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นางสาวสุวิมล ทองแกมแก้ว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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QUALITY ASSESSMENT AND ANTIMICROBIAL ACTIVITIES OF *PSIDIUM GUAJAVA* LEAVES

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	QUALITY ASSESSMENT AND ANTIMICROBIAL ACTIVITIES OF *PSIDIUM GUAJAVA* LEAVES
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Field of Study	Public Health Sciences
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สุวิมล ทองแกมแก้ว : การประเมินมาตรฐานและฤทธิ์การต้านจุลชีพจากใบฝรั่งขี้นก (QUALITY ASSESSMENT AND ANTIMICROBIAL ACTIVITIES OF *PSIDIUM GUAJAVA* LEAVES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : อ.ดร.ชนิดา พลานุเวช, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร.นิจศิริ เรืองรังษี, 91 หน้า.

ฝรั่งพันธุ์ขี้นกเป็นฝรั่งพันธุ์พื้นเมืองของไทย มีชื่อวิทยาศาสตร์ว่า *Psidium guajava* L. อยู่ในวงศ์ MYRTACEAE การศึกษานี้ได้เก็บรวบรวมใบฝรั่งขี้นกจาก 15 พื้นที่ในประเทศไทย โดยมีจุดประสงค์เพื่อจัดทำข้อกำหนดทางเภสัชเวทของใบฝรั่งขี้นก และองค์ประกอบทางเคมีของน้ำมันหอมระเหยจากใบฝรั่งขี้นก จากผลการศึกษาแสดงให้เห็นถึงเอกลักษณ์ทางเคมีฟิสิกส์ของใบฝรั่งขี้นก พบว่าปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด น้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณสารสกัดด้วยเอทานอล ปริมาณสารสกัดที่ละลายด้วยน้ำ ปริมาณความชื้นและปริมาณน้ำมันหอมระเหยมีค่าเป็นร้อยละ 7.88±0.50, 7.03±0.31, 1.42±0.25, 8.61±0.78, 10.21±0.52, 8.71±0.47 และ 1.54±0.17 โดยน้ำหนักแห้งตามลำดับ นอกจากนี้โครงสร้างทางมหทรรศน์และจุลทรรศน์ของใบ พบขนชนิดเซลล์เดียวและต่อมน้ำมันจำนวนมาก ขนของใบฝรั่งขี้นกพบทั้งสองด้านของใบคือด้านหลังใบและท้องใบ โดยเฉลี่ยใน 1 ตารางมิลลิเมตร มีค่าเป็น 24.21±3.75 และ 46.20±4.68 ตามลำดับ ต่อมน้ำมันโดยเฉลี่ยใน 1 ตารางมิลลิเมตร มีค่าเป็น 38.94±3.96 สร้างลายพิมพ์เคมีของสารสกัดด้วยเอทานอลจากใบฝรั่งขี้นกด้วยเทคนิคทินเลเยอร์โครมาโทกราฟี โดยใช้ตัวทำละลายไดคลอโรมีเทนต่อเอทิลอะซิเตทต่อเมทานอลต่อกรดอะซิติก 8:4:2:0.1 ตรวจวัดภายใต้แสงขาวและแสงอัลตราไวโอเลตความยาวคลื่น 254 และ 365 นาโนเมตรและทำปฏิกิริยาเกิดสีกับกรดซัลฟิวริก การศึกษาองค์ประกอบทางเคมีของน้ำมันระเหยในใบฝรั่งขี้นกซึ่งถูกสกัดโดยวิธีการกลั่นด้วยน้ำพบองค์ประกอบทางเคมีอย่างน้อย 40 ชนิด โดยวิธีแกสโครมาโทกราฟี-แมสสเปกโทรเมทรี องค์ประกอบหลักคือ คารีโอฟิลลีน (ร้อยละ 28.21±2.91) ลิโมนีน (ร้อยละ 7.17±5.47) แอลฟาโคพาอีน (ร้อยละ 8.06±3.58) อะโรมาเดรนดรีน (ร้อยละ 6.86±3.86) และโกลบูรอล (ร้อยละ 5.90±2.89) พบว่าน้ำมันระเหยใบฝรั่งขี้นกมีฤทธิ์ฆ่าเชื้อแบคทีเรียชนิดแกรมบวก ได้แก่ *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* และ *Micrococcus luteus*

ภาควิชา วิทยาลัยวิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต..........

สาขาวิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก..........

ปีการศึกษา 2556 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม..........

5578960253 : MAJOR PUBLIC HEALTH SCIENCES

KEYWORDS : PHARMACOGNOSTIC SPECIFICATION / KHEE NOK CULTIVAR / ESSENTIAL OIL CONSITUENTS/ ANTIMICROBIAL ACTIVITY

SUWIMOL TONGKAMKAEW : QUALITY ASSESSMENT AND ANTIMICROBIAL ACTIVITIES OF *PSIDIUM GUAJAVA* LEAVES. ADVISOR : CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., 91 pp.

Khee Nok cultivar is the indigenous variety of *P.guajava* L. (Myrtaceae) in Thailand. Its leaves were collected from 15 places throughout Thailand and investigated for the pharmacognostic specification of leaves and chemical constituents of the leaf essential oil. The results demonstrated the contents of loss on drying, total ash, acid insoluble ash, ethanol-soluble extractive value, water-soluble extractive value, moisture and volatile oil content as 7.88±0.50, 7.03±0.31, 1.42±0.25, 8.61±0.78, 10.21±0.52, 8.71±0.47 and 1.54±0.17 by dried weight, respectively. Anatomical and histological structures of the leaf were illustrated. Unicellular trichomes and oil glands were abundant. Trichomes were found in both dorsal and ventral region. The average trichome numbers in 1 mm^2 of dorsal and ventral area were 24.21±3.75 and 46.20±4.68 respectively. The average oil gland numbers in 1 mm^2 was count in the same way and revealed the average of 38.94±3.96. Thin layer chromatographic fingerprint of ethanolic extract was achieved using dichloromethane: ethyl acetate: methanol: acetic acid 8:4:2:0.1 as mobile phase and visualization under day light, UV 254 nm, 365 nm and staining with sulfuric acid reagent. The leaf essential oil was extracted by hydrodistillation. At least 40 chemical constituents of the essential oil were identified by GC/MS. The major constituents were caryophyllene (28.21±2.91%), limonene (7.17±5.47%), copaene<alpha-> (8.06±3.58%), aromadendrene (6.86±3.86%) and globulol (5.90±2.89%). The essential oil was investigated for antimicrobial activity by agar diffusion assay and demonstrated bactericidal activity against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Micrococcus luteus*.

Department : College of Public Health Sciences Student's Signature ....

Field of Study : Public Health Sciences Advisor's Signature ....

Academic Year : 2013 Co-advisor's Signature ....

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LIST OF ABBREVIATIONS

ATCC	=	American Type Culture Collection, Maryland, USA
°C	=	Degree celsius
CFU	=	Colony forming unit
cm	=	Centimeter
DMSO	=	Dimethyl sulfoxide
EI	=	Electron Ionization
GC	=	Gas chromatography
GC/MS	=	Gas chromatography/mass spectrometry
g	=	Gram
m/z	=	Mass-to-charge ratio
mm^2	=	Square millimeter
mg	=	Milligram
MIC	=	Minimal inhibitory concentration
ml	=	Milliliter
mm	=	Millimeter
MS	=	Mass spectrometry
nm	=	Nanometer
NIST	=	National Institute of Standard and Technology
S.D.	=	Standard derivation
TLC	=	Thin layer chromatography
UV	=	Ultraviolet
v/v	=	Volume by volume
µl	=	Microliter

CHAPTER I
INTRODUCTION

Background and significance of the study

The herbal medicines have been popular and developmental in many countries around the world since the World Health Organization urged its member countries to use folk healing practices and herbal medicines as a part of the basic public health projects. The Herbal medicines are used as remedies, over-the-counter drug products and raw materials for the pharmaceutical industry. However the quality of herbal medicine has not been shown enough to confirm the confidence of consumer. Therefore, there are needs of the procedures to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards by following the World Health Organization guidelines [1]. The herbal medicines have been used to prevent and treat of various health ailments for a long time and become more popular over the past decades [2]. More and more people turn to use herbal medicine due to the awareness of western medicine's side effects as well as the belief in herbal medicine efficacy and safety.

Psidium guajava L. (Family Myrtaceae) is an important medicinal plant in traditional Thai medicine [3] especially the indigenous variety which is called “FARANG-KHEE-NOK”. Khee Nok cultivar has been commonly found in Thailand. However, the cultivation is reduced and crossbreeding is concerned. *P. guajava* Khee Nok cultivar is a small tree, which is 10 m high with thin, smooth, patchy, peeling bark. Leaves are opposite, short-petiolate, the blade oval with prominent pinnate veins, 5–15 cm long. Flowers are somewhat showy, petals whitish up to 2 cm long, stamens numerous [4]. Flesh of the fruits is red and also has hard white seeds. The leaves have been used in traditional Thai medicine for treatment of diarrhea, wound, abscess and ingrown nail. Several tropical leaves including *P. guajava* provided distinctive chemical compounds with health benefits for example the antibacterial, antioxidant and anticancer activities [5]. Plant volatile oil consists of a number of small terpenoids and phenolic compounds which demonstrate high antibacterial activity [6].

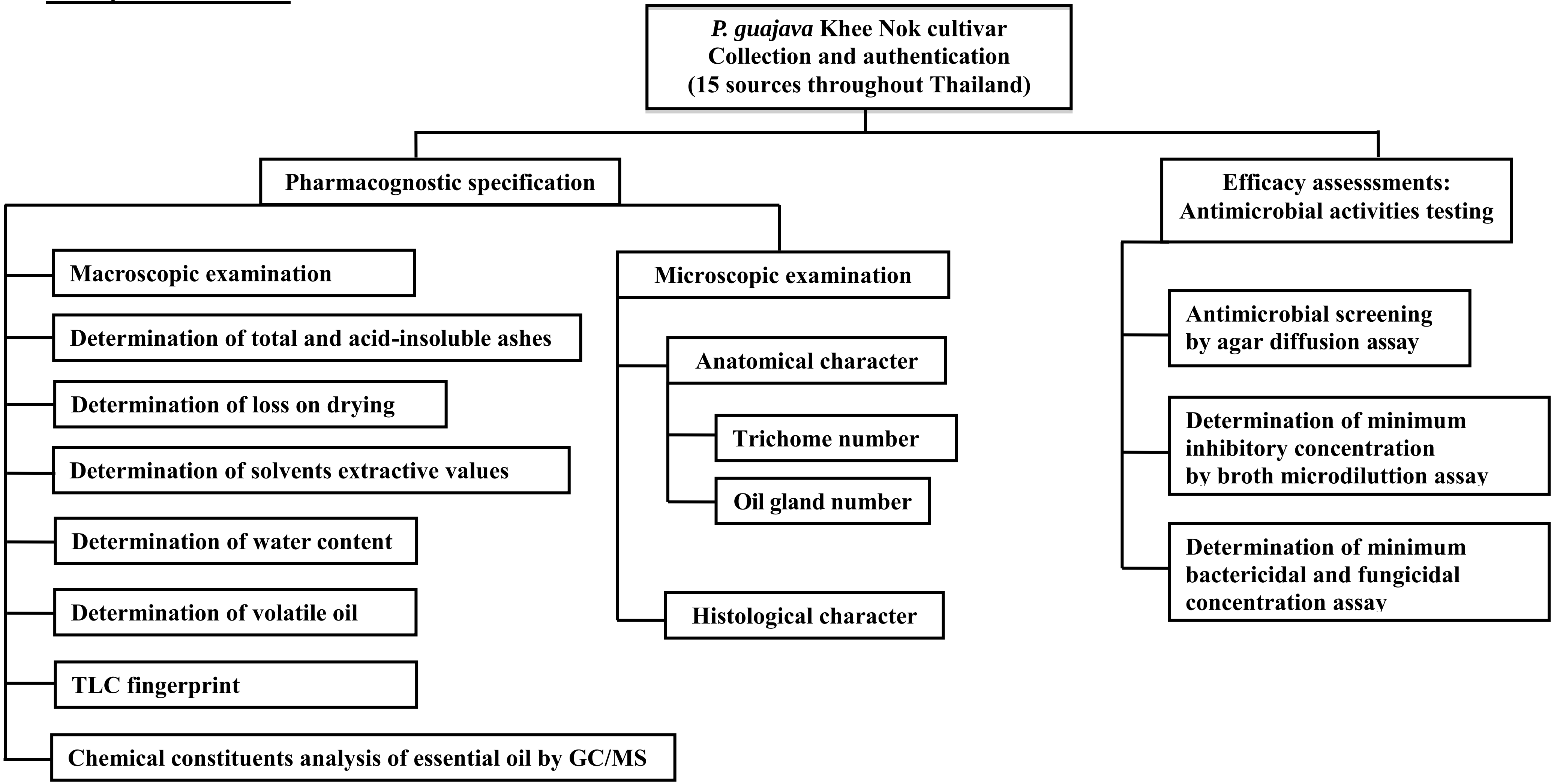
The previous study of the guava plant established lots of its phytochemical components including tannins, phenols, triterpenes, lectins, quercetins, leucocyanidins, sequiterpenes hydro-carbons, caryophyllenes, sterols, gallic acid, guavins A, C and D, carotenoids, vitamins, fibers and fatty acids [7]. Even though *P. guajava* leaves is widely used in traditional Thai medicine, there is no establishment of quality standard of *P. guajava* Khee Nok cultivar.

The purpose of this study was to investigate the standardization parameters by qualitative and quantitative analyses of *P. guajava* Khee Nok cultivar leaves as well as antimicrobial activities of *P. guajava* Khee Nok cultivar leaves essential oils.

Objectives

1. To develop the standardization parameters of Khee Nok cultivar leaves.
2. To develop the microscopic parameters of Khee Nok cultivar leaves.
3. To evaluate the chemical composition of Khee Nok cultivar leaves essential oil.
4. To evaluate the antimicrobial activities of Khee Nok cultivar leaves essential oil.

