the substance is of a pasty character, weigh it in a boat of metal foil. Add a few pieces of porous material and heat the flask gently for 15 minutes. When the toluene begins to boil, distil at the rate of 2 drops per second until most of the water has distilled over, and then increase the rate of distillation to about 4 drops per second. When the water has all distilled over, rinse the inside of the condenser tube with toluene. Continue the distillation for 5 minutes, remove the heat, allow the receiving tube to cool to room temperature, and dislodge any droplets of water which adhere to the walls of the receiving tube. When the water and toluene have completely separated, read the volume of water and calculate the percentage present in the substance using the formular:

$$100(n'-n) / p$$

Where p = the weight in g of the substance to be examined,

n = the volume in ml of water obtain in the first distillation, and

n' = the total volume in ml of water obtained in the second distillation.

3.4.1.4 Total ash content (Thai Herbal Pharmacopoeia, 2007)

Place a 2-4 g sample of the ground substance, accurately weighed, or the quantity specified in the monograph, in a suitable tared crucible (usually of platinum or silica), previously ignited, cooled and weighed. Incinerate the sample by gradually increasing the temperature, not exceeding 450 $^{\circ}$C, until free from carbon; cool and weigh. If a carbon-free ash cannot be obtain in this way, cool the crucible and moisture the residue with about 2 ml of water or a saturated solution of ammonium nitrate. Dry on a water-bath and then on a hot plate and incinerate to constant weight. Calculate the percentage of total ash with reference to the air-dried substance.

3.4.1.5 Acid insoluble ash (Thai Herbal Pharmacopoeia, 2007)

Boil the total ash for 5 minutes with 25 ml of dilute 10% hydrochloric acid, collect the insoluble matter on an ashless filter paper, wash with hot water until the filtrate is neutral, and ignite at about 550 $^{\circ}$C. Calculate the percentage of acid-insoluble ash with reference to the air-dried substance.

3.4.1.6 Ethanol soluble extractive value (Thai Herbal Pharmacopoeia, 2007)

Macerate 5 g of the air-dried drug, coarsely powdered and accurately weighed, with 100.0 ml of ethanol of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and then allowing to stand for 18 hours. Filter rapidly, taking precautions against loss of ethanol, evaporate 20.0 ml of the filtrate to dryness in a tared, flat-bottomed, shallow dish and dry at 105 $^{\circ}$C to constant weigh. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried drug.

3.4.1.7 Water soluble extractive value (Thai Herbal Pharmacopoeia, 2007)

Proceed as directed in Ethanol-soluble Extractive but using 0.25% chloroform water in place of ethanol.

3.4.1.8 Hexane soluble extractive value (Thai Herbal Pharmacopoeia, 2007)

By soxhlet apparatus weigh the sample 2 g in a thimble with 100 ml of hexane heat the flask when the hexane begin to boil for 5 hours, allow to cool to room temperature and rinse the solvent in a crucible, accurately weighed, transfer the hexane solution to tared porcelain dish and allow it to evaporate on the water bath. The dry kept in dessicator for 18 hours and weigh.

3.4.1.9 Chloroform soluble extractive value (Thai Herbal Pharmacopoeia, 2007)

Proceed as directed in hexane but using chloroform in place of hexane.

3.4.1.10 Color test (Thai Herbal Pharmacopoeia, 2007)

Liebermann-Burchard test : macerated 1 g of the sample with 20 ml of ethanol of specified strength in a close flask of 3 days and filter (solution) to 2 ml into the crucible and dryness on the water bath. After that add 1 drop of acetic anhydride and add 1 drop of conc. sulfuric acid and the color are detected.

Kede’s reagent test : macerated 1 g of the sample with 20 ml of ethanol of specified strength in a close flask of 3 days and filter (solution) to 2 ml into the test tube. After that add 2 drop of kedd’s and add 2 drop of potassium hydroxide and the color are detected.

Vanillin-sulphuric acid reagent test : macerated 1 g of the sample with 20 ml of ethanol of specified strength in a close flask of 3 days and filter (solution) to 2 ml into the crucible and dryness on the water bath. After that add 2 drop of (solution I) and add 2 drop of (solution II) and the color are detected.

3.4.1.11 Thin-Layer Chromatography (TLC) technique

For determination of *C. nardus* are modified and carried out the test as describe in the “Thin layer Chromatography” Thai Herbal Pharmacopoeia Volume II 2007, Appendix 3.1, using *siliga gel* GF_{254} as the coating substance and mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 5 μl of each of the following solutions. Prepare solution (A) by macerate 10 g of the sample, in powder, with 200 ml of *petroleum ether* and shaking for 24 hours, filtering, and evaporating to a volume of 2 ml. For solution (B), dilute 2 μl of *citronellal* in 5 μl of *petroleum ether*. After removal of the plate, allow it to dry in air, and examine under ultraviolet light (254 and 366 nm), marking the spots. Spray the plate with *anisaldehyde TS* and heat at 105 °C for 5 minutes.

3.4.1.12 Volatile oil content (European Pharmacopoeia commission, 1980)

Volatile oil from citronella grass was extracted by steam distillation showed as in Figure 3-2 . Which the water contaminated in volatile oil was adsorbed by sodium sulphate anhydrous after distillation. Volatile oil was kept for physical properties (relative density, optical rotation and refractive index) and for identification by GC-MS.

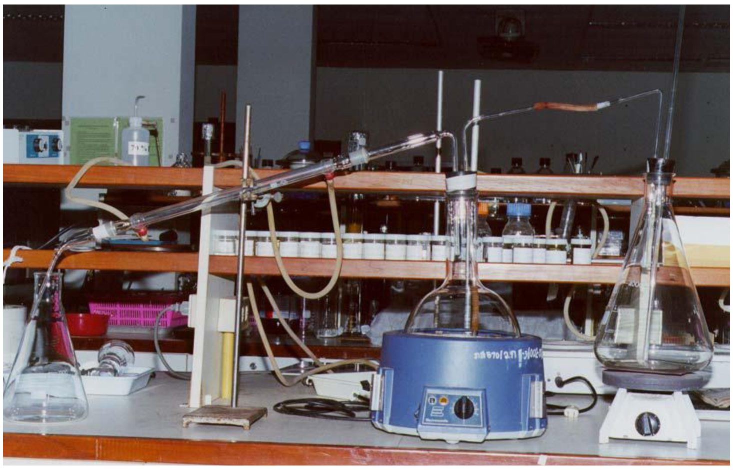


Figure 3-2 Steam distillation apparatus instrument

3.4.2 Monograph of Citronella oils

3.4.2.1 Physical properties

Physical properties of citronella oil were studies such as color, odor, refractive index, optical rotation and relative density.

3.4.2.2 Refractive index (European Pharmacopoeia, 1980)

The refractive index of a medium with reference to air is equal to the ratio of the sine of the angle of refraction of the refracted beam in the given medium. Refractometers normally determine the critical angle. In such apparatus the essential part is a prism of known refractive index in contact with the liquid to be examined.

Figure 3-3 Refractometer

3.4.2.3 Optical rotation (European Pharmacopoeia, 1980)

Optical rotation is the property displayed by certain substances of rotating the plane of polarization polarized light. Determine the zero of the polarimeter and the angle of rotation of polarized light at the wavelength of the D-line of sodium (λ=589.3 nm) at 20$\pm$0.05 $^{\circ}$C. Measurements may be carried out at other temperatures only where the monograph indicates the temperature correction to be made the measured optical rotation. Determine the zero of the apparatus with the tube closed for liquids the zero is determined with the tube empty and for solid filled with the prescribed solvent. Carry out at least five measurements and calculate the average. Calculate the specific optical rotation using the following formulae, dextrorotation and levorotation being designated by (+) and (-), repectively.

Figure 3-4 Optical rotation instruments

3.4.2.4 Relative density (European Pharmacopoeia, 1980)

The relative density of a substance to the mass an equal volume of water, both weighed at 20 $^{\circ}$C. Determine the relative density with precision to the number of decimals prescribed in the monograph, using a pycnometer, a density bottle, a hydrostatic balance or a hydrometer. The thrust of air is disregarded during the weighing ; this may introduce an error of 1 unit in third decimal place.

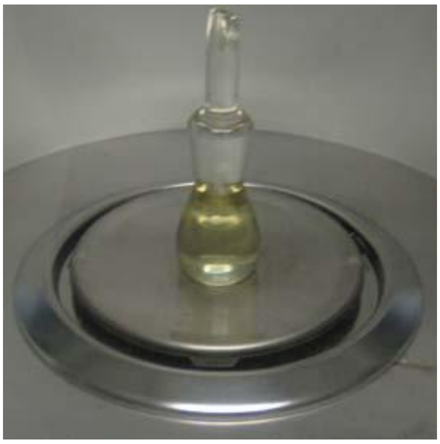


Figure 3-5 Pycnometer

3.4.2.5 Thin-Layer Chromatography (TLC) technique for determination of citronella oil (European Pharmacopoeia, 1980)

Separation of chemical constituents of citronella oils, which are distilled in laboratory and purchased from the market compared with reference standard. Carry out the test as describe in the Thin layer Chromatography using silica gel plate GF_{254} as the coating substance; *toluene* 100 volumes and mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate*, 80 volumes of *toluene* and 20 volumes of *ethyl acetate*, mixture of 90 volumes of *toluene* and 10 volumes of *chloroform* and mixture of 80 volumes of *toluene* and 20 volumes of *chloroform* as the mobile phase. Apply separated:

Test solution :

Dilute citronella oil in 1:10 ml of ethanol and mixed.

Reference solution :

Dilute reference solution in 1:10 ml of ethanol and mixed. Four reference solutions were used as Limonene, Citronellal, Citronellol and Geraniol.

Detection :

Examine in ultraviolet light at 365 nm. After that Spray with anisaldehyde solution and heat at 100-105 $^{\circ}$C for 10 min. Look spots on the TLC after spray and heated.

3.4.2.6 Gas chromatography – mass spectrometry, GC-MS

In this study, the chemical constituents of citronella oil from steam distillation (10 samples) and purchased products (8 samples) were compared by using GC-MS, which the conditions are as followed :

Gas Chromatography (Hewlett Packard U.S.A. company):

Inlet temperature : 260 °C, Split ratio 1 : 50

Oven temperature : Initial temperature 80 °C, 2 min,

Ramp to 150 °C at 3 °C/min.

Ramp to 185 °C at 2 °C/min.

Ramp to 250 °C at 10 °C/min.

Column : HP Innowax 30 m x 0.25 mm I.D., 0.25 μm

Mass Spectrometer (Hewlett Packard U.S.A. company) :

Ionization mode : Electron Ionization

Acquisition mode : Scan, 35-500 amu

Transferline temperature : 260 °C

Solvent delay time : 3.0 min.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Monograph of *Cymbopogon nardus* (L.) Rendle

4.1.1 Description of Species *C. nardus*

Parennial from a stout rootstock. Culms tufted, robust, up to 2.5 m tall, 1-2 cm in diam. Leaves sheaths reddish purple at base, smooth, glabrous; leaf blades dark green or dark brown when dry, drooping for 1/3 of their length, 50-150 cm long 1-3 cm wide, glabrous, abaxial surface scaberulous, adaxial surface smooth, base narrow, apex long acuminate; ligule 2-3 mm. Spathate panicle large, narrow, congested, interrupted, 60-90 cm; spatheoles reddish brown, 1.2-2.5 cm; racemes 1-1.5 cm; rachis internodes and pedicels ciliate on margins; pedicel of homogamous pair not swollen. Sessile spikelet oblong-lanceolate, 3-4.5 × 1-1.2 mm; lower glume flat or slightly concave, reddish brown or purplish upward, sharply 2-keeled, keels narrowly winged, obscurely 0-3-veined between keels; upper lemma linear, entire or slightly 2-lobed, mucronate or very shortly awned. Pedicelled spikelet 3.5-7 mm (Figure 4-1)

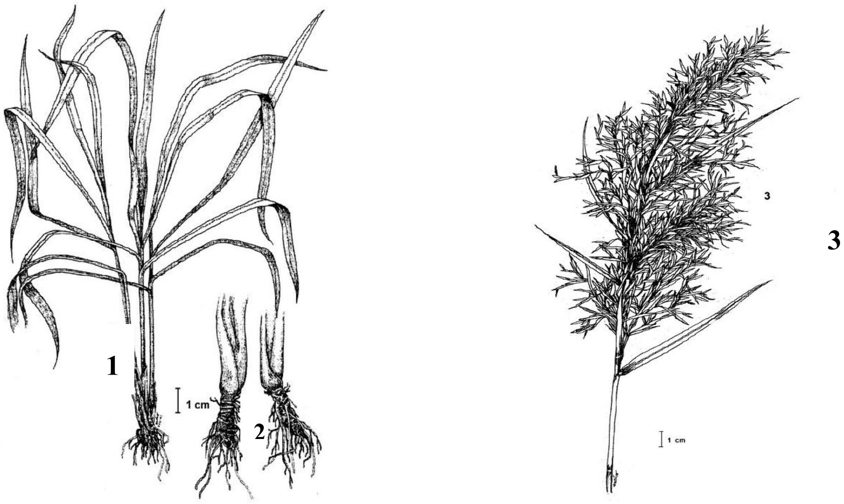


Figure 4-1 *C. nardus*

1. the whole plant 2. rhizome 3. inflorescence

4.1.1.1 Macroscopical characters

The crude drug (Figure 4-2) consists of culm and leaf-blades which occurs in pieces about 2-5 cm in length, and 0.5-1 cm wide. The dried leaf-blades are pale green, thin and scaberulous. The crude drug has characteristic odor.

Figure 4-2 *C. nardus*

1. plant; 2. leaf; 3. stem; 4. inflorescence; 5. rhizome; 6. crude drug

2.

3.

4.

4.

5.

5.

Figure 4-2 *C. nardus* (continued)

6.

6.

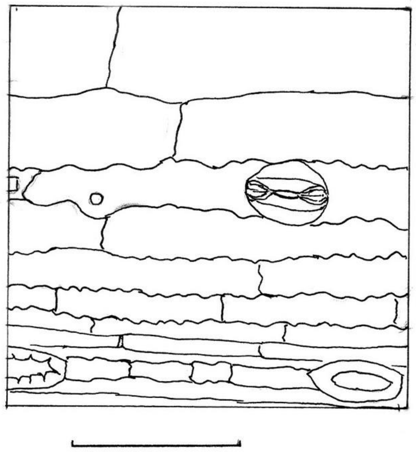
6.