Conceptual Framework



CHAPTER II
REVIEW LITERATURE

Taxonomy

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: *Psidium*

Species: *Psidium guajava*

The plants of the genus *Psidium* were commonly used as folk medicine around the world. The genus *Psidium* belongs to the family Myrtaceae.

…Psidium guajava L. is a *shallow-rooted shrub or small tree, up to 10 m tall, branching from the base and often producing suckers. Bark smooth, green to red-brown, peeling off in thin flakes. Young twigs 4-angled and ridged, pubescent. Leaves opposite, glandular; petiole 3—10 mm long; blade elliptical to oblong, 5—15 cm x 3—7 cm, glabrous above, finely pubescent beneath, veins prominent below. Flowers solitary or in 2—3-flowered cymes, axillary, ca. 3 cm in diameter; calyx lobes 4—6, 1—1.5 cm long, irregular, persistent; petals 4—5, white, 1—2 cm long; stamens numerous, 1—2 cm long; ovary 4—5-locular; style 1.5—2 cm long, stigma capitate. Fruit a berry, globose, ovoid or pyriform, 4—12 cm long, surmounted by the calyx lobes; exocarp green to yellow; mesocarp fleshy, white, yellow, pink or red, with stone cells, sour to sweet and aromatic. Seeds usually numerous, embedded in pulp, yellowish, bony, reniform, 3—5 mm long…*"…[8].

Figure 1 Khee nok cultivar *P. guajava* L.

…Microscopic characteristics of P. guajava L *—The surface view shows nearly straight anticlinal epidermal cell walls on both surfaces, those on upper surface thickened; abundant stomata and covering trichomes on lower surface; oil glands on both surfaces, but more frequent on lower surface. Transverse section shows small epidermal cells with straight anticlinal walls, upper epidermal layer 2–3 cells thick with a few oil glands (schizogeneous); palisade tissue multi-layered; numerous oil glands in the mesophyll region; midrib region shows collenchymatous cells; bicollateral vascular bundle is distinctly horse-shoe shaped and surrounded by lignified pericyclic fibres; xylem elements are generally lignified; uniseriate trichomes smooth-walled and abundant on lower epidermis…*[9].

In addition, Meenakshi V. *et al*. (2012) reported that the leaves of *P. guajava* were hypostomatic, stomata were paracytic and guard cells were kidney shaped with thick inner and outer ledge. Subsidiary cells are rectangular with thin walls. Epidermal cells are irregular with thick walls. Trichomes are simple and unbranched [10].

Traditional Thai medicinal uses

The leaves have been used in traditional Thai medicine for diarrhea. Six to eight fresh mature leaves are chewed up and eaten followed by drinking water or boiled in 2 cups of water then simmered down to 1 cup or infused in 2 cups of water for 15-20 min. Pulverized fresh mature leaves can be used as poultice to stop breeding. Fresh leaves are also chewed to treat bad breath [11].

Phytochemistry

P. guajava leaves were reported for many chemical constituents. The major chemical constituents contain tannins, essential oils, flavonoids and terpenes. Flavonoids reported are quercetin, guajaverin (quercetin-3-*O*-arabinoside), other quercetin glycosides, kaempferol, avicularin and its 3-*L*-4 pyranoside with strong antibacterial action. Tannins reported are gallocatechin, ellagic acid and guavins A, C and D. Terpenes reported are present including ursolic, oleanolic acids and their 20-hydroxy derivatives, nerolidiol, crataegolic acid and guaijavolic acids [3, 9].

The main constituents in essential oil were α-pinene, β-pinene, β-bisabolene, menthol, 1,8-cineol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, caryophyllene oxide, limonene, β-copanene, humulene, selinene, cardinene, uvaol, ursolic acid, curcumene 3-*O*-β-*D*-glucopyranoside, 2α-hydroxy ursolic acid, isoneriucoumaric acid and oleanolic acid [12, 13, 14]. 1,8-Cineol and α-pinene are the principal monoterpenes, caryophyllene and bisabolene are representative of the sesquiterpenes [13, 14].

Figure 2 presented the structures of quercetin, guajaverin (quercetin-3-*O*-arabinoside), ursolic acids, oleanolic acids, ellagic acid, α-pinene and caryophyllene [9].

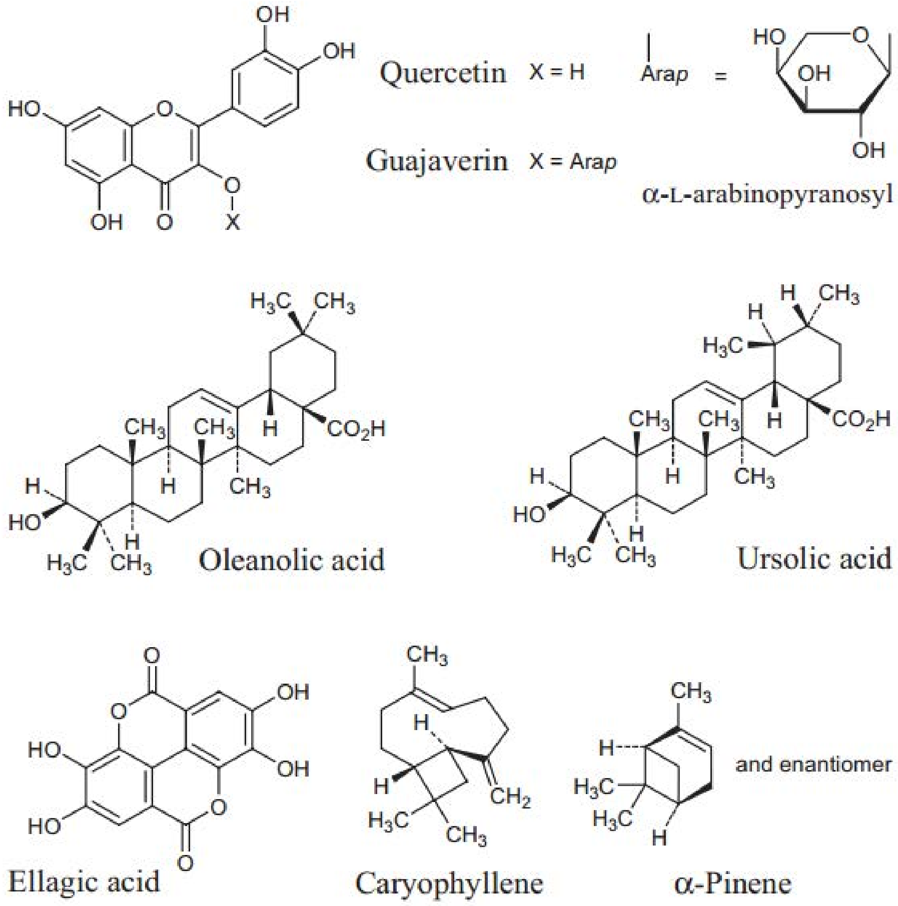


Figure 2 Chemical consituents structure of *P. guajava* leaves

Biological activities

The previous studies of *P. guajava* leaves were reported for its medicinal properties. They were antidiarrheal [15], antimicrobial, antitussive [16], antimalarial [17], antigenotoxic [18], antimutagenic [18], anti-allergic [19], anti-hyperglycemic, anti-inflammatory and antinociceptive activities [3, 20]. *P. guajava* also showed the properties of cardiovascular effect, hypotensive effects, acne lesions healing, wound healing [3, 20], hepatoprotective effects [21], antioxidant [22], free radical scavenger, radio protective effect [23], analgesics effect [24] and dental plaque reduction [25]. The ethnomedical uses of *P. guajava* were demonstrated in **Table 1** [3].

Table 1 Ethnomedical uses of *P. guajava* L. [3]

Region	Part used	Medical use	Preparation
Colombia	Leaves	Gastroenteritis, diarrhea, dysentery, rheumatic pain, wounds, ulcers, and toothache	Decoction and poultice
Indigenous Maya, Nahuatl, Zapotec and Popoluca of the region, Tuxtlas, Veracruz	Leaves	Cough, diarrhea	Decoction or infusion
Latin America, Mozambique	Leaves	Diarrhea, stomachache	Infusion or decoction
Mexico	Shoots, leaves, bark and leaves mixed, ripe fruits	Febrifuge, expel the placenta after childbirth, cold, cough hypoglycemic, affections of the skin, caries, vaginal hemorrhage, wounds, dehydration, fever, respiratory disturbances	Decoction, poultice
Latin America, Central and West Africa, and Southeast Asia	Leaves	Gargle for sore throats, laryngitis and swelling of the mouth, and it is used externally for skin ulcers, vaginal irritation and discharge	Decoction
Cook Islands	Leaves	Sores, boils, cuts, sprains	Infusion or decoction

Table 1 Ethnomedical uses of *P. guajava* L. [3] (cont.)

Region	**Part used**	**Medical use**	**Preparation**
Panama, Cuba, Costa Rica, M´exico, Nicaragua, Panam´a, Per´u, Venezuela, Mozambique, Guatemala, Argentina	Leaves	Anti-inflammatory	Externally applied hot on inflammations
South Africa	Leaves	Diabetes mellitus, hypertension	Infusion or decoction
Caribbean	Leaves	Diabetes mellitus	Infusion or decoction
China	Leaves	Diarrhea, antiseptic, Diabetes mellitus	Infusion or decoction
Philippines	Leaf, bark, unripe fruit, roots	Astringent, ulcers, wounds, diarrhea	Decoction and poultice
India	Leaves	Febrifuge, antispasmodic, rheumatism	Decoction or infusion
Ghana	Shoots	convulsions, astringent	
Senega	Shoots, roots	Diarrhea, dysentery	Infusion or decoction
Kinshasa, Congo	Leaves, bark	Diarrhea, antiamoebic	Infusion or decoction, tisane
Panama, Bolivia and Venezuela	Bark and leaves	Dysentery, astringent, used as a bath to treat skin ailments	Decoction

Table 1 Ethnomedical uses of *P. guajava* L. [3] (cont.)

Region	Part used	Medical use	Preparation
Peru	Flower buds, leaves	Heart and constipation, conjunctivitis, cough, diarrhea, digestive problems, dysentery, edema, gout, hemorrhages, gastroenteritis, gastritis, lung problems, shock, vaginal discharge, vertigo, vomiting, worms	Infusion or decoction
Fiji	Leaves, roots, ripe Fruit	Diarrhea, coughs, stomach-ache, dysentery, toothaches, indigestion, constipation	Juice, the leaves are pounded, squeezed in salt water
New Guinea, Samoa, Tonga, Niue Futuna, Tahiti	Leaves	Itchy rashes caused by scabies	Boiled preparation
Trinidad	Leaves	Bacterial infections, blood cleansing, diarrhea, dysentery	Infusion or decoction
USA	leaf	Antibiotic and diarrhea	Decoction
Brazil	Ripe fruit, flowers, and leaves	Anorexia, cholera, diarrhea, digestive problems, dysentery, gastric insufficiency, inflamed mucous membranes, laryngitis, mouth (swelling), skin problems, sore throat, ulcers, vaginal discharge	Mashed, Decoction

Pharmacognostic specification [1]

Macroscopic and microscopic examination

Herbal materials are categorized according to sensory, macroscopic and microscopic characteristics. Visual inspection is the simplest and fastest means by which to establish identity, purity and quality. Macroscopic determination of herbal materials bases on the shape, size, color, the surface texture, fracture characteristics and appearance of the cut surface. The microscopic examination of the herbal material is necessary for identifying the histological character, cells and structural features.

Determination of loss on drying

This test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Drying can be carried out either by heating or using a desiccator. The desiccation method is especially useful for materials that melt to a sticky mass at elevated temperatures.

Determination of ashes

Ash remaining following combustion of herbal material is determined by two different methods. There are the total ash and acid insoluble ash. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter adhering to the plant surface. Total ash represents carbonate, phosphate, silica and silicates such as sand and soil in the sample. Acid - insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This method can measure the silica presented in the sample, especially as sand and siliceous earth. Determination of ashes can indicate the contamination, adulteration or substitution in preparing the crude drug.

Determination of extractive values

Extractive values are determined for amount of active constituents in a given amount of medicinal plant material when extracted with specified solvents. These values provide an indication of the extent of polar, medium polar and non-polar components present in the plant material.

Determination of water content

Water content or moisture content is determined for the amount of water in plant material. It is based on the fact that water and water immiscible solvent such as toluene or xylene will form a binary azeotropic mixture. Azeotropic method provides the direct measurement of the water present in the material being examined. The water in sample is distilled together with an immiscible solvent. The distilled water are separated and the water volume is recorded.

Determination of volatile oil content

Volatile oil is characterized by its odour, oil-like appearance and ability to volatilize at room temperature. It is usually composed of a mixture of chemicals, for example, monoterpenes, sesquiterpenes and their oxygenated derivatives. Aromatic compounds predominate in certain volatile oils. Because they are considered to be the "essence" of the herbal material, and are often biologically active. The volatile oil is also known as "essential oil". The term "volatile oil" is preferred because it is more specific and describes the physical properties.

The volatile oil is immiscible with water, so it can be distilled with water and condensed to separate layer which can be collected and recorded the volume.

Thin layer chromatographic identification

Thin layer chromatography is particularly valuable for the qualitative determination of chemical mixtures. Separation by TLC is effected by could be directly compared and identified by the fluorescence or color reaction of the developed spots. Silica gel 60 F_{254} precoated TLC plates (Merck, Germany) 0.063–0.200 mm will be used and developed in 10 cm path. Then the plate was placed into a tank with sufficient suitable solvent to just wet the lower edge of the plate sorbent but not enough to wet the part of the plate where the spots were applied. The solvent front then migrates up the plate through the sorbent by capillary action, a process known as development. Remove the plate, mark the position of the solvent front and allowed the solvent to evaporate at room temperature. The spots were visualized under UV light at 254 nm and 365 nm. The information provided by a finished chromatogram of the separated substances. It was given in the form of the R_f value [26-28].

After development, the plate was dried in an oven or fume hood to evaporate the solvent. Compounds were detected on thin layers by their natural color, natural fluorescence under UV light, quenching of fluorescence on a phosphor containing layer, or as colored, UV absorbing, or fluorescent zones after reaction with an appropriate reagent.

Thin layer chromatography or TLC was a solid-liquid form of chromatography. The stationary phase was normally a polar absorbent and the mobile phase could be a single solvent or combination of solvents. The benefited of TLC was inexpensive, fast, short time for analysis and technique to easy. This method used to analyze the mixtures or plant material by separating the compounds in the mixtures or plant material [26-28].

Qualitative of TLC provided the chromatographic measurement known as an R_f value. The R_f value is the “retardation factor” or the “ratio-to-front” value expressed as a decimal fraction. The R_f value was used to quantify the movement of the materials along the plate. The R_f value was calculated as [28]:

$$R_f = \frac{\text{Distance of the compound from original spot travelled to the developed spot}}{\text{Distance of the solvent from original line travelled to the developed line}}$$

Moreover, TLC provides the mean not only for flexible screening procedures and qualitative analyze but also for demanding quantitative determinations. It is not only instrumental TLC but entire concepts that include widely standardize methodology based on validated method. It is instrument controlled by software if there were used for quantitative analysis included data from scanning densitometry and image analysis method.

TLC was used for qualitative and quantitative method. The qualitative determined by the number of compounds in a mixture and identified substances. Moreover, quantitative method used of content determination to test the substances [28]. Fingerprint determine of method for the quality control plant material that were confirmed by WHO. It's suitable for detect adulterations and identify plant species [29]. Chromatographic methods consist of TLC, HPLC and GC is commonly used for fingerprint. TLC method used for identified and authenticate compounds in plant material and its derivative for obtains a fingerprint profile [30-31].

Gas chromatography-mass spectrometry (GC/MS)

Gas chromatography (GC) and mass spectrometry (MS) make an effective combination for chemical analysis. Gas chromatography is performed by an instrument called a gas chromatograph. It involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert gas or mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. Mass spectrometry is usually combined with gas chromatography, liquid chromatography or ion mobility. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios (*m/z*). The most common data provided by the MS is the mass spectrum which is unique and can be used to identify each chemical compound. This data are compiled to the library database for both of mass spectra manual interpretation or software assisted interpretation [32-34].

Antimicrobial susceptibility testing

The aim of antimicrobial susceptibility testing was to guide the appropriate agents for therapy. Constructive agents were commonly used empirically and the laboratory test serves to explain treatment failures and provide suitable alternative agents' range. Routine testing also provide up-to-date accumulated data from information on the most suitable agents for empirical use can be derived. Antimicrobial susceptibility tests are used to evaluate the *in vitro* activity of new agents [35, 36]. Protocols are present with predictions by the Clinical and Laboratory Standards Institute (CLSI) [37].

Antimicrobial susceptibility tests were depended on diffusion and dilution method. Laboratory procedures involving diffusion susceptibility test were commonly performed in agar media called agar diffusion technique.

Agar diffusion susceptibility testing

In general, agar diffusion test were performed by inoculating a nutrient agar medium in a standardized manner and apply the drug to be studied to the agar surface in some type of reservoirs. The drug was allowed to diffuse around medium. The exposes test organism to a continuous gradient of drug concentration, with concentration diminishing as distance from the reservoir increase. After an appropriate period of incubation, there should be a zone of inhibited growth around the reservoir. The zone inhibition's size may be measured to determine the degree of test organism's susceptibility [38].

The solution of antimicrobial agents may be applied to surface of a seeded agar medium in several different. Agar disc diffusion method use filter paper disc that has been moisten with the drug solution and the applied directly to the agar while still wet, disk may be prepared more accurately if a micropipette is used to load each disc with a measured volume of drug solution [39, 40]. Alternatively, agar will be cut from the seeded agar medium by using a cork borer in specified diameter and then be filling with the drug solution [41, 42].

Minimum inhibitory concentration (MIC)

The susceptibility of organisms to serial dilutions of agents in agar or broth is determined. The MIC was defined as the lowest concentration of the agents that inhibits visible growth. The methods were not widely used for routine testing in clinical laboratories in most countries, although commercial systems with restricted range MIC in microliter format were frequently used in some. The most widespread use of MIC methods was in testing new agents. In clinical laboratories, MIC methods were used to test the susceptibility of organisms, which give equivocal results in diffusion tests, for tests on organisms where disc tests may be unreliable, as with slow-growing organisms, when a more accurate result was required for clinical management, e.g. infective endocarditis. In some case it was necessary to know the minimum concentration of an agent that kills, rather than merely inhibits growth of bacterium and fungal.

The minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC) was determined by sub-culturing from tubes showing no turbidity onto antimicrobial agent-free media and observing for growth after further incubation [38, 43].

Quality control of *P. guajava* leaves

The quality control of *P. guajava* leaves could be efficiently performed to analytical method and appropriate quality specifications have been established.

In 2011, the pharmacognostic specification of *P. guajava* leaves has been reported that loss on drying 8.50% (w/w), total ash 7.65% (w/w), water soluble ash 1.50% (w/w), acid insoluble ash 7.50% (w/w), petroleum ether soluble extractive value 3.32% (w/w), methanol soluble extractive value 24.00% (w/w), acetone soluble extractive value 9.00% (w/w), aqueous soluble extractive value 16.92% (w/w) The anomocytic stomata, oil glands and unicellular trichomes were present. Prismatic and cluster type of crystals of calcium oxalate were found [44]. Mishra *et al*. were report that the data of quality parameters there were amount in both young/ mature leaves, ash value 6.20/7.00, water soluble ash value 16.00/11.36, acid insoluble ash value 57.90/16.00, water soluble extractive value 5.40/5.20, alcohol soluble extractive value 4.90/4.70, moisture content 14.00/10.75 were present nearly. The powder microscopy also reveals the presence of unicellular trichome, anomocytic stomata, xylem vessel and calcium oxalate crystals. It could be useful in the identification and standardization of a crude drug [45].

In 2012, the pharmacognostic specification of *P. guajava* leaves has been reported that total ash not more than 10%, acid insoluble ash not more than 3.67%, water extractive value not more than 40%, ethanol extractive value not more than 16%, methanol extractive value not more than 13.92%, chloroform extractive value not more than 3.12%. Stomata are anomocytic. Guard cells are kidney shaped. Epidermal cells are irregular with thick walls and trichomes are simple and unbranched [46].

Thiago Levi Silva Oliveira *et al*. this research was to determine parameters for the characterization and quality control of leaf powder *P. guajava* L. Determination of particle size distribution 391.548±1.532 µm, total ash 6.266±0.011%, acid-insoluble ash 0.003±0.002%, volatile content 6.666±0.057% and swelling index 4.733±0.152% The powder microscopy of *P. guajava* leaf subjected to the Steinmetz reagent revealed the presence of lower epidermis fragments with stomata, unicellular trichomes and upper epidermis fragments having epidermal cells with straight walls without stomata [47]. In addition, Shikha *et al*. reported that foreign organic matter

1.76±0.31, total ash 8.01±0.50, acid insoluble ash 1.67±0.52, alcohol extractive value 6.05±0.05, water soluble extractive value 12.67±0.32 [48].

The previous reports of Khee Nok Cultivar of *P. guajava* leaves in Thailand were not reported that of the pharmacognostic specification. This study could be investigated that of Khee Nok Cultivar of *P. guajava* leaves from 15 different places throughout Thailand according to WHO Quality Control Methods for Herbal material.

Thin layer chromatographic identification of *P. guajava* leaves

Thin layer chromatography identification of *P. guajava* leaves was performed by using the different solvent extracts of *P. guajava* leaves. TLC profile of the petroleum ether extract of *P. guajava* leaves solvent system benzene: chloroform: ethyl acetate (4:3:3). R_f values: 0.10, 0.74, 0.87, 0.93. TLC profile of the chloroform solvent system: benzene: chloroform: ethyl acetate (3:4:3). R_f values: 0.10, 0.15, 0.21, 0.73, 0.84, 0.93. TLC profiles of the methanol extract solvent system: ethyl acetate: methanol (7:3). R_f values: 0.34, 0.44, 0.50, 0.68, 0.84 [49].

In 2012, high performance thin layer chromatography (HPTLC) identification of *P. guajava* leaves was performed on Siliga gel 60 F_{254} using acetone extract of tender leaves using the solvent system, toluene: acetone: formic acid (38:10:5) for detection the spot [50]. Siti N *et al*. reported that *P. guajava* methanol leaf extracts used hexane: ethyl acetate (7:3) for mobile phase TLC analysis to easily detected and calculated for R_f values the spots [51].

Chemical constituents analysis of *P. guajava* leaves oil by GC/MS

P. guajava leaves oil were isolated and analyzed by gas chromatography with mass spectrometry (GC-MS). The main constituents being α-pinene, β-pinene, 1,8 cineole and β-caryophyllene [52-54]. JP N. *et al*. (2013) reported that the essential oil of *P. guajava* were limonene (3.4-20.7%), β-bisabolol (14.9-20.2%), epi-β-bisabolol (11.7-18.9%), β-caryophyllene (6.1-10.9%), β-bisabolene (7.5-10%) [55].

Chen, *et al*. (2007) identified the essential oil constituents 50 compounds from the leaves of *P. guajava* L. was analyzed by GC/MS. The major constituents were β-caryophyllene (27.7%), α-pinene (14.7%) and 1,8-cineole (12.4%) [56].

In Thailand *P. guajava* leaves oil were studied by GC-MS the major compounds in the volatile oil from the leaves of *P. guajava* were gamma-terpinene and alpha-pinene, respectively present in **Table 2** [57].

Table 2 Chemical constituents of *P. guajava* volatile oil [57]

Compounds	%Area	Retention Time
Monoterpenes		
α -Pinene	23.89	5.65
Sylvestrene	7.85	8.44
Oxygenated monoterpenes		
1,8-Cineol	6.32	8.54
Sesquiterpenes		
E-Caryophyllene	14.30	24.18
Aromadendrene	3.01	24.96
α –Humulene	1.51	25.54
β- Bisabolene	2.19	27.74
Oxygenated sesquiterpenes		
E-Nerodiol	3.33	29.85
Caryophyllene oxide	17.25	30.59
Caryophylla-4(12), 8(13)-dien-5-alpha-ol	2.50	32.58

Antimicrobial activity testing of *P. guajava* leaves oil

The leaves oil of *P. guajava* showed the inhibitory effect of *Bacillus subtilis* and *Staphyllococcus aureus* greater [58]. Higher concentrations of chemical compounds that are active in the essential oils to describe the actions of their strong growth inhibition of *Lactobacillus lactis, Lactobacillus acidophilus, Enterobactor aerogenes, Pseudomonas fluorescens* [59, 60], *Bacillus cereus, Lactobacillus lactis, Enterobactor aerogenes, Glucanobactor oxidans* and *Propionibacterium acnes* [57] but no more activity had been observed against the three fungal organisms [61]. In addition it could be inhibited the growth of *Carnobacterium gallirarum, Escherichia coli, Salmonella enteritidis, Staphylococcus aureus*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Shigella flexneri* [62].

B Wannissorn, *et al*. (2013) testing the antimicrobial activity of *P. guajava* leaves oil cultivated in Thailand by using disc diffusion assay was evaluated against zoonotic enteropathogens including *Salmonella spp., Escherichai coli* 0157*, Campylobacter jejunii* and *Clostridium perferingens.* The previous study reported that the inhibitory effect for *Campylobacter jejunii* and *Clostridium perferingens* but exhibited *Salmonella spp.* and *Escherichai coli* 0157 [63].

CHAPTER III
MATERIALS AND METHODOLOGY

Chemicals

1. Acetic acid (Merck, Germany)
2. Ampicillin sodium (T.P. Drug Laboratories(1969) Co., Ltd., Thailand)
3. Amikacin sulfate (T.P. Drug Laboratories(1969) Co., Ltd., Thailand)
4. Dichloromethane (Labscan, Thailand)
5. Dimethyl sulfoxide (Merck, Germany)
6. Ethanol (Labscan, Thailand)
7. Ethyl acetate (Labscan, Thailand)
8. Hydrochloric acid (Labscan, Thailand)
9. Mueller Hinton agar and broth (Merck, Germany)
10. Methanol, HPLC grade (Labscan, Thailand)
11. Sabouraud Dextrose agar and broth (Merck, Germany)
12. Sodium chloride (Mallinckrodt, USA)
13. Toluene (Labscan, Thailand)

The chemicals were analytical grade.

Materials

1. Filter-paper No.4 (Whatman^{TM}, UK)
2. Filter-paper No.40 ashless (Whatman^{TM}, UK)
3. TLC silica 60 F_{254} 20 x 10 cm and 10 x 10 cm, layer thickness 0.2mm. (Merck, Germany)
4. 96-well microtiter plates (Constar, USA)
5. Zebron ZB 5 cappillary column (30 m x 0.25 mm, 0.25µm) (Phenomenex®, CA, USA)

Instruments and equipments

1. Analytical balance (Satorious, Germany)
2. Autoclave (ALP Co., Ltd., Japan)
3. Azeotropic apparatus
4. Balance readability 0.01 g (Pioneer^{TM} Ohaus Crop.Pine Book, NJ, USA)
5. Balance readability 0.0001 g (SI-234, Denver Instrument, Germany)
6. Clevenger apparatus
7. Gas chromatograph (Trace GC Ultra, Thermo Finnigan, USA) equipped with MS detector (DSQ, Thermo Finnigan, USA)
8. Hot air oven (WTB binder No.4940006, Germany)
9. Rotary evaporator (Buchi R210, Switzerland)
10. Spectrophotometer (T60 Visible Spectrophotometer, PG Instruments Limited, UK)
11. Ultraviolet fluorescence analysis cabinet (Spectronics coporation, Spectroline® MODEL CC-80, USA)

Methods

1. Plant materials

The leaves of *P. guajava* Khee Nok cultivar were collected from 15 places throughout Thailand and authenticated by Ruangrungsi N. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. The leaves were air dried and pulverized for pharmacognostic studies.