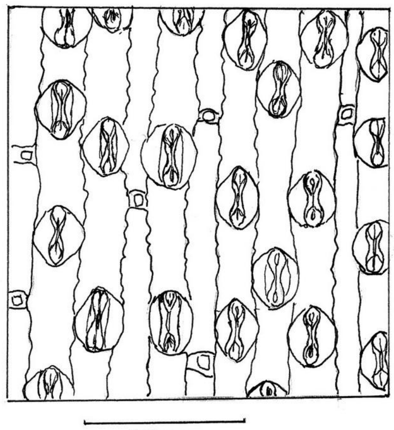
1.0 cm

Figure 4-2 *C. nardus* (continued)

4.1.1.2 Microscopical characters

Surface preparations show that stomata are present on both surface, but are more numerous on the lower one. The stomata are grass type; consist of bone shape guard cell and 2 subsidiary cells with their long axes parallel to the pore. The epidermal cells with wavy outline. Oil globules are usually present in parenchyma (Figure 4-3a).

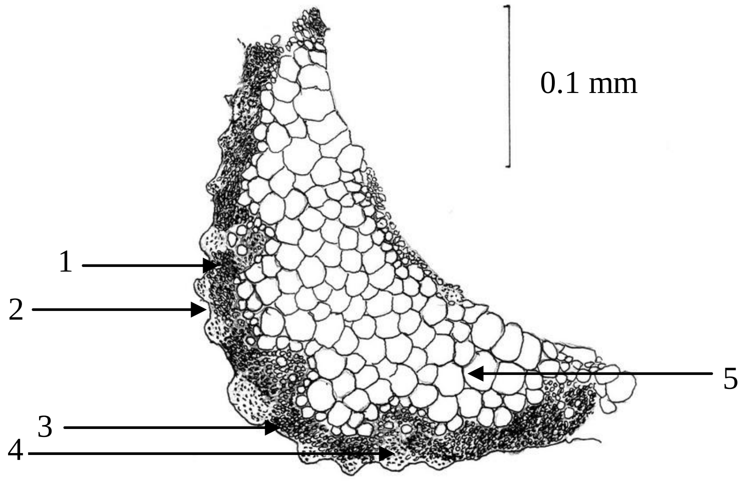




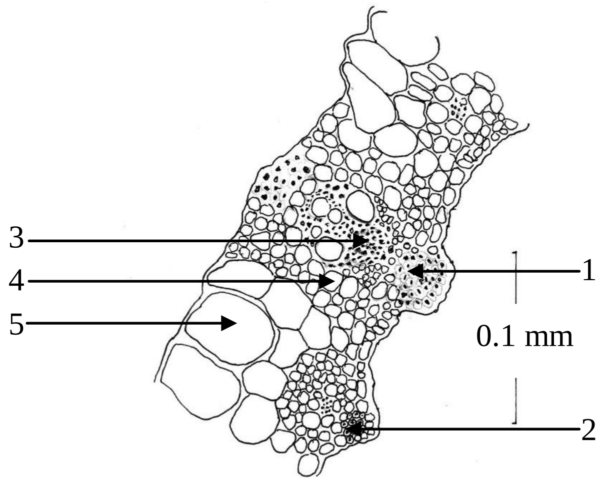
Upper Epidermis of the Lamina **Lower Epidermis of the Lamina**

Figure 4-3a Epidermal cells of the leaf of *C. nardus*

A transverse section of leaf-blade shows that it has a bifacial structure. The upper epidermis; a single layer of rectangular cells, with few unicellular trichome. Mesophyll consisting of several layers of parenchyma rich in chloroplastids probably distinctly radiate around most of the individual vascular bundle. A zone of collenchyma, found beneath upper epidermis in the vein region. Vascular bundles surrounded by sclerenchymatous layer of bundle sheath. Xylem composed of pitted and annular vessels. Lower epidermis, a single layer of rectangular cells with collenchyma underneath and bulliform cells (Figure 4-3b).



Transverse section of the Midrib T.S. (x100)



Transverse section of Lamina T.S. (x400)

Figure 4-3b Transverse section of the leaf of *C. nardus*

1. collenchyma
2. mesophyll
3. vascular bundle
4. parenchyma
5. bulliform cell

Citronella grass in powder possesses the diagnostic microscopical characters of the unground drug (Figure 4-3c and 4-3d).

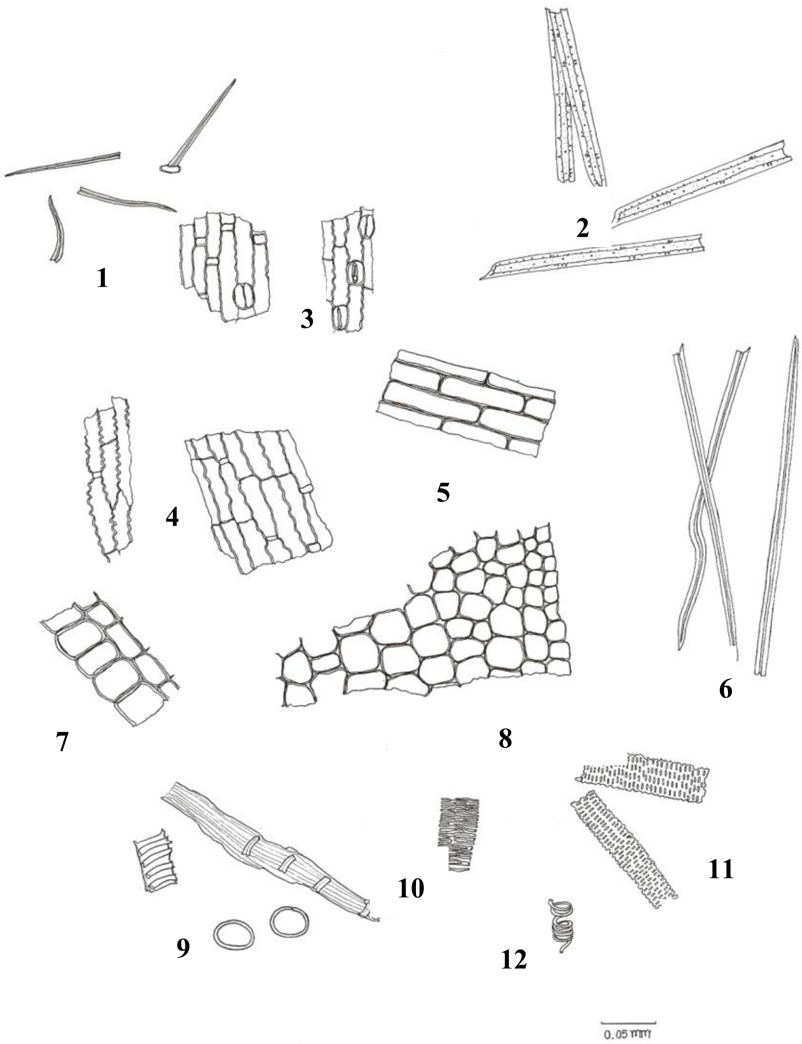


Figure 4-3c Powdered drug of the leaf of *C. nardus*

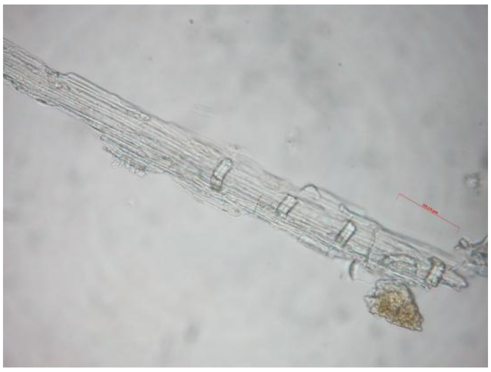
1. unicellular trichome
2. sclereids
3. lower epidermis in surface view showing grass type stomata
4. upper epidermis
5. parenchyma in surface view
6. fragment of fiber
7. bulliform cells
8. parenchyma in sectional view
9. annular vessels
10. reticulated vessel
11. pitted vessels
12. spiral vessel

1. **1.**

Figure 4-3d Powdered drug images of the aerial part of *C. nardus*

1. annular vessel
2. fragment of fiber
3. bulliform cell
4. lower epidermis in surface view showing grass type stomata
5. parenchyma in longitudinal view
6. parenchyma in sectional view
7. pitted vessel
8. reticulated vessel
9. sclereid
10. spiral vessel
11. unicellular trichome
12. upper epidermis

1.



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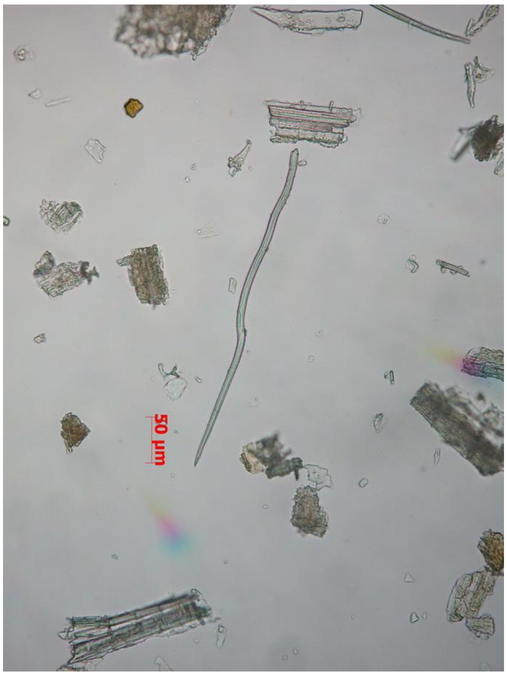

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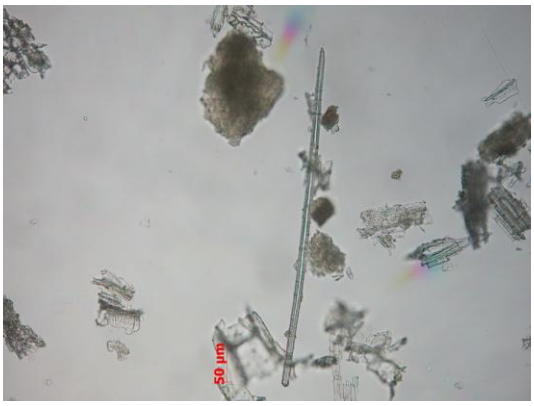

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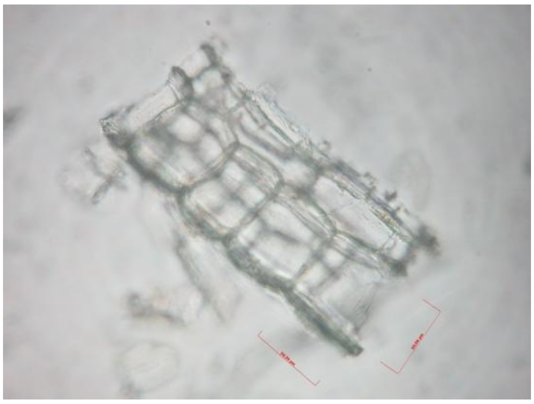
Figure 4-3d (continued)



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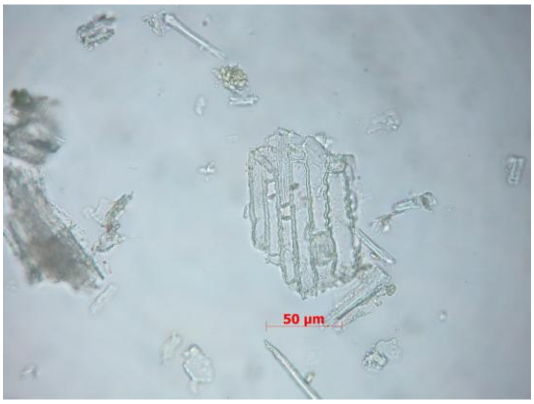

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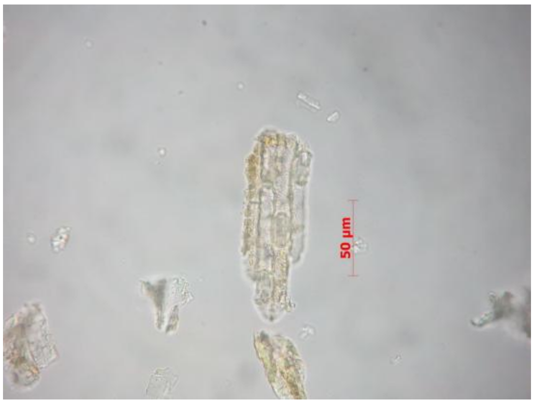

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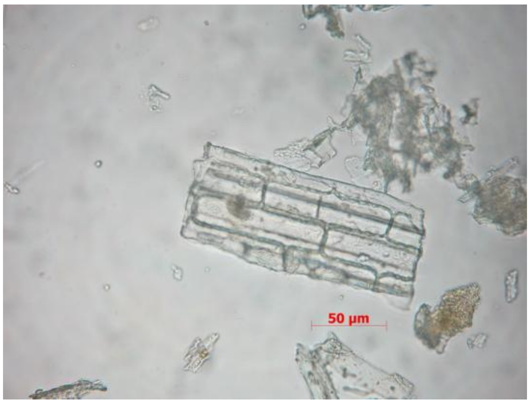

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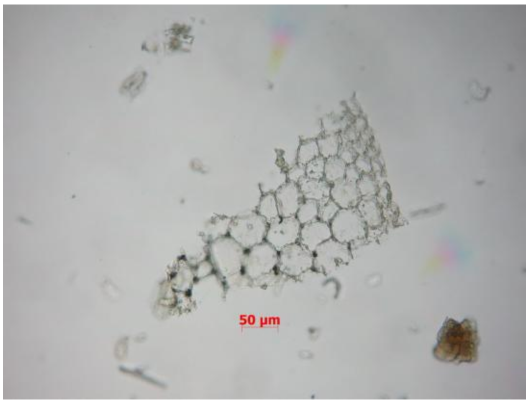
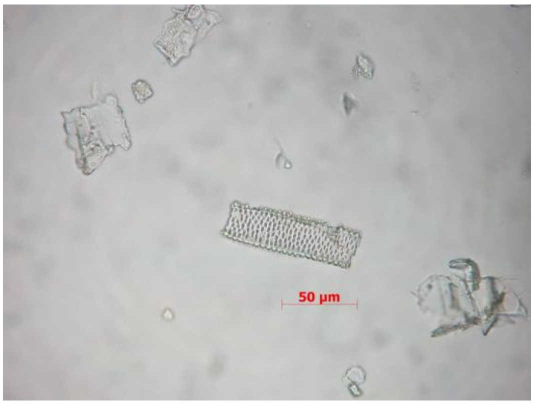


6.

Figure 4-3d (continued)

7.