2. Standardization of *P. guajava* leaves

2.1 Characteristic feature

Macroscopic examination and microscopic examination were examined according to World Health Organization (WHO) guideline standard methods [1].

2.1.1 Macroscopic examination

Visual characters were observed and the whole plant was illustrated by hand drawing for its shape, size and botanical morphology.

2.1.2 Microscopic examination

Anatomical and histological characters of transverse section and powder of mature leaves were investigated under photomicroscope observation under objective lens with a 10X, 20X and 40X magnifications.

2.1.2.1 Anatomical character

Transverse section of leaf

Midrib from mature leaf of *P. guajava* Khee Nok cultivar was thinly transverse sectioned with razor by hand, separately placed a complete piece on the glass slide and cover with a cover glass. The midrib was investigated under photomicroscope observation with objective lens of 10X, 20X and 40X magnifications and eyepiece lens of 10X magnification to evaluate the fine details and recorded the image by digital camera and illustrated by hand drawing.

Trichome number and oil gland number

Mature leaf was cut into small pieces, soaked in sodium hypochlorite solution to remove the chlorophyll, washed 2-3 times. Then boil in chloral hydrate solution until it was clear. Then investigate the trichomes and oil glands in both dorsal epidermis and ventral epidermis of leaf section by wet mounting in glycerin and examining under the photomicroscope with objective lens of 10X magnification and eyepiece lens of 10X magnification. The images were record using program

AxioVision version. The numbers of trichomes and oil glands within 1 square millimeter of leaf section were counted per 1 field. Thirty fields were investigated. Trichome number and oil gland number were expressed as mean and SD.

2.1.2.2 Histological character

The powdered air-dried *P. guajava* Khee Nok cultivar leaves were mounted with water on a glass slide and examined under the microscope.

2.2 Determination of loss on drying

Exactly 3 g of the powders of *P. guajava* Khee Nok cultivar leaves were weighed using 4-digit analytical balance into pre-weighed crucible. Dried the sample at 105°C in an hot air oven for about 6-8 hours until constant weight. Calculate the loss of weight in a percentage of dried material. Each source was done in triplicate.

2.3 Determination of ash values

The ash remaining from combustion of herbal materials were determined by two different methods, which measured total ash and acid-insoluble ash. Each source was done in triplicate.

2.3.1 Determination of total ash

Incinerated exactly 3 g of the powdered air-dried *P. guajava* Khee Nok cultivar leaves in previously dried and pre-weighed crucible by gradually increasing the heat to 600°C until white, cooled in desiccator and weighed, calculated the content of total ash in a percentage of dried material. Each source was done in triplicate.

2.3.2 Determination of acid-insoluble ash

Twenty five milliliters of hydrochloric acid (70 g/l) were added to the crucible which contained the total ash, covered with the watch-glass and boiled for 5 minutes. Rinsed the watch-glass with 5.0 ml of hot water and added this liquid to the crucible. Collected the insoluble matter on an ashless filter-paper No.40 and washed with hot water until the filtrate was neutral. Transfered the filter-paper containing the insoluble matter to the original crucible, dried on a hot-plate and incinerated to constant weight. Allowed the residue to cool in desiccator then weighed without delay and calculated the content of acid-insoluble ash in a percentage of dried material. Each source was done in triplicate.

2.4 Determination of extractive values

This method determined the amount of active compositions in a given amount of *P. guajava* Khee Nok cultivar leaves when extracted with specified solvent. Each source was done in triplicate.

2.4.1 Determination of alcohol soluble extractive value

Exactly 4 g of coarse powdered air-dried of *P. guajava* Khee Nok cultivar leaves in a glass-stopper conical flask, macerated with 70 ml of 95% ethanol for 6 hours, frequently shake then allowed standing for 18 hours. Filtered, washed the marc with ethanol and adjusted the filtrate to 100 ml with ethanol, transferred 25 ml of the filtrate to a pre-weighed beaker and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in desiccator for 30 minutes, weighed without delay and calculated the content of extractable matter in a percentage of dried material. Each source was done in triplicate.

2.4.2 Determination of water soluble extractive value

Four grams of the powder of *P. guajava* Khee Nok cultivar leaves were accurately weighed and macerated with 70 ml of water under shaking for 6 hours then standing for 18 hours. Filtered, washed the marc with water and adjusted the filtrate to 100 ml with water, transferred 20 ml of the filtrate to a pre-weighed beaker and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in desiccator for 30 minutes, weighed without delay and calculated the content of extractable matter in a percentage of dried material. Each source was done in triplicate.

2.5 Determination of water content

Weighed exactly 50 g of the powder of *P. guajava* Khee Nok cultivar leaves by 2-digit analytical balance, and transferred to the distilling flask. Added 200 ml of water - saturated toluene and distilled using azeotropic apparatus until the water was completely removed. Allowed the water and toluene layers in the receiving tube to be separated and read off the volume of water. Calculate the content of water as a percentage of dried material. Each source was done in triplicate.

2.6 Determination of volatile oil

The essential oil of *P. guajava* Khee Nok cultivar leaves were determined the clevenger apparatus. Distilled 50 g of powdered air-dried of *P. guajava* Khee Nok cultivar leaves with 600 ml of water. Allowed the water and essential oil layers in the receiving tube to be separated and read off the volume of volatile oil, calculated the content of essential oil as a percentage of dried material. The essential oil from each source was collected for chemical constituents analysis by GC/MS. Each source was done in triplicate.

2.7 Thin layer chromatographic fingerprint

From aforementioned ethanol soluble extractive procedure, transferred 20/25 ml of the filtrate and evaporated to dryness. Re-dissolved the extract in 1 ml of methanol. Applied 3 µl of ethanolic extracted of *P. guajava* Khee Nok cultivar leaves on the silica gel 60 F_{254} TLC plate of 0.2 mm thickness, developed in a mixture of Dichloromethane: Ethyl acetate: Methanol: Acetic acid 8:4:2:0.1 for a path of 10 cm. After development, the plate was visualized under daylight, UV 254 nm, 365 nm and stained with sulfuric acid (10% in methanol) reagent.

2.8 Analysis of chemical constituents of Khee Nok cultivar *P. guajava* leaves essential oil.

The essential oils of *P. guajava* Khee Nok cultivar leaves from 15 sources were investigated by GC/MS using Finnigan trace GC Ultra connected to Finnigan DSQ Quadrupole detector and BPX5 fused silica column. The injector temperature was 180°C. One microliter of the oil (1:100 in HPLC grade methanol), was injected by splitter (1:100) into capillary column. The oven temperature was 60°C for 1 min, and then ramped to 240°C with the rate of 3°C/min. Helium was used as carrier gas (flow rate at 1 ml/min). MS was performed by EI positive mode at 70 eV ionization voltages. The constituents of the oil were identified by matching their mass spectrum and retention indices with Adams EO Mass Spectral library and NIST05 Mass Spectral library. The percentage ratio of each constituent was computed from GC peak areas.

3. Antimicrobial activities testing

3.1 Microorganisms

Bacillus subtilis ATCC 6633, *Candida albicans* ATCC 10230, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538P and *Saccharomyces cerevisiae* ATCC 9763 were obtained from the Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Micrococcus luteus ATCC 9341, *Bacillus cereus* ATCC 11778 and *Enterobacter aerogenes* ATCC 13048 were obtained from the Department of Microbiology, Faculty of Sciences and Technology, Suan Sunandha Rajabhat University.

Salmonella typhi (Isolates), *Salmonella typhimurium* ATCC 13311, *Shigella*sp. (Isolates) and *Straphylococcus epidermidis* (Isolates) were obtained from the Department of Microbiology, Faculty of Sciences, Chulalongkorn University.

3.2 Agar diffusion test

The essential oil of *P. guajava* Khee Nok cultivar leaves and its dilution (25 and 50 %v/v) in dimethyl sulfoxide (DMSO) were tested for antibacterial activities. Tested microorganisms were grown on Mueller Hinton agar (for bacteria) and Sabouraud Dextose agar (for fungi) then incubated at 37°C for 24 hours. The turbidity of culture was modulated to about 0.5 McFarland standards and suspended in 0.85% sodium chloride. The assay was performed using the double agar layer technique [43, 64]. Briefly, added 100 µl of the suspension to 5 ml of sterile seeds agar then poured onto sterile base agar. Allowed all plates dried at room temperature. Applied 20 µl of essential oil, DMSO as a negative control, ampicillin (1 mg/ml) and amikacin (1 mg/ml) as positive control to each sterile disc (6 mm in diameter). Put the disc on agar plate and incubated at 37°C for 24 hours. The diameter of inhibition zone was measured. Each sample was tested in triplicate.

3.3 Determination of the minimum inhibitory concentration (MIC)

MIC was performed by microdilution method in 96 well - microtiter plate. Each well was filled with 50 µl of essential oil or a positive / negative control in broth and 150 µl of inoculate broth and incubated at 37°C, for 24 hours. The experiment was tested in triplicate. MIC was recorded at the last well which was shown in clear solution.

3.4 Determination of minimum bactericidal concentration (MBC)

Streaked clear inoculate broth from MIC test on agar plates, incubated at 37°C, for 24 hours. The lowest concentration that did not permitted any visible growth on the plates after 18-24 hours was recorded as the MBC.

CHAPTER IV
RESULTS

Pharmacognostic specification of *P. guajava* Khee Nok cultivar leaves

Khee Nok cultivar is the indigenous variety of *Psidium guajava* Linn. (Myrtaceae) in Thailand.

Macroscopic examination

Morphological character of *P. guajava* Khee Nok cultivar was illustrated in **Figure 3.**

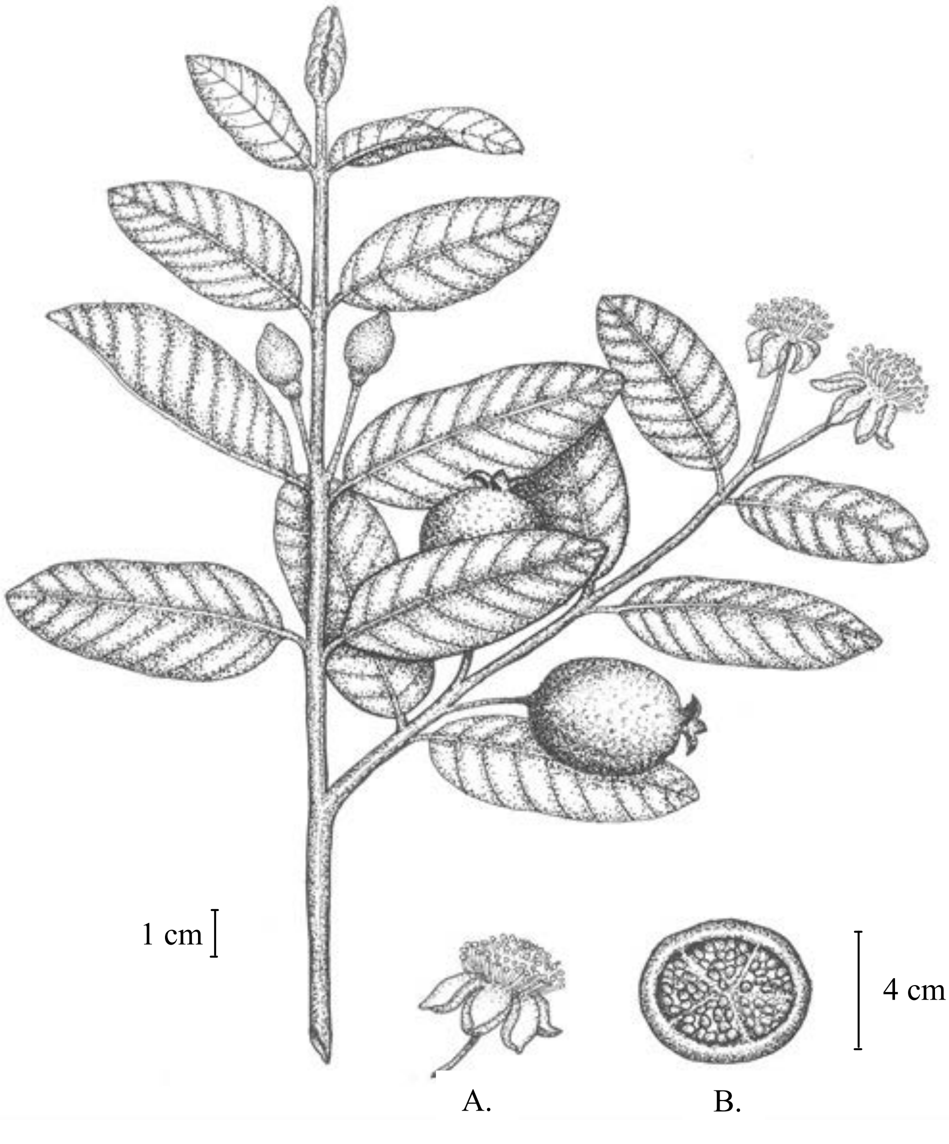


Figure 3 Whole plant of *P. guajava* Khee Nok cultivar

A. Flower B. Fruit

Figure 4 Crude drug of *P. guajava* Khee Nok cultivar leaves

Microscopic examination

Anatomical character of midrib cross section of *P. guajava* Khee Nok cultivar leaves showed unicellular trichome, epidermis, spongy mesophyll, palisade mesophyll, collenchyma, chlorenchyma, parenchyma, xylem vessel, parenchyma ray, phloem tissue and lower epidermis **(Figure 5).**

Histological characterization revealed fragment of fiber, reticulated vessels, unicellular trichome, parenchyma in transverse view, prism crystal of calcium oxalate and stomata **(Figure 6).**

Measurement of trichome was performed in both sides. The trichome number in 1 mm^2 of leaf area was counted for 1 field. Thirty fields were investigated. The average trichome number in dorsal epidermis and ventral epidermis of *P. guajava* Khee Nok cultivar leaf were 24.21±3.75, 46.20±4.68 respectively **(Table 3)**. Oil gland number was count in the same way and revealed the average of 38.94±3.96 **(Table 3)**.

Table 3 The average trichome number and oil gland number of *P. guajava* Khee Nok cultivar leaves (n=30)

The average	**Oil gland number**	**Trichome number**	
		Dorsal	**Ventral**
Min	32	15	38
Max	48	30	54
Mean ± SD	38.94±3.96	24.21±3.75	46.20±4.68

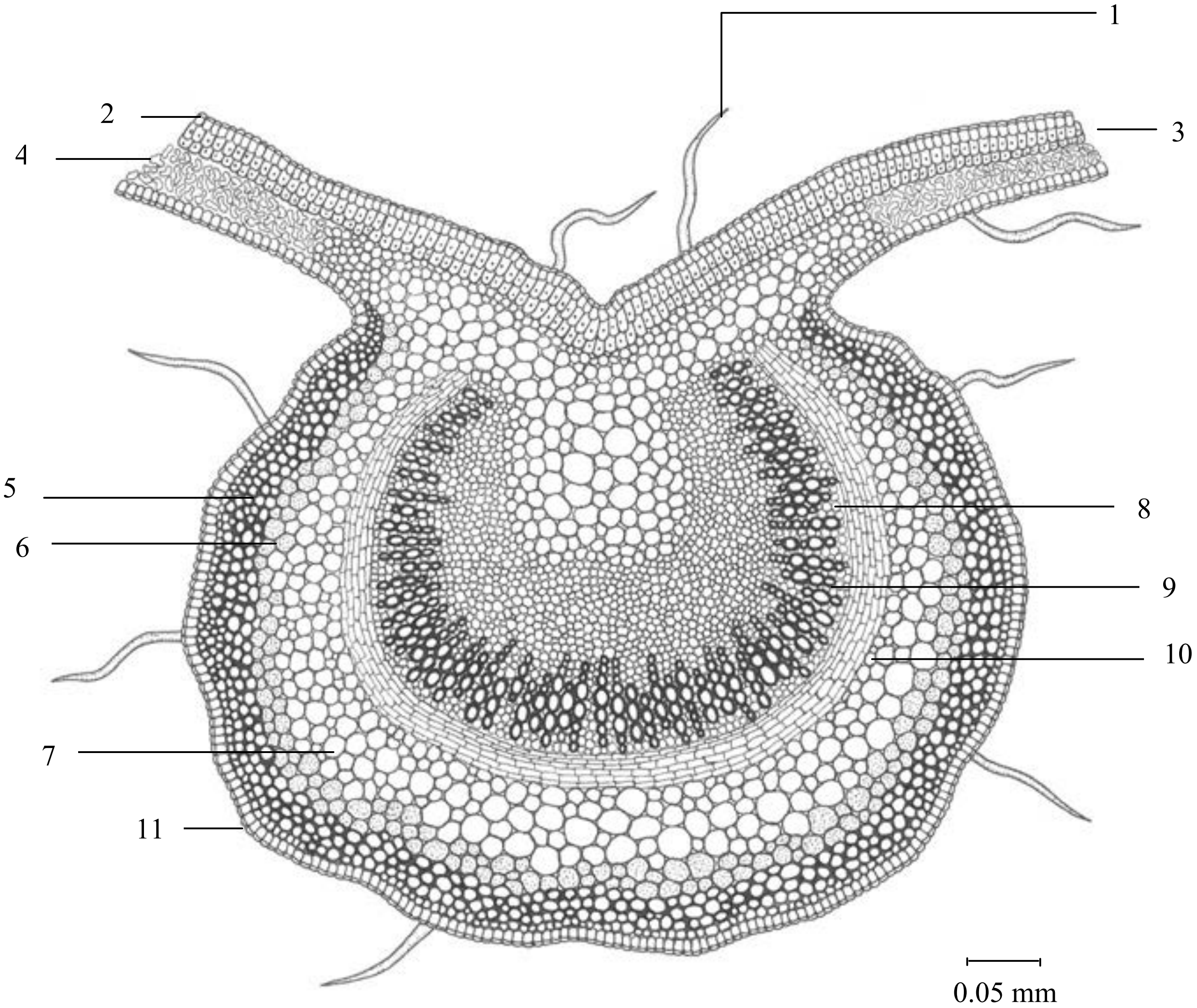


Figure 5 Midrib cross section of *P. guajava* Khee Nok cultivar leaf:

1. Unicellular trichome	2. Epidermis
3. Spongy mesophyll	4. Palisade mesophyll
5. Collenchyma	6. Chlorenchyma
7. Parenchyma	8. Xylem vessel
9. Parenchyma ray	10. Phloem tissue
11. Lower epidermis	

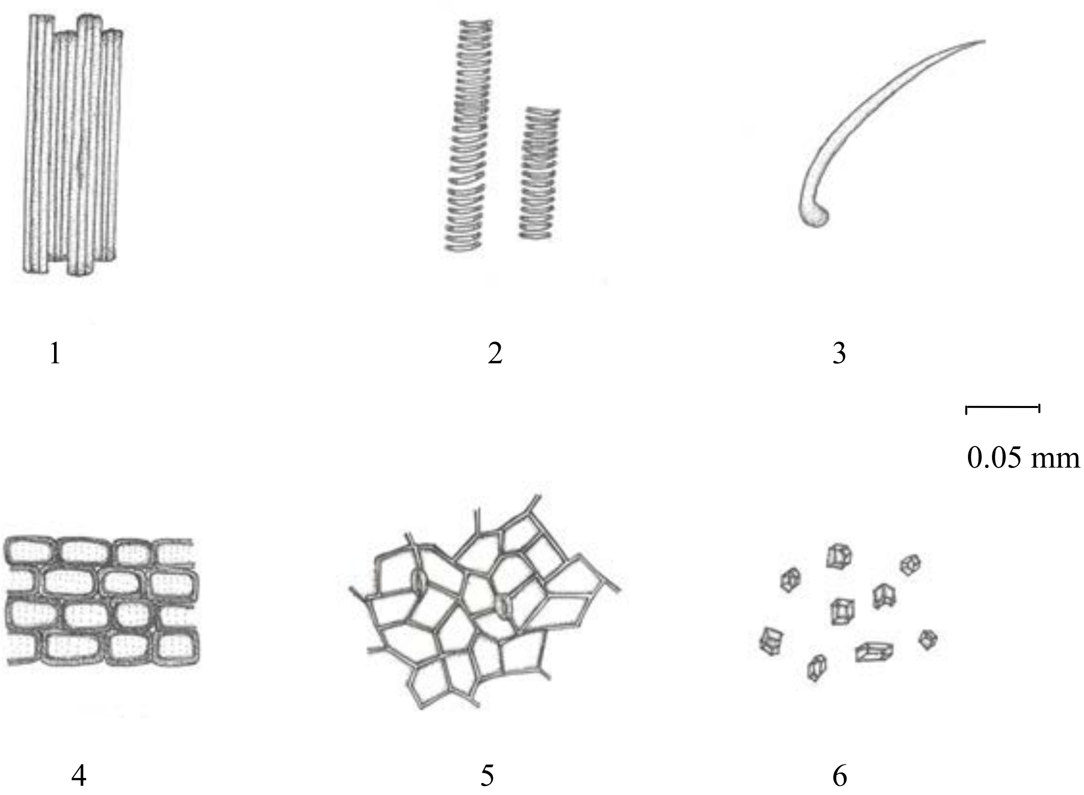


Figure 6 Powder of *P. guajava* Khee Nok cultivar leaf:

1. Fragment of fiber
2. Reticulated vessels
3. Unicellular trichome
4. Parenchyma in transverse view
5. Stomata
6. Prism crystal of calcium oxalate

Quality parameters

The constant numbers due to quality parameters of *P.guajava* Khee Nok cultivar leaves were shown in **Table 4**.

Loss on drying, total ash and acid insoluble ash should be not more than 7.88, 7.03 and 1.42% by dried weight respectively. Ethanol-soluble extractive value, water-soluble extractive value, moisture and volatile oil content should be not less than 8.62, 10.21, 8.71and 1.54% by dried weight respectively.

Table 4 The pharmacognostic parameters of *P. guajava* Khee Nok cultivar leaves

Specification	Content (% by dry weight)		
	Mean	**SD**	**Mean±3SD**
Loss on drying	7.88	0.50	6.391-9.363
Total ash	7.03	0.31	6.101-7.966
Acid-insoluble ash	1.42	0.25	0.672-2.169
Ethanol–soluble extractive value	8.61	0.78	6.260-10.968
Water–soluble extractive value	10.21	0.52	8.643-11.777
Water content	8.71	0.47	7.288-10.132
Volatile oil content	1.54	0.17	1.039-2.045

[a] The parameters were shown as grand mean ± pooled SD. Samples were from 15 different sources throughout Thailand. Each sample was performed in triplicate.

TLC fingerprint

TLC fingerprint of ethanolic extract of *P. guajava* Khee Nok cultivar leaves were shown in **Figure 7.** The mobile phase was dichloromethane: ethyl acetate: methanol: acetic acid 8:4:2:0.1.

The plate was visualized under daylight, UV 254 nm, 365 nm and stained with sulfuric acid reagent.

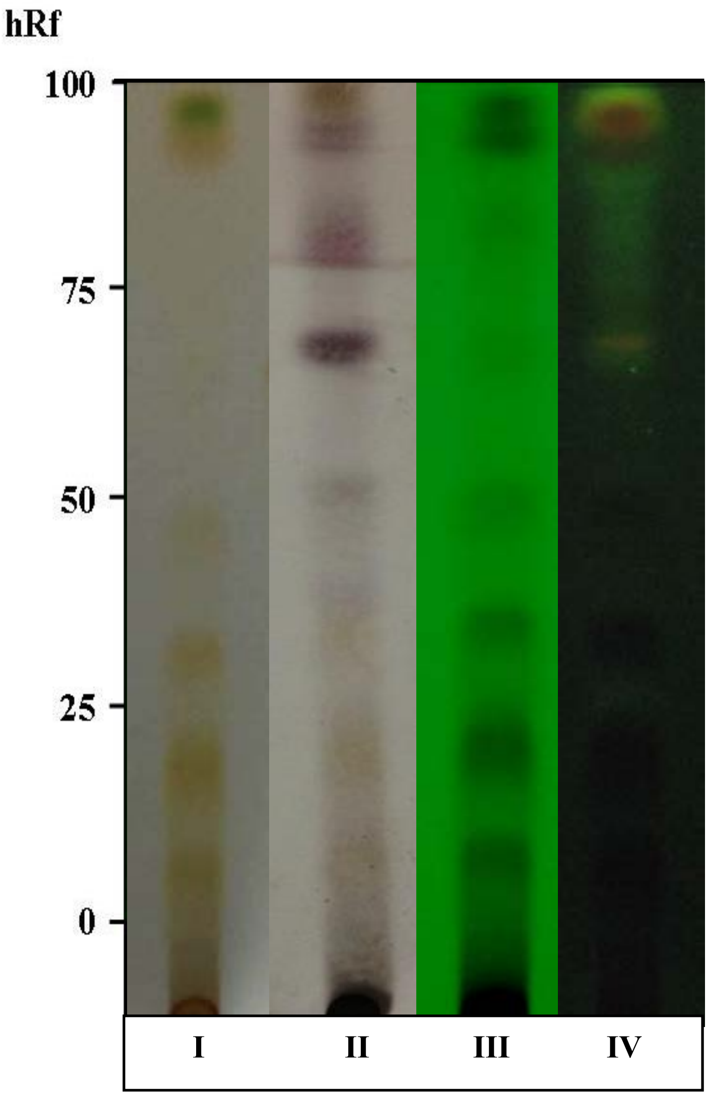


Figure 7 TLC fingerprint of ethanol extract of *P. guajava* Khee Nok cultivar leaves

I = detection under daylight

II = detection with sulfuric acid staining reagent

III = detection under UV light 254 nm

IV = detection under UV light 365nm

GC fingerprint of *P. guajava* Khee Nok cultivar leaves oil

GC chromatogram by MS detector of Uthai Thani province was shown in **Figure 8**.