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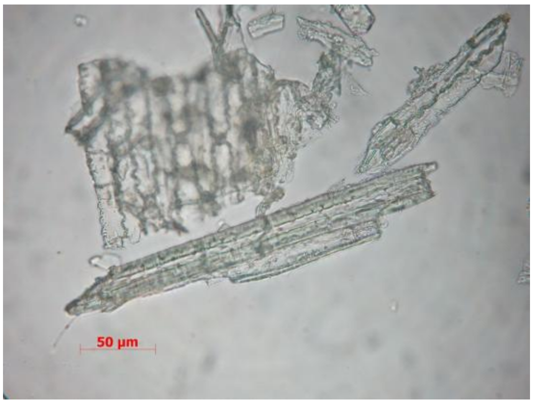


9.

9.

Figure 4-3d (continued)

10.

11.

11.

12.

12.

12.

Figure 4-3d (continued)

4.1.2 Foreign matter

In this study, the results of the foreign matter in the plant materials (15samples) were not more than 2.0 percent w/w.

4.1.3 Water content

The water content was determined by Azeotropic distillation method. And in this study, the result showed the average of water content in *C. nardus* is 2.56 ±0.98 v/w as showed in Table 4-1.

Table 4-1 Determination of water content.

No.	Sample code	Water content (% v/w)
1	CN-PS-0101	2.0
2	CN-PS-0201	1.6
3	CN-PS-0301	2.8
4	CN-PS-0401	2.4
5	CN-PS-0501	3.4
6	CN-PS-0601	3.4
7	CN-PS-0701	3.6
8	CN-PS-0801	1.2
9	CN-PS-0901	1.6
10	CN-PS-1001	1.6
11	CN-PS-1101	3.8
12	CN-PS-1201	2.0
13	CN-PS-1301	3.2
14	CN-PS-1401	1.6
15	CN-PS-1501	4.2
Average		2.56 ±0.98

4.1.4 Total ash content

The average of total ash content in *C. nardus* is 6.49 ± 1.11 w/w. The results are showed in Table 4-2.

Table 4-2 The percentage of total ash content.

No.	Sample code	%Total ash content ± SD
1	CN-PS-0101	6.77±0.04
2	CN-PS-0201	6.12±0.01
3	CN-PS-0301	8.65±0.01
4	CN-PS-0401	8.33±0.00
5	CN-PS-0501	8.09± 0.01
6	CN-PS-0601	6.74± 0.02
7	CN-PS-0701	6.37 ±0.02
8	CN-PS-0801	6.02 ±0.01
9	CN-PS-0901	6.96± 0.02
10	CN-PS-1001	5.11± 0.00
11	CN-PS-1101	5.29± 0.00
12	CN-PS-1201	5.57± 0.00
13	CN-PS-1301	5.78± 0.00
14	CN-PS-1401	5.85± 0.02
15	CN-PS-1501	5.69± 0.00
Average		6.49 ± 1.11

4.1.5 Acid insoluble ash

In this study, the result showed the average of acid insoluble ash in *C. nardus* is 2.46 ± 0.13 w/w as showed in Table 4-3.

Table 4-3 The percentage of acid insoluble ash.

No.	Sample code	%Acid insoluble ash ± SD
1	CN-PS-0101	2.33±0.00
2	CN-PS-0201	2.46±0.00
3	CN-PS-0301	2.49±0.00
4	CN-PS-0401	2.34±0.00
5	CN-PS-0501	2.84±0.00
6	CN-PS-0601	2.38±0.00
7	CN-PS-0701	2.32±0.01
8	CN-PS-0801	2.51±0.01
9	CN-PS-0901	2.47±0.00
10	CN-PS-1001	2.41±0.00
11	CN-PS-1101	2.42±0.01
12	CN-PS-1201	2.38±0.01
13	CN-PS-1301	2.48±0.00
14	CN-PS-1401	2.54±0.00
15	CN-PS-1501	2.53±0.01
Average		2.46%± 0.13

4.1.6 Extractives value

The average of solvent soluble extractive value from ethanol soluble extractive, water soluble extractive, hexane soluble extractive and chloroform soluble extractive of 15 samples of citronella grass are 6.27 ±1.33 w/w, 8.04 ± 1.26 w/w, 3.22 ±1.30 w/w and 7.51 ±3.33 w/w, respectively and are showed in

Table 4-4.

Table 4-4 Solvent soluble extractive values.

No.	Sample code	Solvent soluble extractive value (% w/w)			
		Ethanol soluble extractive	Water soluble extractive	Hexane soluble extractive	Chloroform soluble extractive
1	CN-PS-0101	3.68±0.05	6.93±0.95	1.83±0.00	5.41±0.00
2	CN-PS-0201	4.33±0.09	6.42±1.05	1.24±0.01	5.49±0.01
3	CN-PS-0301	7.13±0.14	8.44±0.34	2.72±0.01	5.45±0.00
4	CN-PS-0401	5.56±0.24	9.62±0.27	3.41±0.01	4.86±0.00
5	CN-PS-0501	6.91±0.05	8.53±0.29	2.30±0.00	5.39±0.00
6	CN-PS-0601	5.57±0.15	6.81±0.93	3.07±0.00	6.65±0.01
7	CN-PS-0701	7.65±0.28	9.58±0.20	2.63±0.01	5.14±0.00
8	CN-PS-0801	6.66±0.10	6.59±0.52	2.50±0.01	5.19±0.01
9	CN-PS-0901	6.59±0.43	9.45±0.29	3.13±0.03	8.23±0.02
10	CN-PS-1001	6.18±0.31	6.27±0.29	4.21±0.01	6.04±0.01
11	CN-PS-1101	7.58±0.33	9.60±0.40	4.97±0.01	9.60±0.02
12	CN-PS-1201	4.11±0.09	8.94±0.41	3.79±0.02	7.34±0.01
13	CN-PS-1301	7.65±0.26	8.51±0.40	2.44±0.00	16.94±0.03
14	CN-PS-1401	6.85±0.05	7.43±0.37	3.45±0.01	8.71±0.03
15	CN-PS-1501	7.52±0.22	7.39±0.25	6.54±0.01	12.22±0.03
Average		6.27%±1.33	8.04%±1.26	3.22%±1.30	7.51%±3.33

4.1.7 Color test of *C. nardus*

The results of Liebermann-Burchard test were shown in Table 4- 5.

Table 4-5 The results of Liebermann-Burchard test.

No.	Sample code	Color
1	CN-PS-0101	Dark green
2	CN-PS-0201	Dark brown
3	CN-PS-0301	Pale brown
4	CN-PS-0401	Green strength brown
5	CN-PS-0501	Dark brown
6	CN-PS-0601	Pale brown
7	CN-PS-0701	Dark brown
8	CN-PS-0801	Dark brown
9	CN-PS-0901	Green
10	CN-PS-1001	Green
11	CN-PS-1101	Green strength brown
12	CN-PS-1201	Green strength brown
13	CN-PS-1301	Green strength brown
14	CN-PS-1401	Green
15	CN-PS-1501	Green strength brown

Table 4-6 The results of Kede reagent test.

No.	Sample code	Color
1	CN-PS-0101	Green
2	CN-PS-0201	Green
3	CN-PS-0301	Green
4	CN-PS-0401	Green
5	CN-PS-0501	Green
6	CN-PS-0601	Green
7	CN-PS-0701	Green
8	CN-PS-0801	Green
9	CN-PS-0901	Green
10	CN-PS-1001	Green
11	CN-PS-1101	Green
12	CN-PS-1201	Green
13	CN-PS-1301	Green
14	CN-PS-1401	Green
15	CN-PS-1501	Green

Table 4-7 The results of Vanillin-sulphuric acid reagent test.

No.	Sample code	Color
1	CN-PS-0101	Green
2	CN-PS-0201	Green
3	CN-PS-0301	Green
4	CN-PS-0401	Green
5	CN-PS-0501	Green
6	CN-PS-0601	Green
7	CN-PS-0701	Green
8	CN-PS-0801	Green
9	CN-PS-0901	Green
10	CN-PS-1001	Green
11	CN-PS-1101	Green
12	CN-PS-1201	Green
13	CN-PS-1301	Green
14	CN-PS-1401	Green
15	CN-PS-1501	Green

4.1.8 Thin layer chromatography (TLC)

From the results of TLC chromatogram (Figure 4-4) and hR_f value of *C. nardus* extract (Table 4-8) and citronellal standard, after detected under UV light 254 nm and 366 nm, both and detected with anisaldehyde TS, they showed totally 16 sports. And the spot with hR_f value 86.0 to 88.7 is corresponding to citronella. Because they had the same hR_f value and the same characteristic properties when detected under UV 254 and 366 nm, both and detected with anisaldehyde TS. Several other spots of different colors are observed (Table 4-8); see also Figure 4-4.

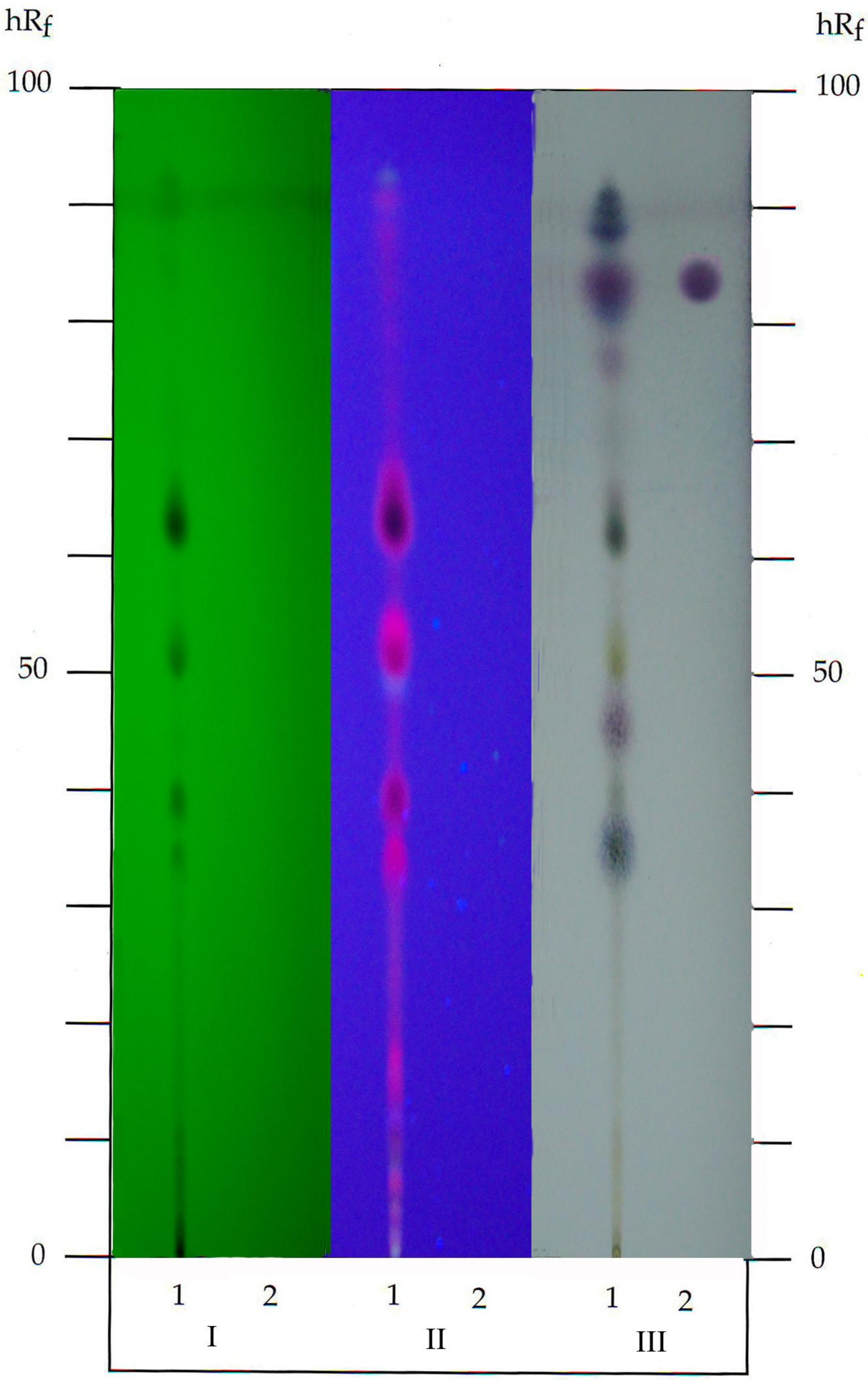


Figure 4-4 Thin layer chromatography of the *C. nardus*

1 = Sample (CN-PS-1301)

2 = Standard of citronellal

I = detection under UV light (254 nm)

II = detection under UV light (366 nm)

III = detection with anisaldehyde TS

Table 4-8 The hR_f Value from TLC of *C. nardus* extract

Detection with				
Spot	**hR_f Value**	**UV 254**	**UV 366**	**Anisaldehyde TS**
1	3.3-5.3	green	red fluorescence	pink
2	6.0-6.7	yellow	red fluorescence	yellow-orange
3	7.3-8.7	green-blue	red fluorescence	pink-violet
4	14.7-16.0	-	-	pale pink
5	28.7-30.7	-	-	green
6	32.7-36.7	-	-	dark blue
7	45.3-47.3	yellow-orange	red fluorescence	violet
8	49.3-51.3	-	-	brown
9	60.0-62.7	green	red fluorescence	pale violet
10	66.7-68.7	yellow	red fluorescence	pink-violet
11	78.7-80.7	dark green	red fluorescence	dark violet
12	84-85.3	-	-	dark blue
13*	86.0-88.7	-	-	red-violet
14	92.0-92.7	-	-	violet
15	94.0-95.3	-	-	dark pink
16	96.0-96.7	yellow	red fluorescence	brown
*Citronellal		-	-	red-violet
*Citronellal = Standard				

4.1.9 Volatile oil

Volatile oils were extracted by steam distillation method. Fifteen samples of citronella grass were collected from each part of Thailand and distilled for volatile oil products. And the average of volatile oil from 15 samples is 0.10±0.07 v/w. The results are showed in Table 4-9.

Table 4-9 Volatile oil content

Sample code	Weight (g)	Volume(ml)	% v/w
CN-PS-0101	6000	2.0	0.03
CN-PS-0201	3000	1.0	0.03
CN-PS-0301	4000	2.0	0.05
CN-PS-0401	4000	5.0	0.12
CN-PS-0501	4000	4.0	0.10
CN-PS-0601	4000	1.7	0.04
CN-PS-0701	4000	7.0	0.17
CN-PS-0801	3000	0.5	0.01
CN-PS-0901	13000	14.0	0.10
CN-PS-1001	16000	21.0	0.13
CN-PS-1101	6000	14.0	0.23
CN-PS-1201	10000	15.0	0.15
CN-PS-1301	4000	7.0	0.17
CN-PS-1401	1500	2.0	0.13
CN-PS-1501	3500	0.5	0.01

4.2 Monograph of citronella oil

4.2.1 Physical property

Table 4-10 The description of citronella oil**.**

No.	Sample code	Description	Solubility	Sample image
1	CN-BS-0101	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
2	CN-BS-0201	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
3	CN-BS-0202	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
4	CN-BS-0301	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	

Table 4-10 The description of citronella oil (continued).

No.	Sample code	Description	Solubility	Sample image
5	CN-BS-0302	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
6	CN-BS-0401	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
7	CN-BS-0501	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
8	CN-BS-0601	Pale yellow liquid, with a very strong odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	

Table 4-10 The description of citronella oil (continued).

No.	Sample code	Description	Solubility	Sample image
9	CN-PS-0101	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
10	CN-PS-0201	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
11	CN-PS-0301	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
12	CN-PS-0401	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	

Table 4-10 The description of citronella oil (continued)**.**

No.	Sample code	Description	Solubility	Sample image
13	CN-PS-0501	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
14	CN-PS-0601	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
15	CN-PS-0701	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
16	CN-PS-0901	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	

Table 4-10 The description of citronella oil (continued).

No.	Sample code	Description	Solubility	Sample image
17	CN-PS-1001	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	
18	CN-PS-1101	Pale yellow liquid, with a mild odor of citronellal	soluble in ethanol, methanol, ethyl acetate, chloroform, hexane insoluble in water	

All of citronella oils from the purchase product are pale yellow liquid and strong odor. And all of citronella oils from the steam distillation in laboratory are pale yellow liquid and mild odor.

4.2.2 Refractive index

The refractive index of citronella oils from the purchase product and from the plant extract are between 1.458 to 1.484. The refractive index of 18 samples are showed in Table 4-11.

Table 4-11 The refractive index of citronella oils.

No.	Sample code	Refractive index ± SD
1	CN-BS-0101	1.470 ± 0.000
2	CN-BS-0201	1.471 ± 0.000
3	CN-BS-0202	1.471 ± 0.000
4	CN-BS-0301	1.469 ± 0.000
5	CN-BS-0302	1.468 ± 0.000
6	CN-BS-0401	1.458 ± 0.000
7	CN-BS-0501	1.484 ± 0.000
8	CN-BS-0601	1.469 ± 0.000
9	CN-PS-0101	1.483 ± 0.000
10	CN-PS-0201	1.470 ± 0.000
11	CN-PS-0301	1.480 ± 0.000
12	CN-PS-0401	1.475 ± 0.000
13	CN-PS-0501	1.478 ± 0.000
14	CN-PS-0601	1.475 ± 0.000
15	CN-PS-0701	1.470 ± 0.000
16	CN-PS-0901	1.474 ± 0.000
17	CN-PS-1001	1.472 ± 0.000
18	CN-PS-1101	1.476 ± 0.000

4.2.3 Optical rotation

The optical rotation of citronella oils from the purchase product and from the plant product are between -0.054 to 2.344. One sample from Lampang is a lowest and one sample of citronella oil from Chiang Mai province is hightest. The optical rotation of 18 samples are showed in Table 4-12.

Table 4-12 The optical rotation of citronella oils.

No.	Sample code	Average ± SD
1	CN-BS-0101	-0.448 ± 0.031
2	CN-BS-0201	-0.432 ± 0.003
3	CN-BS-0202	-0.426 ± 0.002
4	CN-BS-0301	-0.334 ± 0.043
5	CN-BS-0302	-0.321 ± 0.006
6	CN-BS-0401	-0.146 ± 0.007
7	CN-BS-0501	2.344 ± 0.000
8	CN-BS-0601	-0.324 ± 0.002
9	CN-PS-0101	0.124 ± 0.000
10	CN-PS-0201	0.238 ± 0.0054
11	CN-PS-0301	-0.087 ± 0.0008
12	CN-PS-0401	-0.192 ± 0.0031
13	CN-PS-0501	-0.070 ± 0.003
14	CN-PS-0601	0.122 ± 0.002
15	CN-PS-0701	0.212 ± 0.013
16	CN-PS-0901	0.125 ± 0.001
17	CN-PS-1001	0.141 ± 0.001
18	CN-PS-1101	-0.054 ± 0.004

4.2.4 Relative density

The relative density of citronella oils from the purchase product and from the plant extract are between 0.878 to 0.902. The relative density of 18 samples are showed in Table 4-13.

Table 4-13 The relative density of citronella oils.

No.	Sample code	Relative density ± SD
1	CN-BS-0101	0.886 ± 0.000
2	CN-BS-0201	0.886 ± 0.000
3	CN-BS-0202	0.889 ± 0.000
4	CN-BS-0301	0.888 ± 0.000
5	CN-BS-0302	0.883 ± 0.000
6	CN-BS-0501	0.902 ± 0.000
7	CN-BS-0601	0.887 ± 0.000
8	CN-PS-0901	0.882 ± 0.002
9	CN-PS-1001	0.878 ± 0.000
10	CN-PS-1101	0.885 ± 0.000

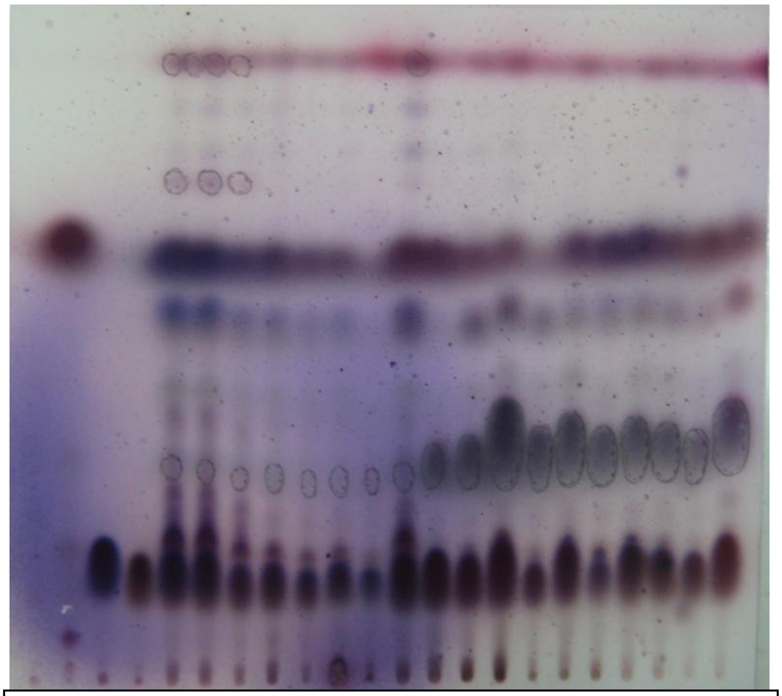
4.2.5 Thin layer chromatography of citronella oil

Eighteen samples of citronella oil are from the purchase products and extraction in order to use for study of the chemical constituents in citronella oil on the TLC-fingerprint profile. Five of solvents system are used to be the mobile phase and four reference standards are as Limonene (A), Citronellal (B), Citronellol (C) and Geraniol (D). The results are showed in Table 4-14 and Figure 4-5 - 4-9.

Table 4-14 The hR_f value of reference standards in different solvent systems.

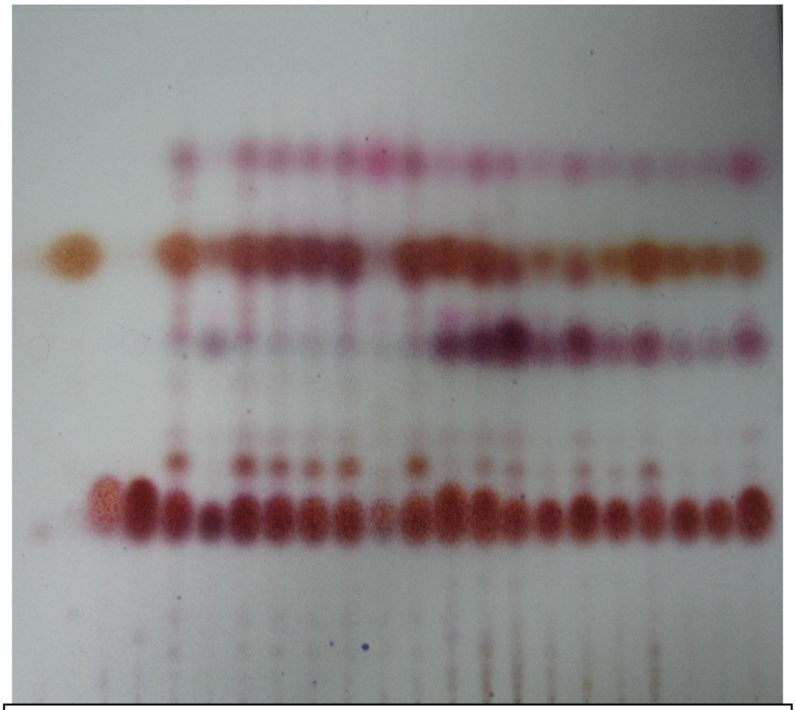
Reference standard	hR_f value				
	1 Toluene	2 EtOAc:Toluene	3 EtOAc:Toluene	4 $CHCl_3$:Toluene	5 $CHCl_3$:Toluene
*Limonene (A)	N/A	N/A	N/A	N/A	N/A
Citronellal (B)	61-63	66-68	59-61	58-60	42-44
Citronellol (C)	15-17	27-29	29-31	22-24	29-31
Geraniol (D)	13-15	25-27	28-30	21-23	28-30

*Limonene did not detect with anisaldehyde solution



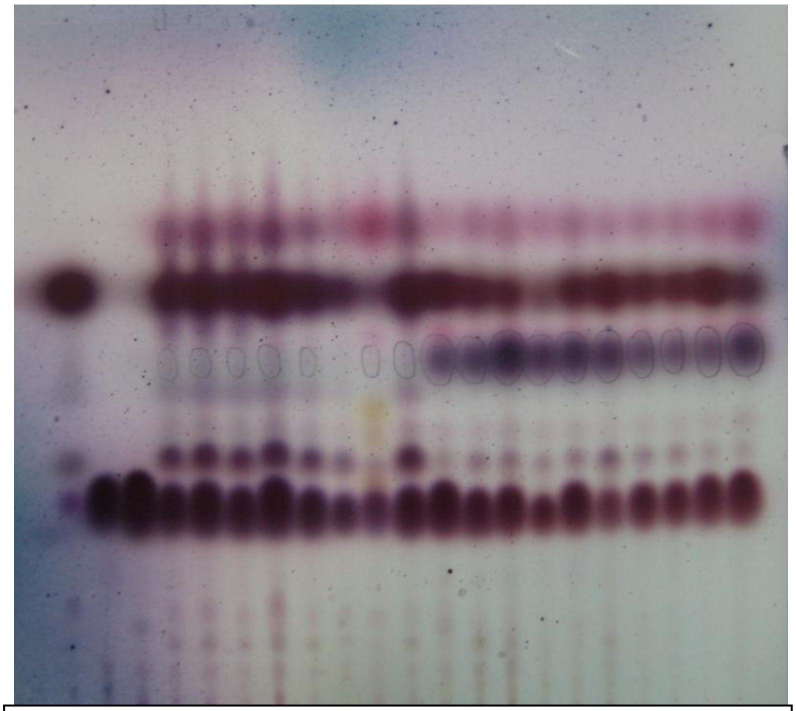
A B C D 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure 4-5 TLC-Fingerprint of citronella oil using toluene as mobile phase



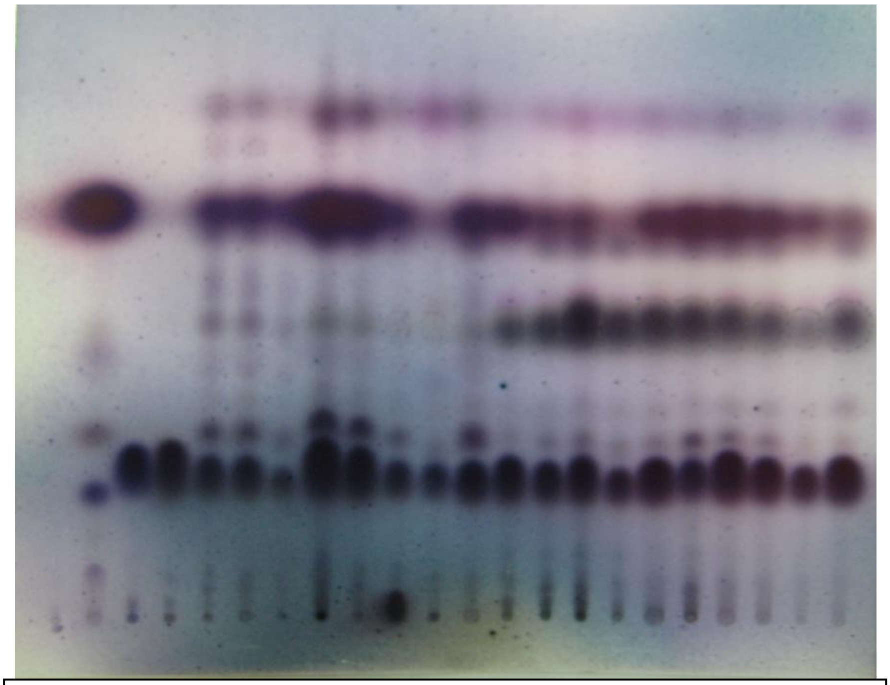
A B C D 1 2 3 4 5 6 7 8 9 10 11 12 1314 15 161718

Figure4-6 TLC-Fingerprint of citronella oil using ethyl acetate and toluene (1:9) as mobile phase