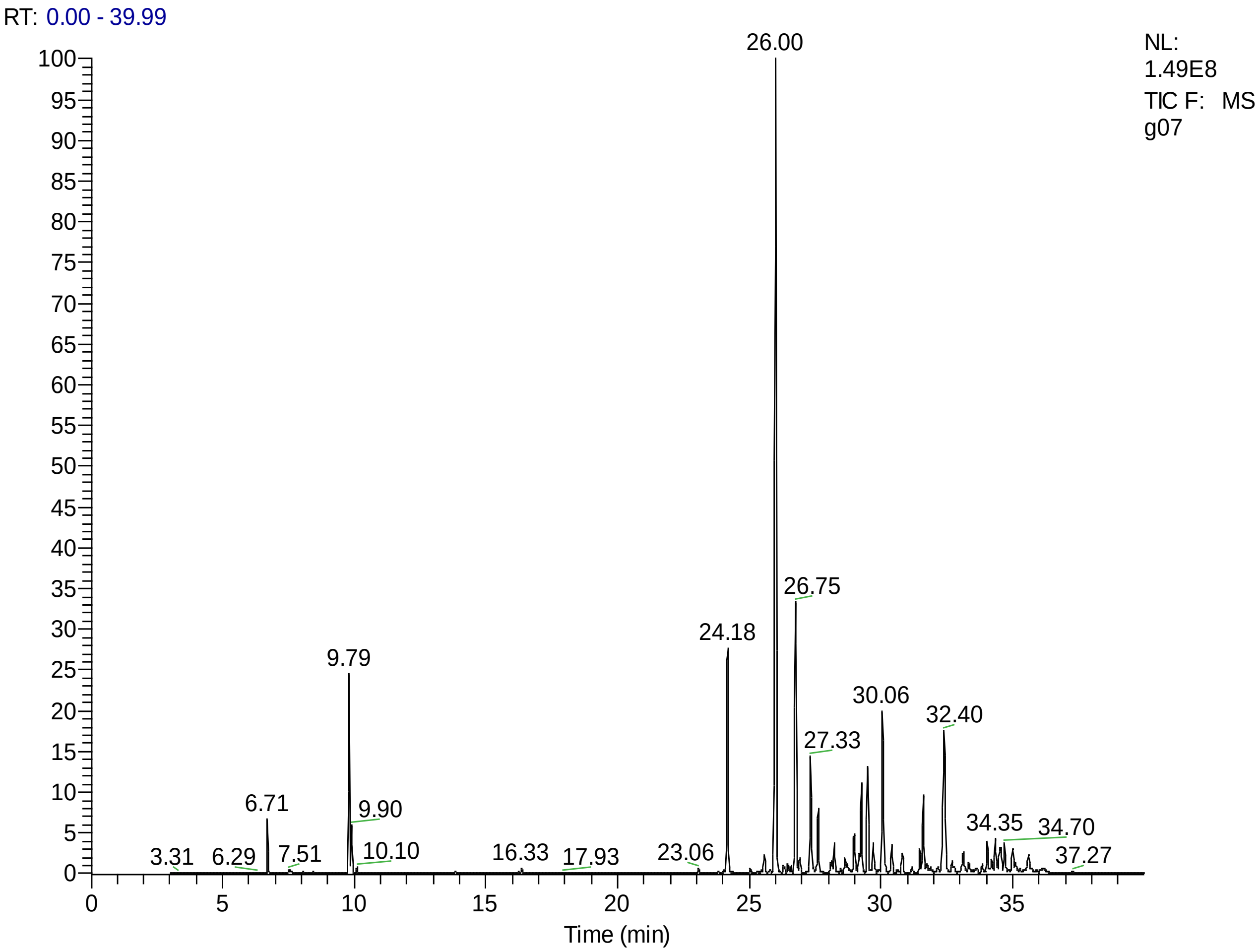


Figure 8 GC fingerprint of *P. guajava* Khee Nok cultivar leaves oil

Chemical constituents analysis of essential oil by GC/MS

The chemical constituents of Khee Nok cultivar essential oil analysed by GC/MS consisted of at least 40 compounds as shown in **Table 5.**

Table 5 The chemical constituents of the essential oil of *P. guajava* Khee Nok cultivar leaves

RT	Chemical Composition	Area %	Kovat's index
6.71	Pinene<alpha->	2.66±2.95	939
9.79	Limonene	7.17±5.47	1029
9.90	1,8-cineol	1.80±0.77	1031
24.18	Copaene<alpha->	8.06±3.58	1376
25.57	Gurjunene<alpha->	0.36±0.22	1409
26.00	Caryophyllene(E-)	28.21±2.91	1419
26.46	Gurjunene<beta->	0.29±0.23	1433
26.75	Aromadendrene	6.86±3.86	1441
26.91	Eudesma-6,11-diene<cis->	0.33±0.26	1489
27.33	Humulene<alpha->	3.11±0.42	1454
27.62	Caryophyllene<9-epi-(E)->	1.91±0.53	1466
28.12	Cadina-1(6),4-diene<trans->	0.16±0.23	1476
28.24	Muurolene<gamma->	0.96±0.26	1479
28.63	Selinene<beta->	0.63±0.21	1490
28.98	Viridiflorene	0.56±0.59	1496
29.17	Muurolene<alpha->	0.36±0.32	1500
29.25	Bisabolene<(Z)-alpha->	2.56±1.31	1507
29.50	Bisabolene<beta->	3.39±1.76	1505
29.71	Cadinene<gamma->	0.81±0.32	1513
30.06	Cadinene<delta->	5.19±2.18	1523
30.41	Cadina-1,4-diene<trans->	0.90±0.49	1534
30.81	Calacorene<alpha->	0.55±0.16	1545
31.16	Elemol	0.47±1.08	1549
31.47	Epiglobulol	0.58±0.38	-
31.59	Nerolidol<E->	2.49±1.33	1563

Table 5 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves (cont.)

RT	Chemical Composition	Area %	Kovat's index
32.40	Globulol	5.90±2.89	1590
32.69	Viridiflorol	0.40±0.29	1592
33.13	Ledol	0.74±0.54	1602
33.33	Humulene epoxide II	0.26±0.21	1608
33.83	Eudesmol<beta->	0.36±0.46	1650
34.04	Muurola-4,10(14)-dien-1-beta-ol	1.17±0.64	1631
34.35	Caryophylla-4(12),8(13)-dien5-beta-ol	1.55±0.53	1640
34.53	Murrolol<epi-alpha->	1.09±0.69	1642
34.70	Muurolol<alpha->	1.01±0.31	1646
34.99	Cadinol<alpha->	0.61±0.45	1654
35.11	Unidentified	0.29±0.33	-
35.59	Caryophyllene<14-hydroxy-9-epi-(E)->	1.05±0.49	1669
34.35	Caryophylla-4(12),8(13)-dien5-beta-ol	1.55±0.53	1640

The main component was E-caryophyllene (28.21±2.91% peak area)

Antimicrobial activities

The essential oil of *P. guajava* Khee Nok cultivar leaves and its dilution (25 and 50 %v/v in DMSO) were tested for antimicribial activities by agar disc diffusion method. Diluted oil showed no inhibition zone at all. *P. guajava* Khee Nok cultivar leaves oil showed inhibition zone against only gram positive bacteria.

Table 6 Inhibition zone by agar disc diffusion method

Microorganism	Inhibition zone (mm)*		
	P. guajava **Khee Nok cultivar leaves oil**	**Ampicillin**	**Amikacin**
Basillus cereus	8.83±0.29	27.83±0.29	16.83±0.29
Bacillus subtilis	9.67±0.58	14.67±0.29	13.17±0.29
Staphylococcus aureus	8.67±0.58	32.17±0.29	9.33±0.58
Staphylococcus epidermidis	10.00±0.00	25.67±0.58	12.33±0.58
Micrococcus luteus	9.67±0.58	43.33±0.58	11.67±0.58
Pseudomonas aeruginosa	NA	NA	9.33±0.58
Enterobacter aerogenes	NA	9.67±0.58	14.00±0.00
Salmonella typhi	NA	23.00±0.00	9.33±0.58
Salmonella typhimurium	NA	27.83±0.29	9.67±0.58
Shigella Spp.	NA	26.33±0.58	17.83±0.29
Escherichia coli	NA	18.17±0.29	8.17±0.29
Candida albicans	NA	NA	NA
Saccharomyces cerevisiae	NA	NA	NA

*means ± SD, NA = no activity, Ø 6 mm of disc. The tests were done in triplicate.

MIC and MBC tests showed bactericidal potential of *P. guajava* Khee Nok cultivar leaves oil against *Basillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Micrococcus luteus*. There were no microbial activities against tested gram-negative bacteria and fungi **(Table 7)**.

Table 7 MIC and MBC by Broth microdilution method

Microorganism	***P. guajava* Khee Nok cultivar leaves oil**		**Ampicillin**		**Amikacin**	
	MIC (%v/v)	**MBC (%v/v)**	**MIC (µg/ml)**	**MBC (µg/ml)**	**MIC (µg/ml)**	**MBC (µg/ml)**
Basillus cereus	100%	100%	0.97	6.25	3.12	6.25
Bacillus subtilis	50%	50%	25.00	25.00	6.25	12.50
Staphylococcus aureus	25%	50%	0.97	6.25	12.50	25.00
Staphylococcus epidermidis	100%	100%	0.97	6.25	6.25	25.00
Micrococcus luteus	50%	100%	0.97	3.12	12.50	12.50

The tests were done in triplicate.

* 25 and 50% of essential oil in DMSO.

100% means undiluted oil.

CHAPTER V
DISCUSSION AND CONCLUSION

The quality evaluation is an importance for identification, authentication and standardization of medicinal plant. World Health Organization (WHO) guideline, “Quality Control Method for Medicinal Plant Material” was widely distributed for recommended procedures to ensure the quality of medicinal plant products. The procedures also include modern control techniques and suitable standard compounds as reference [1]. *P. guajava* Khee Nok cultivar is an important medicinal plant in traditional Thai medicine [3] and this plant is used for herbal medicines in primary health care in Thailand.

The standardization of herbal medicines is necessary in Thailand because traditional Thai medicine uses many herbal materials for drug treatment. The pharmacognostic specification is a tool to confirm the confidence of plant material use. In this study, the pharmacognostic specifications of *P.guajava* Khee Nok cultivar leaves were conducted according to WHO guideline [1].

The macroscopic examinations based on the morphological featured such as shape, size, color, texture and other characteristics were used to distinguish various species or evaluate their quality. They were preceded by observing, touching, smelling, tasting and testing by some other ways. Microscopic observations based on optical system by the microscope referred to size, shape and relative structure of the different cells and internal features in plant materials. The cross section of midrib presented the qualitative component of the leaf cells as well as quantitative parameter such as trichrome number and oil gland number which could identify the unique character of this crude drug. Microscopically inspection of crude drugs was essential for the identification of grounded or powdered materials [45]. It can provide supporting evidence for the standardization and evaluation of herbal drugs. This study provided the specification of powdered plant material, macroscopic and microscopic characters. It could be used to confirm the nature and characteristic of crude drug [8, 9, 44].

P.guajava Khee Nok cultivar is a small tree, which is 10 m high with thin, smooth, patchy, peeling bark. Leaves are opposite, short-petiolate, the blade oval with prominent pinnate veins, 5–15 cm long. Flowers are somewhat showy, petals whitish up to 2 cm long, stamens numerous [4]. Flesh of the fruits is red and also has hard white seeds. The macroscopic character of Khee Nok cultivar is same as the others but the color of fresh fruit is different for the red mesocarp in this cultivar instead of the white one [8].

Anatomical character of midrib transverse section of *P.guajava* Khee Nok cultivar leaf in this study demonstrated cell structure as other report [9] which included epidermis, oil glands, palisade cell, collenchyma cells, parenchyma cells, fiber, xylem, xylem ray, phloem tissue, lower epidermis and unicellular trichomes. Histological character of this cultivar leaves powder showed the fragment of fiber, reticulated vessels, unicellular trichome, parenchyma in transverse view, prism crystal of calcium oxalate which in accordance with the previous studies [10, 44, 45]. The previous study of stomata in other cultivar reported anomocytic stomata type [44]. The stomata of *P.guajava* Khee Nok cultivar leaves were found to be anomocytic. The trichome number and oil gland number of *P.guajava* Khee Nok cultivar leaves were revealed firstly in this study. The trichome number of ventral epidermis of leaf was 2 times higher than dorsal epidermis. The previous studies also reported abundant trichomes on lower epidermis [9, 10].

The physico-chemical parameters of crude drug are important for the quality control for plant material. These are loss on drying, total ash, acid-insoluble ash, ethanol insoluble-extractive value, water-insoluble extractive value, water content and volatile content. Higher ashes can indicate the contamination, unconcern, adulteration or substitution in plant material. The values of solvent extractable matters provide an indication of the extent of polar, medium polar and non-polar components present in the plant material. Water content or moisture content in crude drug is a crucial factor for crude drug deterioration and contamination by microbial growth. These parameters of *P. guajava* Khee Nok cultivar leaves were found to be less than previous reports of the other cultivars [44-47].

Thin layer chromatographic examination revealed the fingerprint of chemical constituents which could be used for identification and authentication of crude drug. This study demonstrated that a mixture of dichloromethane: ethyl acetate: methanol: acetic acid 8:4:2:0.1 was efficacious mobile phase. The plate could be visualized under daylight, UV 254 nm, 365 nm and staining with stained with sulfuric acid reagent.

Gas chromatography-mass spectrometry is the most benefit implements for the essential oil analysis of *P. guajava* Khee Nok cultivar leaves [32-34]. At least 40 essential compounds were identified in *P. guajava* Khee Nok cultivar leaves oil. The major constituents were E-caryophyllene (28.21±2.91%), limonene (7.17±5.47%), copaene<alpha-> (8.06±3.58%), aromadendrene (6.86±3.86%) and globulol (5.90±2.89%). This result was similar to the previous studies in essential oil of *P. guajava* leaves but the percent peak areas were different [52-57]. The previous study of the essential oil of *P. guajava* other cultivar in Thailand reported E-caryophyllene 14.30%, aromadendrene 3.01%, α–humulene 1.51%, β-bisabolene 2.19%, α–Pinene 23.89% and 1,8-cineol 6.32% [57].

The anti-infectious usage of *P. guajava* leaves oil is a common practice in traditional medicine [11]. In this study, the antimicrobial activity of essential oil from *P. guajava* Khee Nok cultivar leaves was tested against eleven bacteria and two fungi. The oil showed inhibition zone against only gram positive bacteria. The large inhibition zone of 10.00±0.00 mm was found against *Staphylococcus epidermidis.* MIC and MBC tests showed that the oil had the promising bactericidal activity against tested gram-positive bacteria especially *Bacillus subtilis* and *Staphylococcus aureus*. There was no antibacterial activity against tested gram-negative bacteria. The previous study also showed that guava leaves essential oil was highly active against gram positive bacteria [58]. Wannissorn, *et al*. (2013) tested the antimicrobial activity of *P. guajava* leaves oil in Thailand by using disc diffusion assay and reported the inhibitory effect on gram negative bacteria *Salmonella spp* and *Escherichai coli* [63]. *P. guajava* Khee Nok cultivar leaves oil was shown to be not active on fungal organisms. This finding was similar to previous study of other cultivars of *P. guajava* leaves oil [58-63].

Conclusion

This study revealed the pharmacognostic specification, microscopic examination especially trichome number and oil gland number capable to authenticate *P. guajava* Khee Nok cultivar leaves crude drug. In addition, the chemical constituents and antimicrobial activities of *P. guajava* Khee Nok cultivar leaves oil were demonstrated.