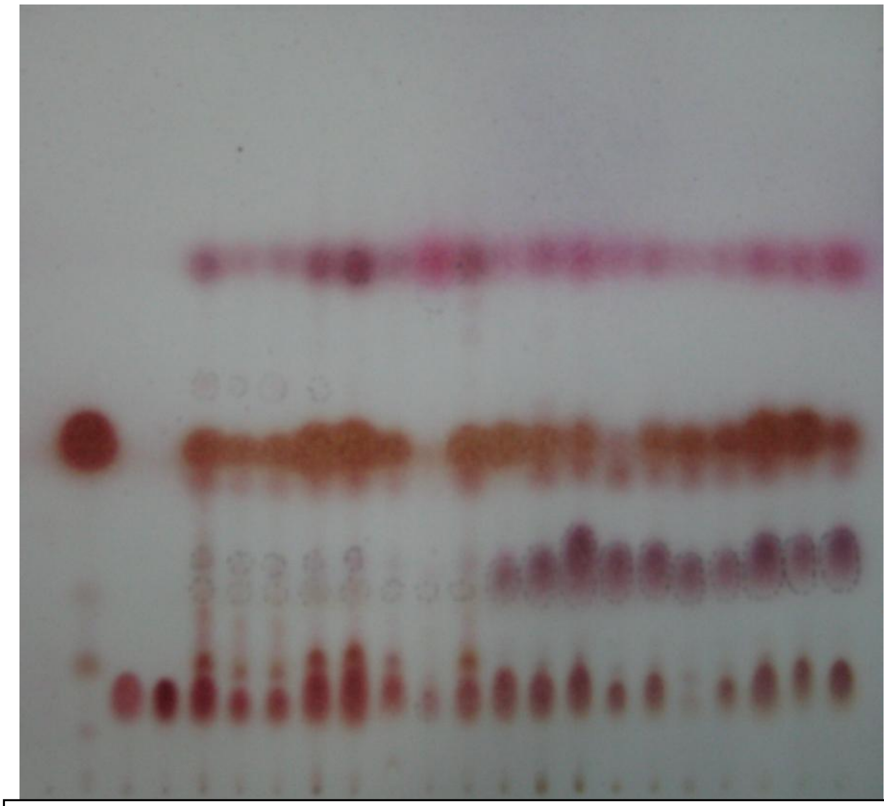
A B C D 1 2 3 4 5 6 7 8 9 10 11 121314 15 16 1718

Figure4-7 TLC-Fingerprint of citronella oil using ethyl acetate and toluene (1:4) as mobile phase



A B C D 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure 4-8 TLC-Fingerprint of citronella oil using chloroform and toluene (1:9) as mobile phase



A B C D 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure 4-9 TLC-Fingerprint of citronella oil using chloroform and toluene (1:4) as mobile phase

4.2.6 Gas chromatography – mass spectrometry

The results from GC-MS are showed in Table 4-15 and 4-16. From the results showed that the chemical constituents of citronella oil from purchase products and from steam distillation in laboratory were different.

Table 4-15 The chemical constituents in citronella oils.

No.	Sample code	Time (min)	Relative area (%)	Chemical constituents
1	CN-BS-0101	4.014	2.318	α-Pinene
		10.917	39.73	Citronellal
		17.901	2.068	Germacrene-d
		19.671	3.21	Δ-Cadinene
		20.335	10.313	Citronellol
		23.127	18.437	Geraniol
		30.623	3.953	Valencene
		33.67	1.406	Eugenol
2	CN-BS-0201	10.891	37.076	Citronellal
		17.896	1.837	Germacrene-d
		19.675	3.082	Δ-Cadinene
		20.34	10.212	Citronellol
		23.132	19.643	Geraniol
		33.675	2.194	Eugenol
3	CN-BS-0202	10.915	39.86	Citronellal
		17.879	1.655	Germacrene-D
		19.659	3.339	Δ-Cadinene
		20.323	9.511	Citronellol
		23.136	19.766	Geraniol
		33.669	1.265	Eugenol

Table 4-13 The chemical constituents in citronella oils (continued).

No.	Sample code	Time (min)	Relative area %	Chemical constituents
3	CN-BS-0202	10.915	39.86	Citronellal
		17.879	1.655	Germacrene-D
		19.659	3.339	Δ-Cadinene
		20.323	9.511	Citronellol
		23.136	19.766	Geraniol
		33.669	1.265	Eugenol
4	CN-BS-0301	10.87	33.2	Citronellol
		19.665	4.288	Δ-Cadinene
		20.309	9.236	Citronellol
		23.131	20.966	Geraniol
		33.654	1.64	Eugenol
5	CN-BS-0302	4.339	1.58	Limonene
		11.017	36.193	Citronellal
		14.065	3.406	*trans*-Caryophyllene
		17.848	1.677	Germacrene-d
		19.73	4.696	β-Cadinene
		23.156	23.347	Geraniol
		33.597	2.173	Eugenol
6	CN-BS-0401	4.33	1.039	Limonene
		10.875	26.454	Citronellal
		13.974	2.465	β-Elemene
		19.537	3.446	β-Cadinene
		19.772	2.458	Geranyl propionate
		33.588	1.911	Eugenol

Table 4-13 The chemical constituents in citronella oils (continued).

No.	Sample code	Time (min)	Relative area %	Chemical constituents
7	CN-BS-0501	3.579	1.718	Benzene, ethyl-
		4.448	15.081	Limonene
		5.01	0.47	γ-Terpinene
		5.675	1.128	Terpiolene
		5.777	0.651	α-Terpinolene
		13.6	26.289	Valencene
		14.388	7.356	*trans*-Caryophyllene
		19.552	0.853	β-Cadinene
		23.571	0.396	Benzyl alcohol
		27.948	9.945	Benzene, 1,1'-oxybis
		33.562	0.435	Eugenol
8	CN-BS-0601	4.339	1.824	Limonene
		10.976	28.571	Citronellal
		12.663	0.864	α-Terpinene
		13.093	0.781	Isopulegol
		14.064	3.585	β-Elemene
		17.848	1.574	Germacrene d
		18.472	1.201	α-Murolene
		33.607	2.502	Eugenol
9	CN-PS-0101	39.662	3.363	Hexadecanoic acid, 15-methyl, methyl ester
10	CN-PS-0201	10.879	37.598	Citronellal
		20.297	9.865	Citronellol
		23.212	36.485	Geraniol
		33.622	0.277	Eugenol

Table 4-15 The chemical constituents in citronella oils (continued).

No.	Sample code	Time (min)	Relative area %	Chemical constituents
11	CN-PS-0301	4.324	0.167	d1-Limonene
		14.152	1.851	*trans*-Caryophyllene
		19.296	29.08	Citral
		33.592	1.051	Eugenol
12	CN-PS-0401	10.832	18.534	Citronellal
		14.156	1.849	*trans*-Caryophyllene
		19.596	1.654	α-amorphene
		23.268	39.723	Geraniol
13	CN-PS-0501	4.308	0.116	Limonene
		14.146	2.033	*trans*-Caryophyllene
		19.637	0.339	Δ-Cadinene
		23.227	36.17	Geraniol
		33.576	1.222	Eugenol
		40.284	0.317	Isoeugenol
14	CN-PS-0601	10.852	20.8	Citronellal
		14.104	1.134	*trans*-Caryophyllene
		19.637	2.382	α-amorphene
		23.247	36.733	Geraniol
		33.565	1.144	Eugenol
15	CN-PS-0701	4.313	0.081	Limonene
		10.96	35.557	Citronellal
		40.268	0.283	Isoeugenol

Table 4-15 The chemical constituents in citronella oils (continued).

No.	Sample code	Time (min)	Relative area %	Chemical constituents
16	CN-PS-0901	10.876	23.514	Citronellal
		14.108	1.405	*trans*-Caryophyllene
		23.27	38.595	Geraniol
		33.558	0.957	Eugenol
17	CN-PS-1001	10.95	31.135	Citronellal
		14.141	1.875	*trans*-Caryophyllene
		16.574	1.394	Citronellyl acetate
		19.53	1.357	γ-Cadinene
		23.293	37.594	Geraniol
		33.54	0.502	Eugenol
		40.259	0.186	Isoeugenol
18	CN-PS-1101	14.186	3.554	*trans*-Caryophyllene
		23.359	53.282	*trans*-Geraniol
		33.554	1.144	Eugenol
		40.263	0.224	Isoeugenol

Table 4-16 The identification of citronella oil.

Compound	limonene	citronellal	citronellyl acetate	neral	geranial	geranyl acetate	citronellol	geraniol
European (%)	1.0-5.0	30.0-45.0	2.0-4.0	<2	<2	3.8-8.0	9.0-15.0	20.0-25.0
CN-BS-0101	2.318	39.730	3.594	-	-	1.929	10.313	18.437
CN-BS-0201	2.347	37.076	3.493	-	-	2.159	10.212	19.643
CN-BS-0202	2.626	39.860	2.944	-	-	1.861	9.511	19.766
CN-BS-0301	1.932	33.200	3.791	-	-	3.920	9.236	20.966
CN-BS-0302	1.580	36.193	3.017	-	-	1.870	10.340	23.347
CN-BS-0401	1.039	26.454	3.235	-	-	2.462	9.212	17.250
CN-BS-0501	15.081	4.064	1.648	-	-	-	10.178	-
CN-BS-0601	1.824	28.571	4.429	-	-	3.171	8.801	21.138
CN-PS-0101	-	37.598	-	-	-	-	9.865	36.485
CN-PS-0201	-	19.993	2.036	-	-	-	6.987	29.178
CN-PS-0301	-	6.098	1.850	-	-	6.157	-	20.627
CN-PS-0401	1.985	18.534	1.069	-	12.005	2.791	7.213	39.723
CN-PS-0501	-	6.326	1.449	-	21.447	6.276	-	36.170
CN-PS-0601	-	20.800	1.271	-	12.723	2.583	6.135	36.733
CN-PS-0701	-	35.557	-	-	13.496	5.570	11.644	8.634
CN-PS-0901	-	23.514	1.190	-	8.839	3.013	6.322	38.595
CN-PS-1001	-	31.135	1.390	-	6.324	2.153	7.217	37.594
CN-PS-1101	-	8.224	1.243	-	10.594	3.067	-	53.282

The results showed that the major constituents of citronella oil from purchase products and steam distillation in laboratory were similar, but limonene was found to be a component in citronella oil from purchase product and was not found in citronella oil from steam distillation in laboratory. Also geranial was found to be a component in citronella oil from steam distillation in laboratory but was not found in citronella oil from purchase product. So, the comparison of the chemical constituents in citronella oil from purchase products and steam distillation in laboratory showed that the chemical constituents in citronella oil from purchase products were similar to chemical constituents of citronella oil in European Pharmacopoeia as we knew that volatile oil was from *C. winterianus*. Thus, we could conclude that citronella oil from steam distillation in laboratory was from *C. nardus* that must have geranial as the chemical constituents and did not have limonene. This results would be the first report of the difference of the chemical constituents in citronella oil from *C. winterianus* and *C. nardus*.

Monograph of *Cymbopogon nardus* (L.) Rendle

ตะไคร้หอม (TA KRAI HOM)

Cymbopoginis Nardi Herba

Cymbopogon Nardus Herb

Citronella Grass Herb

Synonyms Cha khai ma khuut; Ta khrai ma khuut; Ta khrai daeng.

Category Insect repellant

Cymbopogon Nardus Herb is the dried stem and leaf of *Cymbopogon nardus* (L.) Rendle. A voucher specimen of this plant was deposited in the herbarium of the Department of Medical Science(DMSc), Ministry of Public Health (Family Gramineae/Poaceae)

Constituents *Cymbopogon nardus* Herb contains volatile oil, of which limonene, citronellal, citronellol, geraniol, citral, citronellyl acetate, geranyl acetate are its major components.

Description of the plant Parennial from a stout rootstock. Culms tufted, robust, up to 2.5 m tall, 1-2 cm in diam. Leaves sheaths reddish purple at base, smooth, glabrous; leaf blades dark green or dark brown when dry, drooping for 1/3 of their length, 50-150 cm long 1-3 cm wide, glabrous, abaxial surface scaberulous, adaxial surface smooth, base narrow, apex long acuminate; ligule 2-3 mm. Spathate panicle large, narrow, congested, interrupted, 60-90 cm. (Figure 1a, 1b)

Description Odor, characteristic, aromatic

Macroscopical Cymbopogon Nardus Herb consists of culm and leaf-blades which occurs in pieces about 2-5 cm in length, and 0.5-1 cm wide. The dried leaf-blades are pale green, thin and scaberulous. The crude drug has characteristic odor. (Figure 1a)

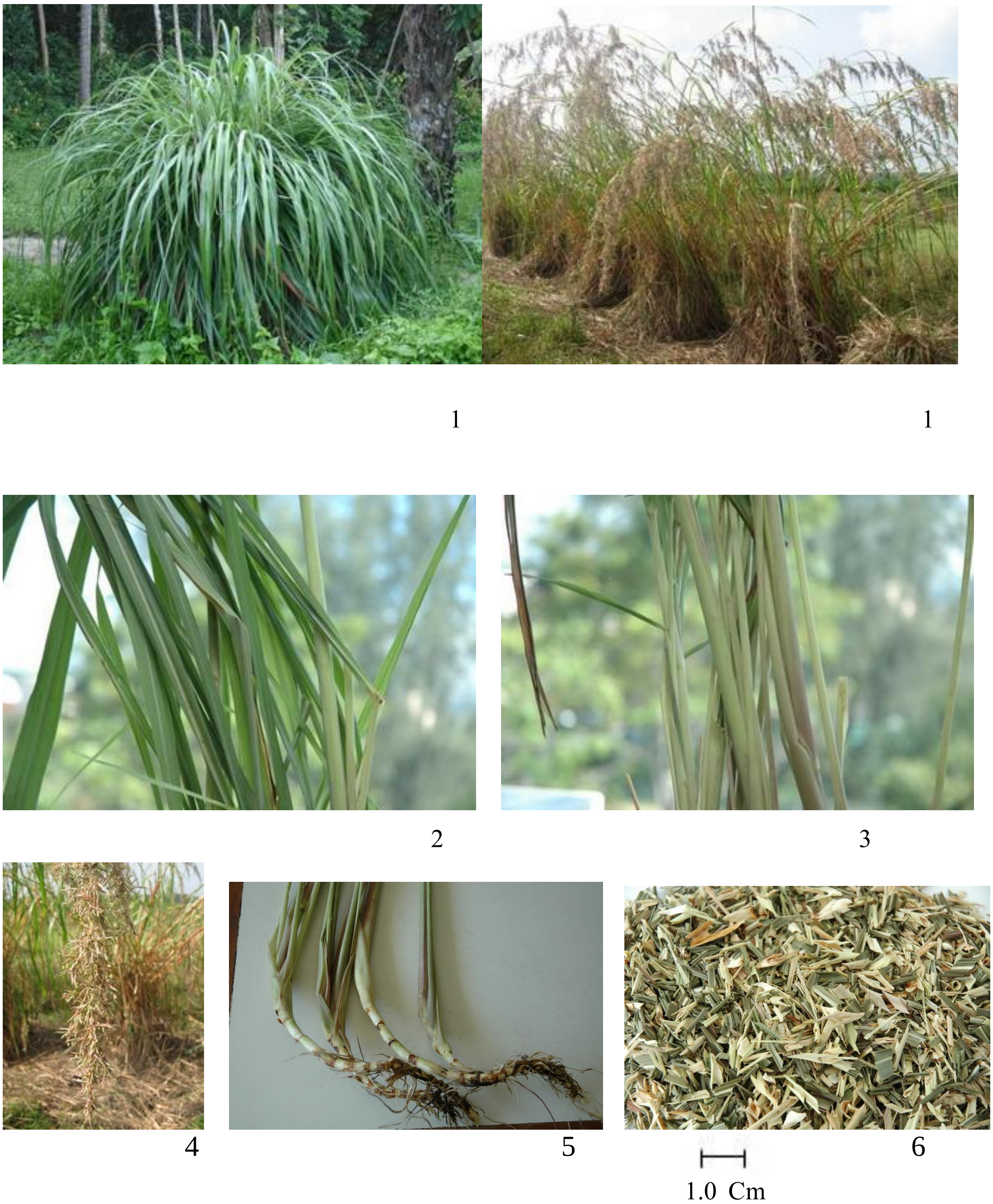


Figure 1a *Cymbopogon nardus* (L.) Rendle

1.the plant ; 2. leaf ; 3. stem ; 4. inflorescence ; 5. rhizome and 6. crude drug