REFERENCES

[1] World Health Organization. WHO Guidelines, Quality control methods for medicinal plant materials. Geneva: World Health Organization, 2011.

[2] Sahoo N., Manchikanti P., and Dey S. Herbal drugs: Standards and regulation. Fitoterapia 81 (2010): 462-471.

[3] Rosa Martha P., Sylvia M., and Rosario V.: *Psidium guajava* A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology 117 (2008): 1–27.

[4] Bontempo P, Doto A, Miceli M, Mita L, Benedetti R et al.: *Psidium guajava* L. anti-neoplastic effects: induction of apoptosis and cell differentiation. Cell Prolif 45(1) (2012): 22-31.

[5] Maridass M.: Antibacterial activity of essential oils of aromatic plants from south India. International Journal of Advances in Pharmaceutical Sciences 1 (2010): 176-180.

[6] Joseph, B., R.M. Priya P.A.M. Helen and S. Sujatha.: Bio-active compounds in essential oil and its effects of antimicrobial, cytotoxic activity from the *Psidium guajava* Leaf. Journal of Advanced Biotechnology 9 (2010): 10-14.

[7] Baby Joseph R., Mini P.: Preliminary Phytochemicals of *Psidium guajava* L. leaf of methanol extract and its cytotoxic study on HeLa cell lines. Journal of Ethnopharmacology 1(2) (2010): 322-327.

[8] Soetopo L. *Psidium guajava* L. Proseabase. Verheij, E.W.M. and Coronel, R.E. (1991) Availble from: http://www.proseanet.org [2013, August 26]

[9] World Health Organization. WHO monographs on selected medicinal plants 4 (2009): 127–139.

[10] Meenakshi V., Bindu G.: Pharmacognostic standardization of leaves of *Psidium guajava*. Journal of Herbal Medicine and Toxicology 6(1) (2012): 105-108.

[11] Plant genetic conversation project. Herbal medicine lists and use (1996) [Online]. Availble from: http://www.rspg.or.th/plants_data/herbs/herbs_07_6.htm [2013, August 2]

[12] Zakaria, M., Mohd, M.A. Traditional Malay medicinal plants. Penerbit Fajar Bakti Sudan Berhard (1994): 129–132.

[13] Joseph, B., R.M. Priya.: Phytochemical and biopharmaceutical aspects of *Psidium guajava* (L.) essential oil: A review. Journal of Medicinal Plants Research 5 (2011): 432-442.

[14] Begum, S., Hassan, S.I., Siddiqui, B.S.: Two new triterpenoids from the fresh leaves of *Psidium guajava*. Planta Medical 68 (2002): 1149–1152.

[15] Lutterodt GD.: Inhibition of gastrointestinal release of acetylcholene by quercetin as a possible mode of action of *Psidium quajava* leaf extracts in the treatment of acute diarrhoeal disease. Journal of Ethnopharmacology 25(3) (1989): 235-47.

[16] Jaiarj P, Khoohaswan P, Wongkrajang Y, et al.: Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. Journal of Ethnopharmacology 67(2) (1999): 203-212.

[17] Saotoing P, VroumsiaT, T., et al.: Medicinal plants used in traditional treatment of malaria in Cameroon. International Journal of the Physical Sciences 3(3) (2011): 104–117.

[18] Grover, I.S., Bala, S.: Studies on antimutagenic effect of guava (*Psidium guajava*) in Salmonella typhimurium. Mutatation Research 300 (1993): 1–3.

[19] Seo, N., Ito, T., et al.: Anti-allergic *Psidium guajava* extracts exert an antitumor effect inhibition of Tregulatory cells and resultant augmentation of Th1 cells. Anticancer Research 25 (2005): 3763–3770.

[20] K.A.Sanda, H.A.Grema et al.: Pharmacological Aspect of *Psidium guajava*:An Update. International Journal of Pharmacology 7(3) (2011): 316-324.

[21] Roy, C.K., Kamath, J.V., Asad, M.: Hepatoprotective activity of *Psidium guajava* Linn. Indian Journal of Experimental Biology 44 (2006): 305–311.

[22] Suganya T, Fumio I., Siriporn O.: Antioxidant active principles isolated from *Psidium guajava* grown in Thailand. Scientia Pharmaceutica 75 (2007): 179-193.

[23] Hui C., Gow Y.: Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chemistry 101 (2007): 686–694.

[24] Sirivan A, Rith W, Sujimon T. et al.: The development of anti-acne products from *Eucalyptus globulus* and *Psidium guajava oil.* Journal of Health Research 22(3) (2008): 109-113.

[25] Razak, F.A., Othman, R.Y., Rahim, Z.H.: The effect of Piper betle and *Psidium guajava* extracts on the cell-surface hydrophobicity of selected early settlers of dental plaque. Journal of Oral Science 48 (2006): 71–75.

[26] Soponar, F., Mot, A.C., and Sârbu, C.: Quantitative determination of some food dyes using digital processing of images obtained by thin-layer chromatography. Journal of Chromatography A (2008): 295-300.

[27] Sherma, J. Thin Layer Chromatography. In Cazes J. (ed.), Ewing's Analytical Instrumentation Handbook New York : Marcel Dekker (2005): 995-1014

[28] Shewiyo, D.H. *et al.*: HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. Journal of Pharmaceutical and Biomedical Analysis 66 (2012): 11-23.

[29] Cieśla, Ł. Biological Fingerprinting of Herbal Samples by Means of Liquid Chromatography. Chromatography Research International (2012): 1-9.

[30] Hajimehdipoor, H., *et al*. Fingerprint Study of *Thymus* spp. by TLC. Journal of Medicicnal Plant 8 (2009): 19-24.

[31] Rafi, M., Rohaeti, E., Miftahudin, A., and Darusman, L.K. Differentiation of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar* by thin layer chromatography fingerprint analysis. Indonesian Journal of Chemistry 11 (2011): 71-74.

[32] Parkman, O., David. Mass spectrometry desk reference. Pittsburgh: Global View Pub. (2000): ISBN 0-9660813-2-3.

[33] McMaster, M and McMaster C. GC/MS: a practical user's guide. New York: VCH Publisher, 1998.

[34] Watson, J. and Sparkman, O. Introduction to Mass spectrometry: Instrumentation, Application, and Strategies for Data interpretation. 4th ed. Chichester: John Wiley&Sons, 2007.

[35] Collins, C.H., Patricia, M. Lyne and Grange, J.M. Microbiological Methods. 7th ed. London: Butterworth-Heinemann. 1995.

[36] Richard., S., Lyne, S.M., and Avery, C.G. Antimicrobial susceptibility testing protocol. USA.: CRC Press, 2007.

[37] Clinical and Laboratory Standards Institute. Performance standards antimicrobial susceptibility testing. Seventeennth Informational Supplement (M100-S17) 1 (2007): 16-18.

[38] Lorian, V. Antibiotics in laboratory medicine, 3rd ed. London: Williams & Wilkins. 1991.

[39] Baurer, A.W., D.M. Perry, and Kirby, W.M.M. Single disc antibiotic sensitivity testing of Staphylococi. A.M.A. Arch. Intern. Med 104 (1959): 208-216.

[40] Baurer, A.W., Kirby, W.M.M., Sherris, J.C., and Turck, M.: Antibiotic sensitivity testing by a standardized single disk method. American Journal of Clinical Pathology 36 (1966): 493-496.

[41] Bell , S. C., Grundy, W. E.: Preparation of agar wells for antibiotic assay. Journal of Applied Microbiology 16 (1968): 1611-1612.

[42] Parekh, J., Chanda, S.V.: *In vitro* Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turkish Journal of Biology 31 (2007): 53-58.

[43] Mathabe, M.C., Nikolova, R.V., Lall, N. and Nyazema, N.Z.: Antibacterial activities of medicinal plants used for the treatment of diarrhea in Limpopo Province, South Africa. Journal of Ethnopharmacology 105 (2006): 286-293.

[44] Mital K, Sumitra C.: Phytochemical and Pharmacognostic Evaluation of Leaves of *Psidium guajava* L. (Myrtaceae). Pharmacognosy Journal 3(23) (2011): 41-45.

[45] Mishra, *et al*.: A pharmacognostical approach for study of *Psidium guajava* linn Research. International Journal of Current Pharmaceutical Research 3(4) (2011): 55-57.

[46] Meenakshi V, Bindu G.: Pharmacognostic standardization of leaves of *Psidium guajava.* Journal of Herbal Medicine and Toxicology 6(1) (2012): 105-108.

[47] Thiago Levi Silva Oliveira *et al*.: Quality control of *Psidium guajava* L. cv. 'Pedro Sato' leaf powder and obtaining its standard ethanolic extract. Journal of Pharmacy Research 5(4) (2012): 2338-2343.

[48] Shikha. *et al*.: Preliminary screening of some of the emerging antidiabetic plants for quality control. International Journal of Pharmacy and Pharmaceutical Sciences 4(3) (2012): 331-333.

[49] Jaydeep S, Sujoy P, *et al*.: Thin layer chromatographic profiling and evaluation of analgesic activity of *Psidium guajava* leaf extracts in mice. Journal of Advanced Pharmacy Education and Research 3 (2011): 177-183.

[50] Bindu A R., Remya K., *et al*.: High performance thin layer chromatographic method for quantitative determination of quercetin in tender leaves of *Psidium guajava*. Pharmacognosy Journal 17(2) (2010): 21-23.

[51] Siti N., *et al.*: TLC profiling of *Psidium guajava* L. methanol leaf extracts. International Journal of Advanced Biological Research 2(3) (2012): 552-553.

[52] J.D. da Silva, A.I.R. Luz., *et al*.: Essential oils of the leaves and stems of four *Psidium spp.* Flavour and Fragrance Journal 18 (2003): 240 – 243.

[53] F. Adam, I. Vahirua-Lechat, *et al*.: Menut,Aromatic plants of French Polynesia. V. Chemical com- position of essential oils of leaves of *Psidium guajava L.*and *Psidium cattleyanum* Sabine. Journal of Essential Oil Research 23 (2011): 98 – 101.

[54] R. Porat, Z., *et al*. Dag, Aroma volatile compositions of high- and low-aromatic guava varieties. Journal of the Science of Food and Agriculture 91 (2011): 2794– 2798.

[55] JP N., *et al*.: Chemical Compositions and Physical Characteristics of Volatile Extracts of Leaves of *Psidium Guajava* Linn and Lantana Camara Linn of Benin. Research Journal of Pharmaceutical, Biological and Chemical Sciences 4(1) (2013): 28–37.

[56] Chen HC, Sheu MJ., *et al.*: Chemical composition of the leaf essential oil of *Psidium guajava* from Taiwan. Journal of Essential Oil Research 19 (2007): 345-347.

[57] Sirivan, A., Rith, W.S., Ujimon, T., Panida, V., Paisarn, K., Prapan, S., Nijsiri, R.: The development of anti-acne products from *Eucalyptus globulus* and *Psidium guajava* oil. Journal of Health Research 22 (2008): 109-113.

[58] Baby Joseph R, Mini P.: In vitro antimicrobial activity of *Psidium guajava* L. leaf essential oil and extracts using agar well diffusion method. International Journal of Current Pharmaceutical Research 2(3) (2010): 28-32.

[59] Jaiarj, P., Khoohaswan P, *et al.*: Anticough and antimicrobial activities of *Psidium guajava* Linn. Leaf extract. Journal of Ethnopharmacology 67 (1999): 203-12.

[60] Andrade-Neto, M, Alencar, *et al.*: Volatile constituents of *Psidium pohlianum* Berg and *Psidium guyanensis* Pers Journal of Essential oil research 6 (1994): 299-300.

[61] Baby Joseph, Mini Priya R, *et al*. Bio-active compounds in essential oil and its effects of antimicrobial, cytotoxic activity from the *Psidium guajava* L. leaf Journal of Advanced Biotech 9(10) (2010): 10-14.

[62] L *et al*. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa,Congo. Phytomedicine 6 (1999): 59–66.

[63] B Wannissorn, *et al*. Antibacterial properties of essential oils from Thai medicinal plants Fitoterapia 76 (2005): 233 – 236.

[64] Okeke MI, Iroegbu CU, Eze EN, *et al*. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. Journal of Ethnopharmacology 78 (2001): 119-127.

APPENDICES

APPENDIX A

Data of pharmacognostic specifications of *P. guajava* Khee Nok cultivar leaves

Table 8 Pharmacognostic specification s (% by weight) of *P. guajava* Khee Nok cultivar leaves

Sources	No.	Loss on drying	Total ash content	Acid-insoluble ash content	Ethanol insoluble extractive	Water insoluble extractive	Water content	Volatile content
1. Kamphaengphet	1	6.496	7.025	1.069	6.726	7.589	5.799	2.000
	2	6.654	7.013	1.345	6.459	7.762	5.797	1.600
	3	6.865	7.001	1.383	6.828	8.343	5.597	2.000
2. Kalasin	1	7.184	6.934	1.363	7.836	9.624	7.000	1.000
	2	7.227	6.812	1.504	7.698	9.533	6.400	1.200
	3	7.890	6.860	1.359	8.791	9.862	7.798	1.000
3. Khonkaen	1	8.586	8.913	2.293	7.536	9.435	8.600	1.400
	2	8.738	8.901	2.201	8.052	8.947	8.998	1.400
	3	8.356	8.995	2.166	7.628	9.608	8.800	1.600
4. Tak	1	8.805	6.917	1.182	8.356	11.826	8.400	1.333
	2	8.667	6.930	1.323	11.512	11.198	9.000	1.333
	3	8.512	6.941	1.331	8.980	10.331	9.400	1.666
5. Phichit	1	8.936	5.867	1.093	7.449	8.775	8.398	1.200
	2	7.381	6.092	1.596	7.940	7.968	8.398	1.200
	3	9.208	5.554	1.423	7.305	8.744	8.598	1.000

Table 8 Pharmacognostic specification s (% by weight) of *P. guajava* Khee Nok cultivar leaves (cont.)