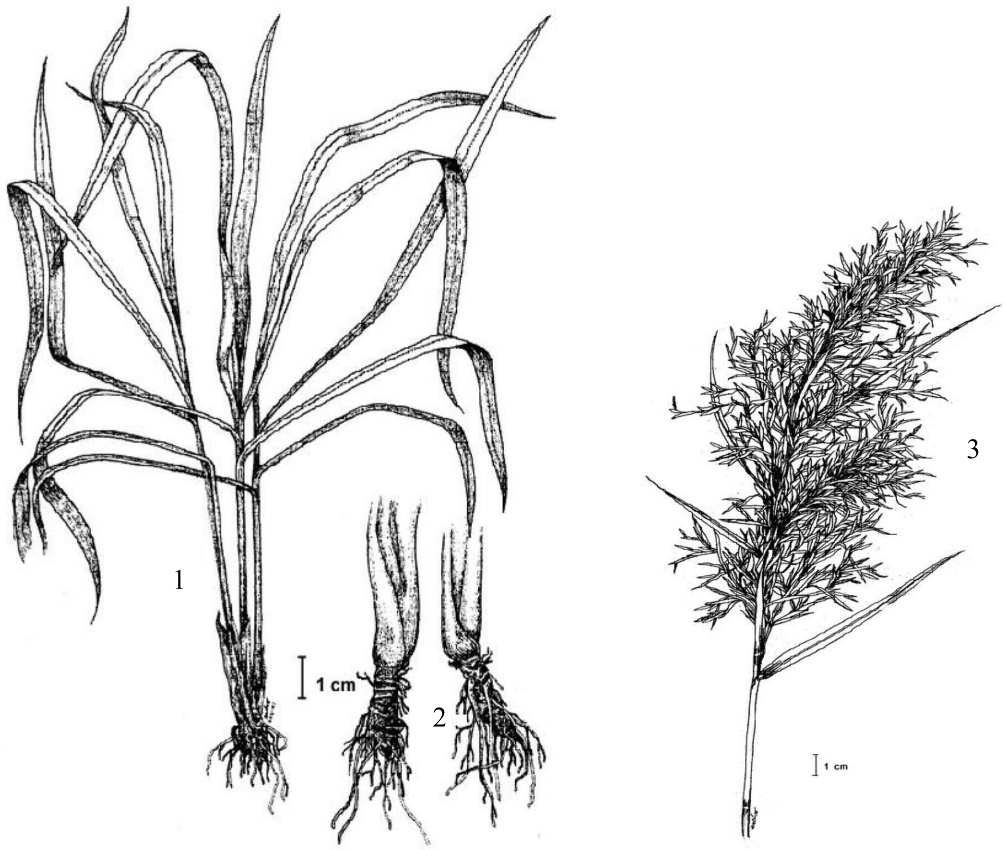


Figure 1b *C. nardus*

1.the plant ; 2. rhizome ; 3. inflorescence

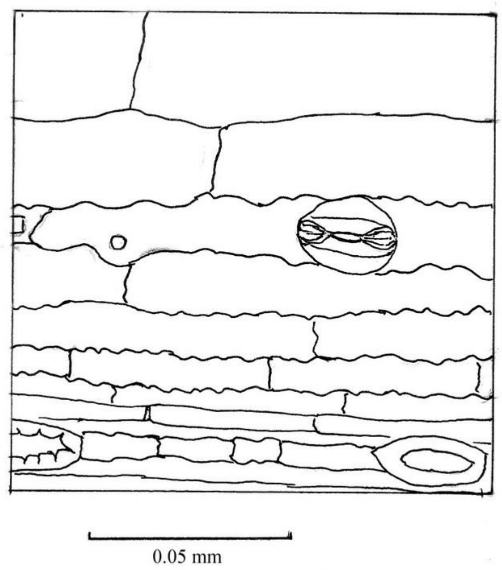
Microscopical Surface preparations show that stomata are present on both surface, but are more numerous on the lower one. The stomata are grass type; consist of bone shape guard cell and 2 subsidiary cells with their long axes parallel to the pore. The epidermal cells with wavy outline. Oil globules are usually present in parenchyma. (Figure 2a). A transverse section of leaf-blade shows that it has a bifacial structure. The upper epidermis; a single layer of rectangular cells, with few unicellular trichome. Mesophyll consisting of several layers of parenchyma rich in chloroplastids probably distinctly radiate around most of the individual vascular bundle. A zone of collenchyma, found beneath upper epidermis in the vein region. Vascular bundles surrounded by sclerenchymatous layer of bundle sheath. Xylem composed of pitted and annular vessels. Lower epidermis, a single layer of rectangular cells with collenchyma underneath and bulliform cells (Figure 2b, 2c, 2d)

Storage Dried plant powder was kept in well-closed container and protected from light at 4 °C.

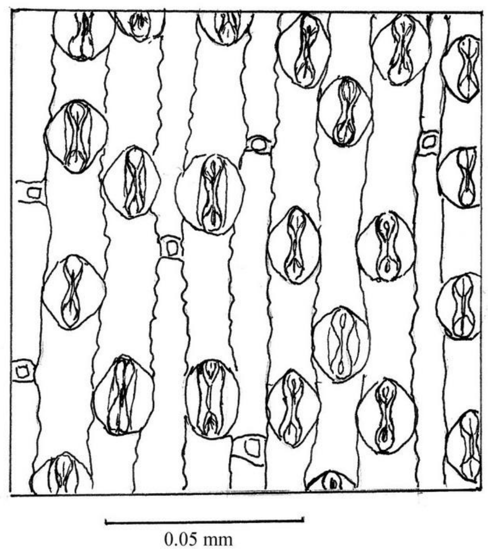
Identification

A. Liebermann-Burchard test : macerated 1 g of the sample with 20 ml of ethanol of specified strength in a close flask of 3 days and filter (solution) to 2 ml into the crucible and dryness on the water bath. After that add 1 drop of acetic anhydride and add 1 drop of conc. sulfuric acid: a green to brown color is produced.

B. Carry out the test as described in the "Thin layer chromatography" THP Volume II, 2007, Appendix 3.1, using *siliga gel* GF_{254} as the coating substance and mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 5 μl of each of the following solutions. Prepare solution (A) by macerate 10 g of the sample, in powder, with 200 ml of *petroleum ether* and shaking for 24 hours, filtering, and evaporating to a volume of 2 ml. For solution (B), dilute 2 μl of *citronellal* in 5 μl of *petroleum ether*. After removal of the plate, allow it to dry in air, and examine under ultraviolet light (254 and 366 nm), marking the spots. Spray the plate with *anisaldehyde TS* and heat at 105 °C for 5 minutes. The chromatogram obtained from solution (A) shows a red-violet spot with anisaldehyde *TS* (hR_f value 86.0 to 88.7) corresponding to citronella. Several other spots of different colors are observed (Table 1); see also Figure 3.

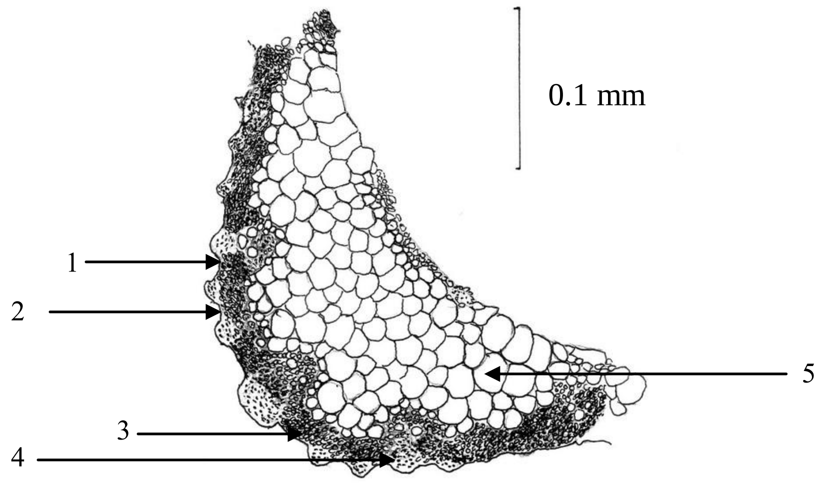


Upper Epidermis of the Lamina

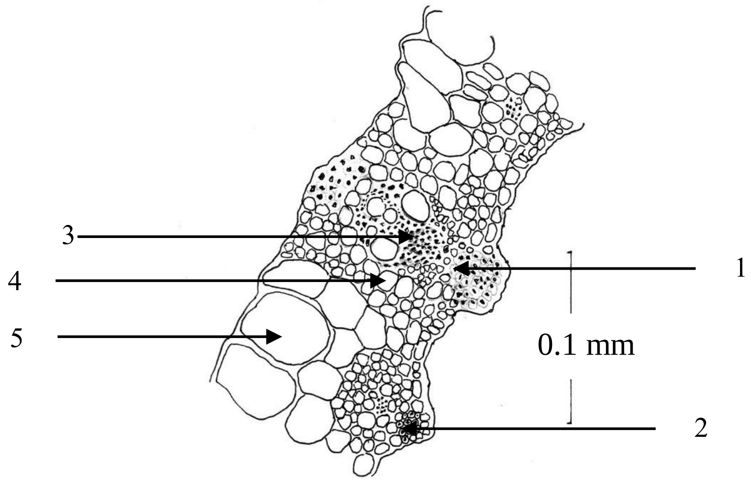


Lower Epidermis of the Lamina

Figure 2a Epidermal Cell of the Leaf of *C. nardus*



Transverse section of the Midrib T.S. (x 100)



Transvers Section of Lamina T.S.(x400)

Figure 2b Transverse Section of the Leaf of *C. nardus* Rendle

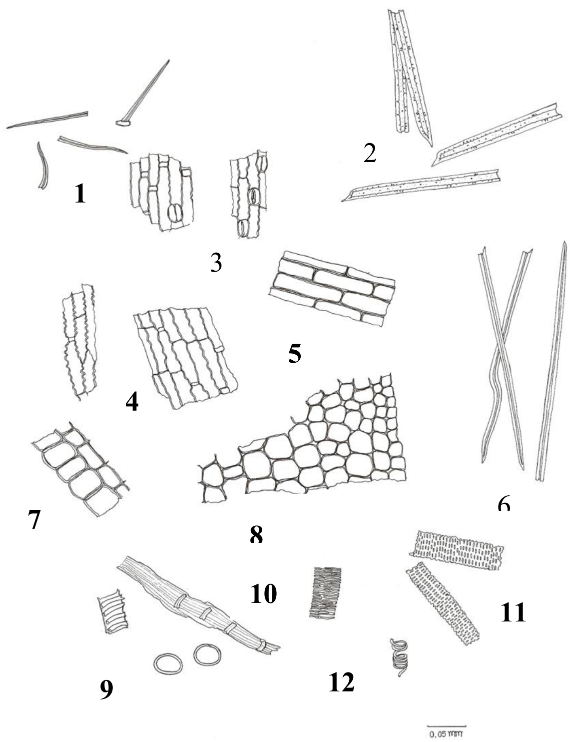


Figure 2c Powdered drug of the leaf of *C. nardus*

1. unicellular trichome
2. sclereids
3. lower epidermis in surface view showing grass type stomata
4. upper epidermis
5. parenchyma in surface view
6. fragment of fiber
7. bulliform cells
8. parenchyma in sectional view
9. annular vessels
10. reticulated vessel
11. pitted vessels
12. spiral vessel

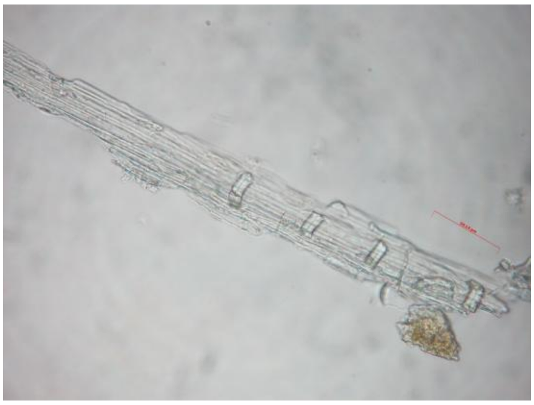
1.

1.

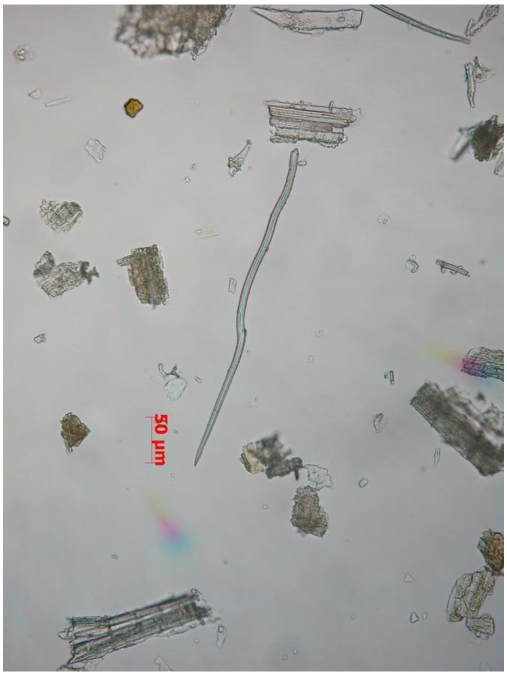
Figure 2d Powdered drug images of the aerial part of *C. nardus*

1. annular vessel
2. fragment of fiber
3. bulliform cell
4. lower epidermis in surface view showing grass type stomata
5. parenchyma in longitudinal view
6. parenchyma in sectional view
7. pitted vessel
8. reticulated vessel
9. sclereid
10. spiral vessel
11. unicellular trichome
12. upper epidermis

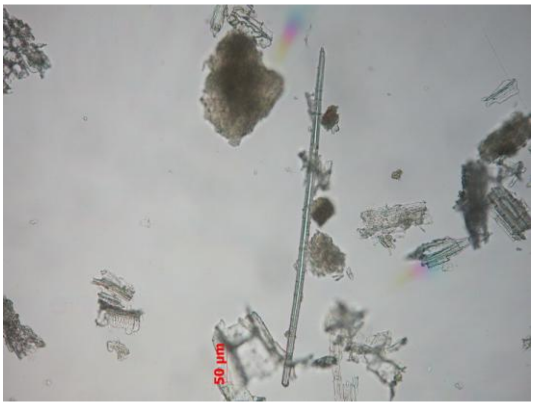
1.



1.



2.

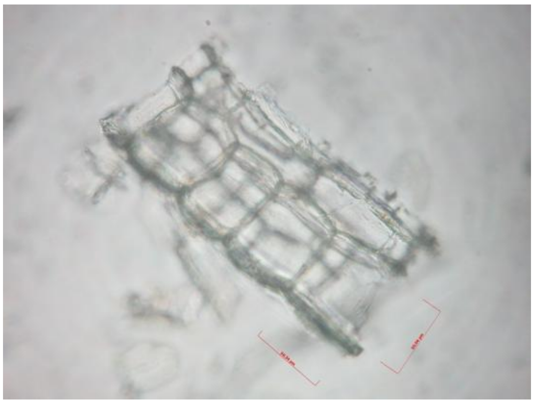


2.

2.

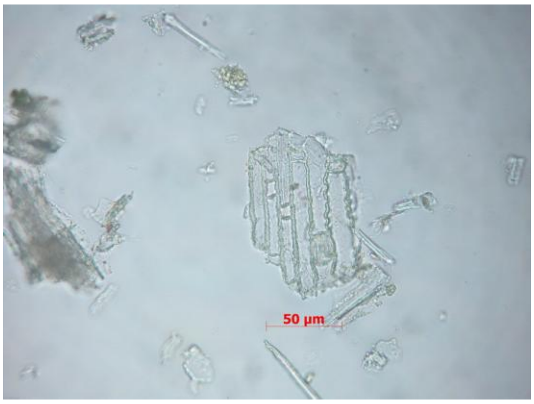
2.

Figure 2d (continued)



3.

4.



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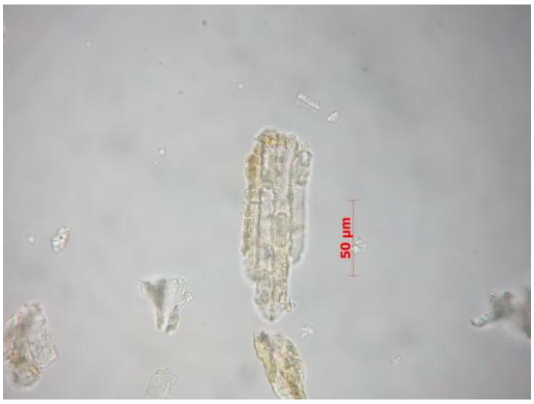

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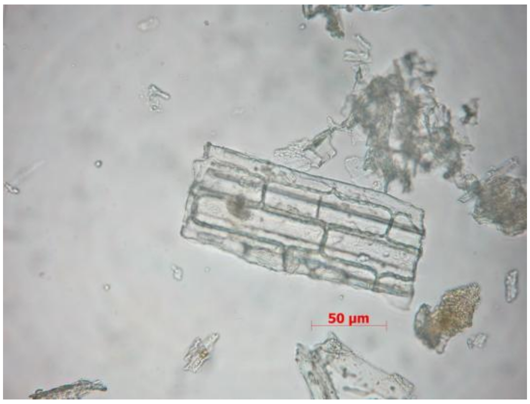
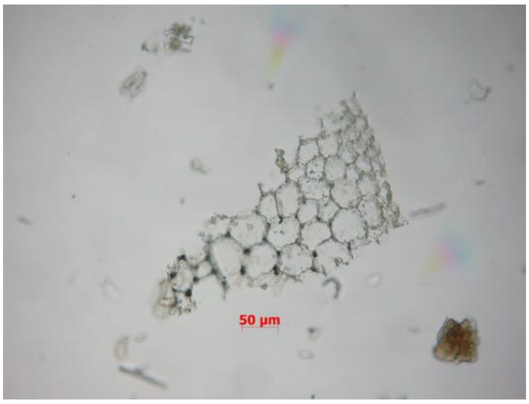


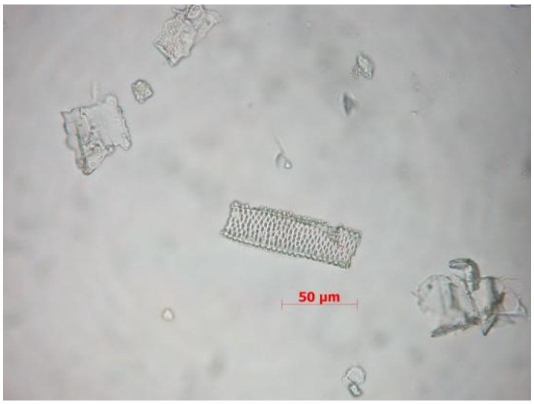
5.



6.

Figure 2d (continued)

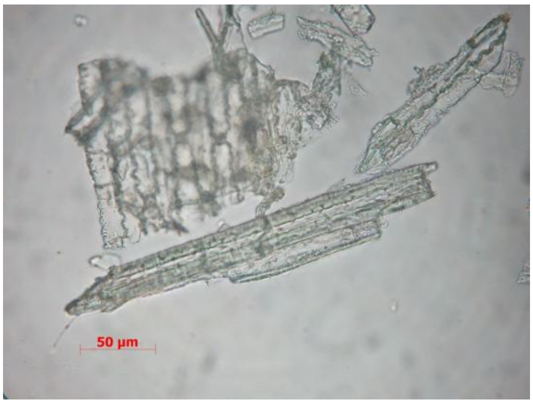
7.



7.

8.

9.



9.

9.

Figure 2d (continued)

10.

11.

11.

11.

12.

12.

Figure 2d (continued)

12.

Figure 2d (continued)

Table 1 hR_f values of components in petroleum ether of the aerial part of *C. nardus*

Detection with				
Spot	**hR_f Value**	**UV 254**	**UV 366**	**Anisaldehyde TS**
1	3.3-5.3	green	Red fluorescence	pink
2	6.0-6.7	yellow	Red fluorescence	yellow-orange
3	7.3-8.7	green-blue	Red fluorescence	pink-violet
4	14.7-16.0	-	-	pale pink
5	28.7-30.7	-	-	green
6	32.7-36.7	-	-	dark blue
7	45.3-47.3	yellow-orange	Red fluorescence	violet
8	49.3-51.3	-	-	brown
9	60.0-62.7	green	Red fluorescence	pale violet
10	66.7-68.7	yellow	Red fluorescence	pink-violet
11	78.7-80.7	dark green	Red fluorescence	dark violet
12	84-85.3	-	-	dark blue
13*	86.0-88.7	-	-	red-violet
14	92.0-92.7	-	-	violet
15	94.0-95.3	-	-	dark pink
16	96.0-96.7	yellow	Red fluorescence	brown

*Citronellal

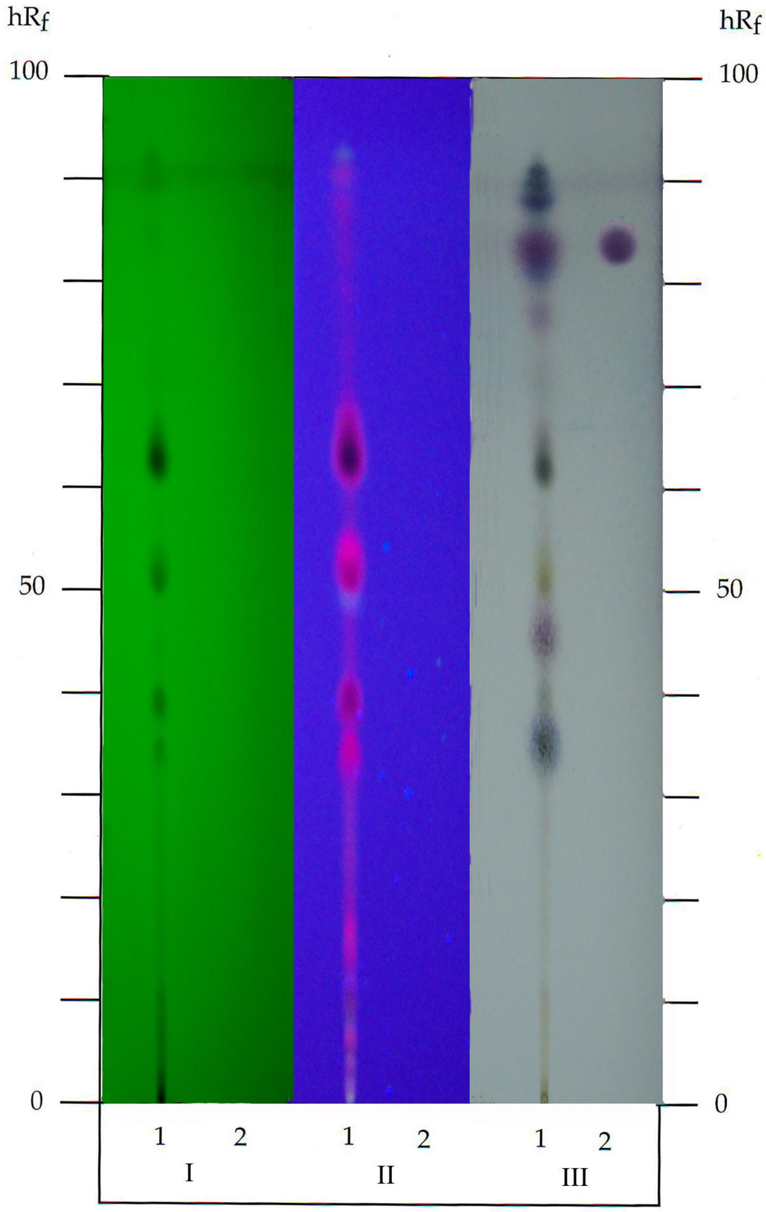


Figure 3 Thin layer chromatography of *C. nardus*.

1 = Sample

2 = Standard of citronellal

I = detection under UV light (254 nm)

II = detection under UV light (366 nm)

III = detection with anisaldehyde TS

Foreign matter: Not more than 2.0 per cent w/w.

Water: Not more than 4.0 per cent v/w (Azeotropic Distillation Method).

Total ash: Not more than 8.0 per cent w/w.

Acid insoluble ash: Not more than 3.0 per cent w/w.

Ethanol soluble extractive: Not less than 5.0 per cent w/w.

Water soluble extractive: Not less than 7.0 per cent w/w.

Hexane soluble extractive: Not less than 2.0 per cent w/w.

Chloroform soluble extractive: Not less than 4.0 per cent w/w.

Volatile oil: Not less than 0.03 per cent v/w.

Monograph of Citronella Oil

Thai name **น้ำมันตะไคร้หอม** (NAM-MAN TA-KHRAI HOM)

Definition

Oil obtained by steam distillation from the fresh plants of *Cymbopogon nardus* (L.) Rendle.

Characters

Pale yellow liquid, with a very strong odor.

Solubility Soluble in ethanol, methanol, ethyl acetate, chloroform, hexane; insoluble in water.

Identification

A. Thin-layer chromatography

Test solution : Dilute citronella oil in 1:10 ml of ethanol and mixed.

Reference solution : Dilute reference solution in 1:10 ml of ethanol and mixed. Four reference solutions were used as Limonene, Citronellal, Citronellol and Geraniol.

Plate : TLC silica gel plate GF_{254}

Mobile phase : toluene (100 v/v), toluene : ethyl acetate(90:10 v/v), toluene : ethyl acetate(80:20 v/v), toluene : chloroform (90:10 v/v) and toluene : chloroform (80:20 v/v).

Application : 5 µl, as bands.

Development : over a path of 15 cm.

Drying : in air.

Detection : examine in ultraviolet light at 365 nm. Spray with anisaldehyde solution and heat at 100-105 $^{\circ}$C for 5-10 min.

Result :

Top of the plate	
Citronellal : a violet zone	A zone similar in color to the citronellal zone An orange zone (citronellol-geraniol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Result : the characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in chromatogram obtain with the reference solution.

Chromatographic profile. Gas chromatography – mass spectrometry which the conditions are as followed :

Gas Chromatography (Hewlett Packard U.S.A. company):

Inlet temperature : 260 °C, Split ratio 1 : 50

Oven temperature : Initial temperature 80 °C, 2 min,

Ramp to 150 °C at 3 °C/min.

Ramp to 185 °C at 2 °C/min.

Ramp to 250 °C at 10 °C/min.

Column : HP Innowax 30 m x 0.25 mm I.D., 0.25 µm

Mass Spectrometer(Hewlett Packard U.S.A. company):

Ionization mode	:	Electron Ionization
Acquisition mode	:	Scan, 35-500 amu
Transferline temperature	:	260 °C
Solvent delay time	:	3.0 min.

Determine the percentage content of each of these components.

-limonene: 1.98 per cent,

-citronellal: 6.0 per cent to 38.0 per cent,

-citronellyl acetate: 1.0 per cent to 2.0 per cent,

-neral: less than 2.0 per cent,

-geranial: 6.0 per cent to 21.0 per cent,

-geranyl acetate: 2.0 per cent to 6.0 per cent,

-citronellol: 6.0 percent to 12.0 per cent,

-geraniol: 8.0 per cent to 53.0 per cent,

Tests

Relative density : 0.878 to 0.902.

Refractive index : 1.458 to 1.484.

Optical rotation : -0.054° to 2.34°.

Storage

In a well-filled container, protected from light.

CHAPTER 5

CONCLUSION

In this study, fifteen sample of *Cymbopogon nardus* (L.) Rendle (citronella grass) and eighteen samples of citronella oils were collected from several parts of Thailand in order to do the monographs of this plants and its volatile oil. The monographs of *C. nardus* and volatile oil are following Thai Herbal Pharmacopoeia 2007 and European Pharmacopoeia 1980, respectively. The quality of herbal products is a matter of concern for protection the consumer from the adulterated or substandard drugs. The strict control of the herbal drugs is there for essential to ensure reproducible quality of herbal preparations. From the results we could do the monographs of *C. nardus* and citronella oil, which will be the first report of their monograph in Thailand. This monograph will be useful for quality control and standardization of raw material (citronella grass) and its product (citronella oil). Since, the good quality of Thai herbal medicines will be shared in the world market.

REFERENCES

กนก อุไรสกุล. 2545. **การใช้สมุนไพรบางชนิดยับยั้งการเจริญเติบโตของไรแดงกุหลาบ**. รายงานการวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ.

กิตติพันธ์ ตันตระรุ่งโรจน์. 2543**. การพัฒนาตำรับยากันยุงสมุนไพร.** รวมบทคัดย่องานวิจัยการแพทย์แผนไทยและทิศทางการวิจัยในอนาคต สถาบันการแพทย์แผนไทย กระทรวงสาธารณสุข กรุงเทพมหานคร.

เนาวรัตน์ ศุขะพันธ์ สมคิด แก้วมณี ไพโรจน์ เปรมปรีดิ์ สุชาติ อุปถัมภ์ และยุพา รองศรีแย้ม. 2536. **ประสิทธิภาพการแสดงฤทธิ์ของสารสกัดสมุนไพรในการขับไล่ยุง**. การประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 19 หาดใหญ่ สงขลา. 480-1.

พเยาว์ เหมือนวงษ์ญาติ. 2530**. คู่มือการใช้สมุนไพร**. เรือนแก้วการพิมพ์ กรุงเทพมหานคร.

เพ็ญนภา ทรัพย์เจริญ. 2549. **สวนสมุนไพรในงานมหกรรมพืชสวนโลก.** สามเจริญพาณิชย์ กรุงเทพมหานคร.

วสุ วิฑูรย์สฤษฏ์ศิลป์ และ ศุทธิชัย พจนานุภาพ. 2545. **แชมพูสมุนไพรสำหรับสัตว์เลี้ยง** โครงการพิเศษ คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล กรุงเทพมหานคร.

วีณา วิรัจฉริยากุล. 2534. **ยาและผลิตภัณฑ์ธรรมชาติ**. ภาควิชาเภสัชวินิจฉัย คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล กรุงเทพมหานคร.

วุฒิ วุฒิธรรมเวช**.** 2540**. สารานุกรมสมุนไพร** โอ. เอส. พริ้นติ้ง เฮาส์ กรุงเทพมหานคร.

ศศิธร วสุวัต**.** 2533. **ประสิทธิภาพป้องกันยุงกัดของครีมตะไคร้หอม.** วิทยาศาสตร์และเทคโนโลยี. 5(2):62-67.

สมพร ภูติยานันต์. 2542. **การตรวจเอกลักษณ์ พืชสมุนไพร**. สถาบันการแพทย์แผนไทย กรมการแพทย์ กระทรวงสาธารณสุข กรุงเทพมหานคร.

สันติสุข โสภณสิริ. 2537. **สมุนไพรเพื่อสุขภาพ 2** ปกเกล้าการพิมพ์ กรุงเทพมหานคร.

อรัญ งามผ่องใส สุนทร พิพิธแสงจันทร์ และ วิภาวดี ชำนาญ. 2546. **การใช้สารฆ่าแมลงและสารสกัดจากพืชบางชนิดควบคุมแมลงศัตรูถั่วฝักยาว**. มหาวิทยาลัยสงขลานครินทร์. 2546 (3):307-316.

กลุ่มแม่บ้านเกษตรกรบ้านร้องขี้เหล็ก. 2546. ตะไคร้หอมผงสำเร็จรูป (ออนไลน์). สืบค้นจาก : http://www.thaitambon.com/Tambon.html (17 มีนาคม 2553).

กรมวิทยาศาสตร์บริการ (ออนไลน์). 2553. สืบค้นจาก : http://www.dss.go.th/otop_subhead.html (17 มีนาคม2553).

การงานอาชีพและเทคโนโลยี (ออนไลน์). 2553. สืบค้นจาก : http://www.tryodream.com/index.html (17มีนาคม 2553).

Abena, A.A., Gbenou, J.D., Yayi, E., Moudachirou, M., Ongoka, R.P., Ouamba, J.M., and Silou, T. 2007. Comparative chemical and analgesic properties of essential oils of *Cymbopogon nardus* (L.) Rendle of Benin and Congo. **The African Journal of Traditional**, 4(2):267-272.

Bhusita, W., Manessin, P., Tubtimted, S., and Wangchanachai, G. 2009. Antimicrobial activity of essential oils extracted from Thai herbs and species. **Asian Journal of Food and Agro-Industry**, 2(04):677-689.

Blanco, M.M,. Costa, C.A.R.A., Freire, A.O., Santos, J.G.,Costa, M. 2009. Neurobehavioral effect of essential oil of *Cymbopogon citratus* in mice. **Phytomedicine**, 16: 265-270.

Bower, W.S., Oretego, F., You, X.O., and Evans, P.H. 1993. Insect repellants from Chinese pickly ash *Zanthoxylum bungenanum*. **Journal of Natural Products**, 566: 935-938.

Chogo, J.B., and Crank, G. 1981. Chemical composition and biological activity of the Tanzanian Plant, *Ocimum suave*. **Journal of Natural Products**, 433: 308-311.

Cos, N.D. 1980. Flea treatment composition for animals. **United States Patent** 193, 986.

Dhar, M.L.O.S., Dhar, M.N., Dhawan, B.N., Mehrotra, B.N., Srimal, R.C., and Tandon, J.S. 1973. Screening of Indian plants for biological activity. Part IV. **Indian Journal of Experimental Biology**, 11: 43.

Essam, A.A., Deon,C., Wagdy, M.F.Y., Hoda, A.W., and Abdel-Hamid, M. 2005. A review of botanical phytochemicals with mosquitocidal potential. **Entomology International**, 31: 1149-1166.

European Pharmacopoeia Commission. 1980. **European Pharmacopoeia**, French: Johnson, Council of Europe, Maisonneuve S.A., France. pp. v. 6.4-6.6.

Farnsworth, R.N., and Bunyapraphatsara, N. 1992. **Thai medicinal plants**. Medicinal Plant Information Center. Prachachon, Bangkok, pp. 143-145.

Fuselli, S.R., Garcia de la Rosa, S.B., Eguaras, M.J., and Fritz, R. 2010. *In vitro* antibacterial effect of exotic plants essential oils on the honeybee pathogen *Paenibacillus larvae*, causal agent of American foulbrood. **Journal Agricultural Research**, 8 (3), 651-657.

Heiba, H.I., and Rizk, A.M. 1986. Constituents of *Cymbopogon* species. **Qatar University Science Bull**, 6: 53-75.

Hwang, Y.S., Wu, K.H., Kumamoto, J., Axclrod, H., and Mulla, M.S. 1985. Isolation and identification of mosquito repellents in *Artemisia vulgaris*. **Journal of chemical Ecology**, 119: 1297-1306.

Josphat, C., Matasyoh, I.N., Wagara, J.L. Na., and Anderson, M.K. 2011. Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus* species. **African Journal of Food science**, 5(3): 138-142.

Kazuhiko, N., Najeeb, S., Alzoreky, T., Yoshihashi, H., Nguyen, T.T., and Trakoontivakorn, G. 2003. Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella grass). **Japan Agricultural Research Quarterly**, 37 (4): 249-252.

Ketoh, G.K., Glitho, A.L., and Huignard, J. 2005. Susceptibility of the bruchid *Callosobruchus maculates* (Coleoptera:Bruchidae) and its parasitoid *Dinamus basalis* (Hymenoptera: pteromalidae) to three essential oils. **Journal of Economic Entomology**, 95 (1): 174-182.

Ketoh, G.K., Koumaglo, H.K., Glitho, L.A., Auger, J., and Huignard, J. 2005. Essential oil residual effects on *Callosobbruchus maculates* (Celeoptera: Bruchidae) survival and female reproduction and cowpea seed germination. **Journal of Insect Science**, 25 (2): 129-133.

Koba, K., Sanda, K., Guyon, C., Raynaud, C., Chaumont, J.P., And Nicod, L. 2009. *In vitro* cytotoxic activity of *Cymbopogon citrates* L. and *Cymbopogon nardus* L. essential oils from Togo. **Bangladesh Journal of Pharmacology**, 4:

Marcus, C., and Lichtenstein, E.P. 1979. Biological active components of anise: toxicity and interaction with insecticides in insects. **Journal of Agricultural and Food Chemistry**, 276: 1217-1223.

Masui, K. and Kochi, H. 1974. Camphor and tricyclodecane in deodorants and insect repelling compositions. **Japanese Patent Kokai Tokyo koho**, 100 (239): 4.

Ministry of Public Health. 2007. **Thai Herbal Pharmacopoeia** Volume II: Department of Medical Sciences. Prachachon, Bangkok, pp. 101-152.

Nath, S.C., Sarma, K.K., Vajezikova, I., and Leclercq, P.A. 2002. Comparison of volatile inflorescence oils and taxonomy of certain *Cymbopogon* taxa described as *Cymbopogon flexuosus* (Nees ex Steud.) Wats. **Biochemical Systematics and Ecology**. 30: 151-162.

Paranagama, P., Abesekara, T., Nugaliyadde, L., and Abeywickrama, K. 2003. Effect of the essential oils of *Cymbopogon citratus , C. nardus* and *Cinnamomum zealanicum* on pest incidence and grain quality of rough rice (paddy) stored in an enclosed seed box. **Journal of Food Agriculture and Environment**, 1 (2): 134-136.

Quintans-Junior, L.J., Sauza, T.T., Leite, B.S., Lessa, N.M.N., Bonjadin, L.R., Santos, M.R.V., Alves, P.B., Blank, A.F., and Antoniolli, A.R. 2008. Phytochemical screening and anticonvulsant activity of *Cymbopogon winterianus* Jowitt (Poaceae) leaf essential oil in rodents. **Phytomedicine**, 15: 619-24.

Reinaldo, N.A., Maria, F,. Flavia, N. S. M., and Damiao, P. S. 2011. Essential oils and their constituents: Anticonvulsant activity. **Molecular Journal,** 16: 2726-2742.

Rizk, A.M., Heiba, H.I., and Sandra, P. 1983. Constituents of the volatile oil of *Cymbopogon parkeri*. J Chromatography, 279: 145-50.

Sangwan, N.S., Farooqi, A.H.A., Ahabil, F., and Sangwan, R.S. 2001. Regulation of essential oil production in plants. **Indian Journal of Medical Research**, 34: 3-21.

Schearrer, W.R. 1984. Components of oils of Tancy (*Tanacetum vulgare*) that repel colorado potato beets (*Leptinotarsa decemlineata*). **Journal of Natural Product**, 476: 964-969.

Scriven, R., and Meloan, C.E. 1984. Determining the active component in 1,3,3-trimethyl-2-oxabycyclo [2,2,2] octane (coneole) that repels the American cockroach, *Periphaneta americannna*. **The Ohio Journal of Science**, 843: 85-88.

Silva, M.R., Ximenes, R.M., Costa, J.G.M., Leal, L.K.A.M., Lopes, A.A., and Viana, G.S.B. 2010. Comparative anticonvulsant activities of the essential oils (EOs) from *Cymbopogon winterianus* Jowitt and *Cymbopogon citrat*us (DC) Stapf in mice. **Architecture of Pharmacology**, 381: 415-426.

Simic, A., Rancic, A., Sokovic, M.D., Ristic, M., Grujic-Javanovic, S., Vukojevic, J., and Marin, P.D. 2008. Essential oil composition of *Cymbopogon winterianus* and their antimicrobial activity. **Pharmaceutical Biology**, 46(6): 437-441.

Sinchisri, N., Roongsook, D., and Aeekul, S. 1988. Botanical repellant against the diamondback moth, *Plutella xylostella* L. **Kasetsart Journal** (Wittayasan Kasetsart), 22 (5): 71-74.

Sugiara, M., Ishizuka, T., and Neishi, M. 2002. Flying insect repellents containing essential oils. **Patent: Japanese Kokai Tokkyo Koho Japan**, 173, 404.

Thorsell, W., Mikiver, A., and Tunon, H. 2006. Repelling properties of some plant materials of the tick *Ixodes ricinu* L. **Phytomedicine**, 13: 132-134.

Tunon, H., Thoreell, W., and Bohlin, L. 1994. Mosquito repelling activity of compounds occurring in *Achillea millefolium* L. (Astraceae). **Economic Botany**, 482: 111-120.

Tyaki, B.K., Shahi, A.K., and Kual, B.L. 1998. Evaluation of repellant activities of *Cymbopogon* essential oils against mosquito vectors of malaria, filariasis, and dengue fever in India**.** **Phytomedicine**, 5 (4):324-329.

Vartak, P.H., and Shama, R.N. 1993. Vapour toxicity and repellence of some essential oils and terpenoids to adults of *Ades algypti* (L.) (Doptera: Culicidae). **Indian Journal of Medical Research**, 973: 122-127.

Verma, M.M. 1981. The isolation and identification of a cockroach repellent in bay leaves and a fluorescence method for determination of protein in wheat. **Diss Abst Int B**, 414, 514.

Vijender, S. M., and Mohd, Ali. 2003. Volatile constituents of *Cymbopogon nardus* (Linn.) Rendle. **Flavour and Fragrance of Journal**, 18: 73-76.

Wannisorn, B., Maneesin, P., Tubtimted, S., and Wangchanachai, G. 2009. Antimicrobial activity of essential oils extracted from Thai herbs and species. **Asian Journal of Food and Agro-Industry**, 2(04): 677-689.

APPENDIX

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Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science Chemistry	Yala Rajaphat University	2006

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Nitangsam, N., Puripattanavong, T. and Dej-adisai, S. 2010. Monographs of *Cymbopogon nardus* (L.) Rendle. Thailand Research Symposium 2010**,** Proceedings, 26-31 August, 2010 at Bangkok Convention Center, Central World, Bangkok, Thailand (poster presentation).

Nitangsam, N., Puripattanavong, T. and Dej-adisai, S. 2010. Chemical constituents in citronella oils from the *Cymbopogon nardus* (L.) Rendle. NRCT-JSPS Core University Program on Natural Medicine in Pharmaceutical Science *The 9th Joint Seminar* Natural Medicine Research for the Next Decade: New Challenges and Future Collaboration, Proceedings, 8-9 December, 2010. Faculty of Pharmaceutical Science, Chulalonhkorn University, Bangkok, Thailand (poster presentation).