Sources	No.	Loss on drying	Total ash content	Acid-insoluble ash content	Ethanol insoluble extractive	Water insoluble extractive	Water content	Volatile content
6. Phitsanulok	1	8.256	6.491	1.016	8.874	9.896	8.600	1.200
	2	6.770	7.751	1.541	8.558	9.380	9.000	1.000
	3	7.865	6.560	1.139	8.855	9.977	9.198	1.000
7. Uthai Thani	1	6.722	5.072	1.163	9.259	11.442	7.000	2.000
	2	7.043	5.203	1.170	9.707	10.181	7.998	2.333
	3	6.974	5.084	1.166	10.117	11.674	8.598	2.000
8. Sukhothai	1	9.564	8.864	1.696	9.003	9.548	9.998	1.400
	2	9.438	8.876	1.844	8.461	9.209	9.396	1.600
	3	9.617	8.954	1.909	9.257	10.270	10.600	1.600
9. Nakhon Si Thammarat	1	9.243	5.430	0.966	10.977	11.808	11.000	2.600
	2	9.242	5.488	0.623	8.918	10.264	9.998	2.799
	3	9.352	5.419	1.112	8.128	10.348	10.000	2.800
10. Sakaeo	1	6.214	7.343	1.423	10.209	11.960	8.798	1.999
	2	7.687	7.021	1.405	11.885	11.720	8.398	1.333
	3	7.643	7.040	1.793	9.202	11.144	8.998	1.999

Table 8 Pharmacognostic specification s (% by weight) of *P. guajava* Khee Nok cultivar leaves (cont.)

Sources	**No.**	**Loss on drying**	**Total ash content**	**Acid-insoluble ash content**	**Ethanol insoluble extractive**	**Water insoluble extractive**	**Water content**	**Volatile content**
11. Phrae	1	7.688	5.836	1.222	6.377	9.434	9.798	0.600
	2	7.715	5.452	1.484	7.391	9.595	8.598	0.400
	3	7.401	6.045	1.326	6.220	9.567	8.998	0.400
12. Phetchabun	1	8.977	7.578	0.856	10.937	11.913	9.600	1.599
	2	9.007	7.534	1.669	11.165	11.610	9.998	1.600
	3	8.921	6.346	1.153	11.398	10.559	9.998	1.800
13. Nakonsawan	1	7.090	10.481	2.346	7.780	10.163	8.000	1.000
	2	7.283	10.443	1.163	6.715	10.554	8.800	1.200
	3	7.582	10.398	2.130	7.122	11.255	8.398	1.000
14. Trang	1	5.788	6.557	1.562	9.489	12.085	9.000	1.400
	2	6.767	6.531	1.457	8.035	12.945	9.198	1.200
	3	6.713	6.686	1.840	8.370	12.571	8.200	1.200
15. Lampang	1	7.930	6.202	1.049	8.300	9.563	9.200	2.400
	2	7.983	6.984	1.009	8.758	9.879	9.596	2.600
	3	6.482	6.086	1.052	9.123	9.469	9.996	2.400

APPENDIX B

Trichome number and oil gland number of *P. guajava* Khee Nok cultivar *leaves*

Table 9 Trichome number and oil gland number of *P. guajava* Khee Nok cultivar leaves from Bangkok

Field	Oil gland number	Trichome number	
		Dorsal	Ventral
1	42	18	40
2	43	20	42
3	42	20	39
4	41	19	41
5	43	22	38
6	46	18	39
7	43	23	45
8	46	19	44
9	43	20	46
10	46	22	42
11	43	20	40
12	40	21	39
13	43	21	43
14	43	15	40
15	44	19	42
16	44	17	40
17	42	22	41
18	40	19	39
19	42	20	38
20	43	19	41
21	44	20	42
22	44	19	40
23	45	22	40
24	43	21	41
25	47	17	39
26	42	20	42
27	46	19	40
28	44	19	41
29	48	20	40
30	45	18	39
Mean	43.57	19.63	40.77
SD	1.92	1.73	1.92

Table 10 Trichome number and oil gland number of *P. guajava* Khee Nok cultivar leaves from Phitsanulok

Field	Oil gland number	Trichome number	
		Dorsal	Ventral
1	37	29	46
2	34	30	48
3	39	25	47
4	36	30	45
5	38	29	44
6	36	27	46
7	39	26	48
8	40	25	45
9	38	24	45
10	36	30	43
11	36	26	45
12	35	29	44
13	41	25	48
14	39	28	49
15	37	25	46
16	38	27	49
17	41	29	47
18	40	26	47
19	39	28	44
20	36	27	48
21	38	25	45
22	37	25	46
23	36	28	48
24	39	28	45
25	38	24	49
26	43	25	46
27	37	26	49
28	39	26	49
29	38	28	50
30	40	27	48
Mean	38	26.9	46.63
SD	1.98	1.84	1.88

Table 11 Trichome number and oil gland number of *P. guajava* Khee Nok cultivar leaves from Kamphaengphet

Field	Oil gland number	Trichome number	
		Dorsal	Ventral
1	39	22	50
2	35	28	54
3	37	27	52
4	36	25	50
5	36	23	50
6	32	24	49
7	35	26	51
8	35	29	53
9	39	25	51
10	38	28	52
11	34	25	52
12	32	26	49
13	35	23	47
14	37	28	52
15	35	27	53
16	36	25	53
17	34	28	52
18	33	23	54
19	35	27	50
20	37	29	52
21	35	24	54
22	33	28	52
23	32	26	49
24	35	26	53
25	36	27	48
26	36	24	50
27	34	26	52
28	36	27	52
29	37	28	49
30	34	29	51
Mean	35.27	26.1	51.2
SD	1.85	1.99	1.83

APPENDIX C

GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil

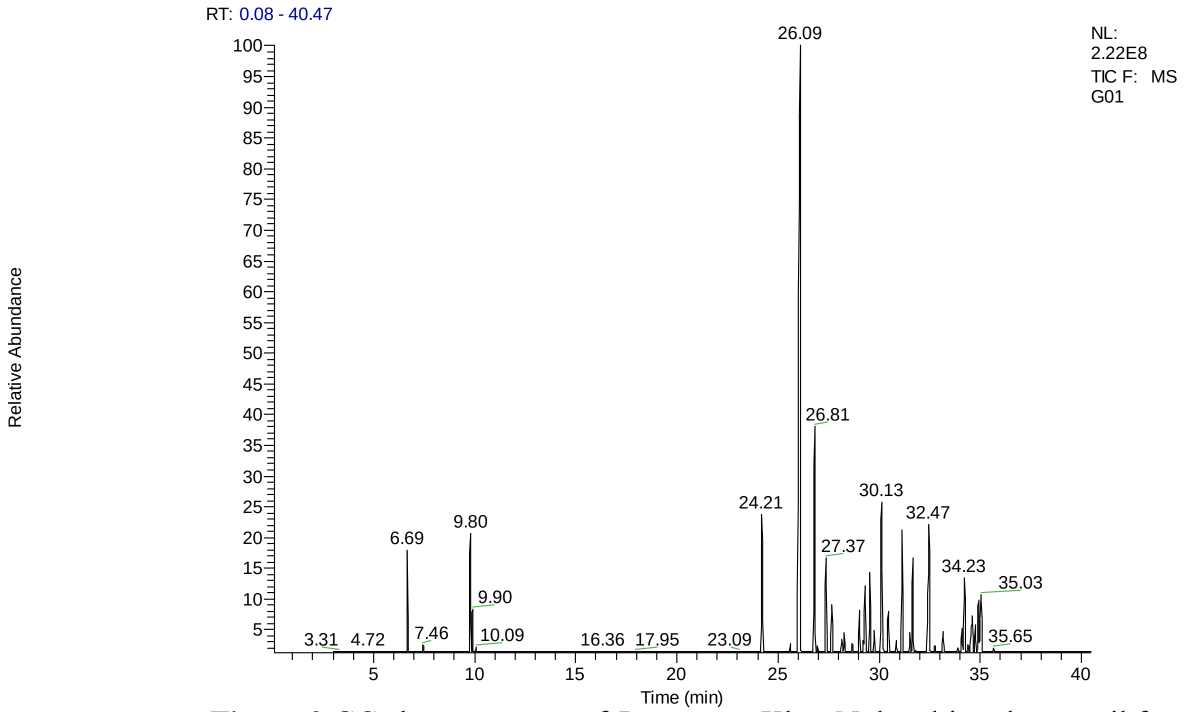


Figure 9 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Kamphaengphet

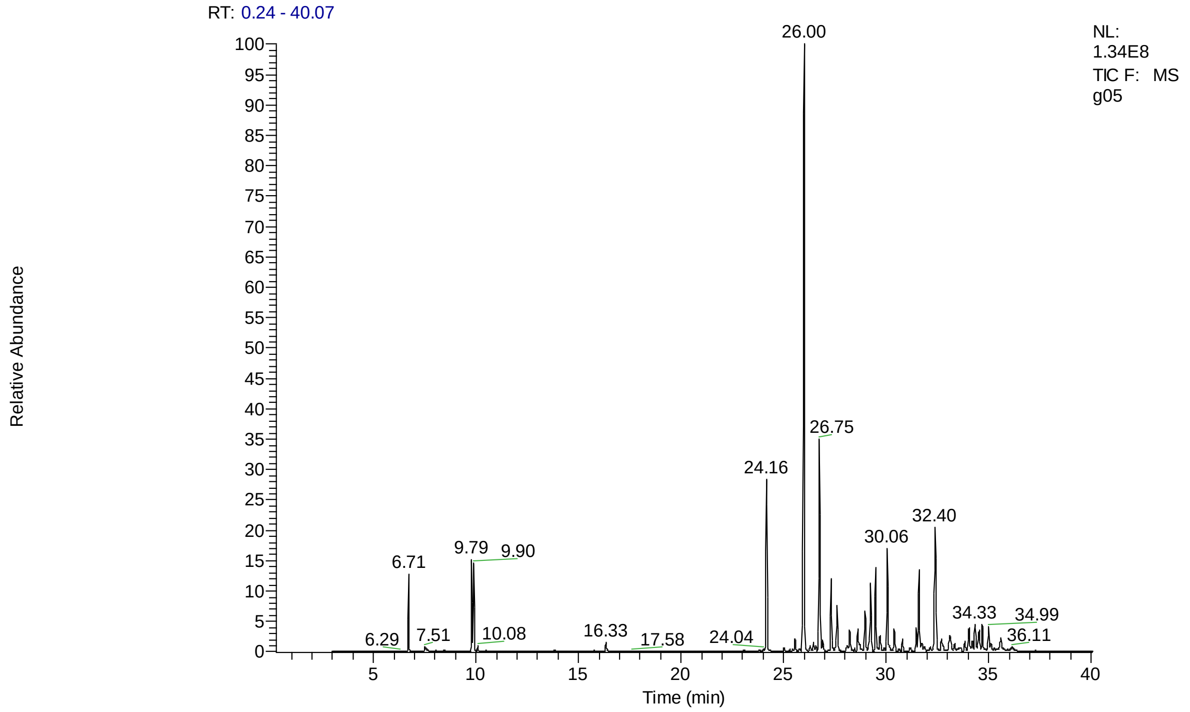


Figure 10 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Kalasin

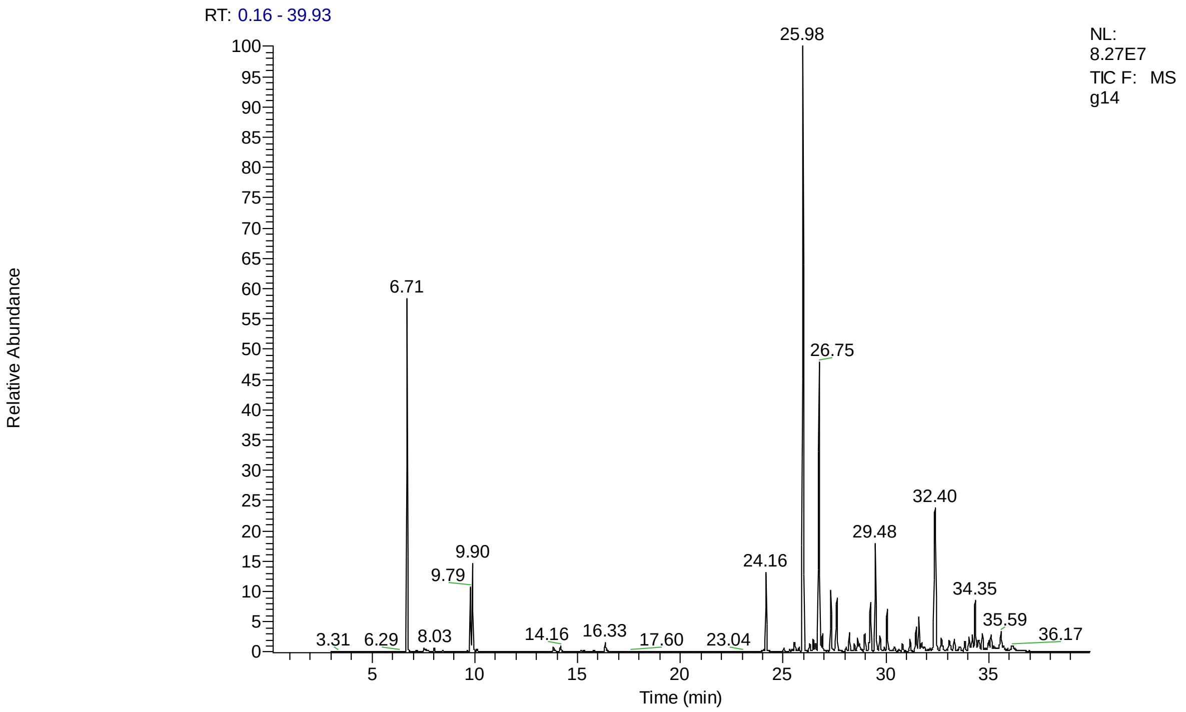


Figure 11 GC chromatogram *P. guajava* of Khee Nok cultivar leaves oil from Khonkaen

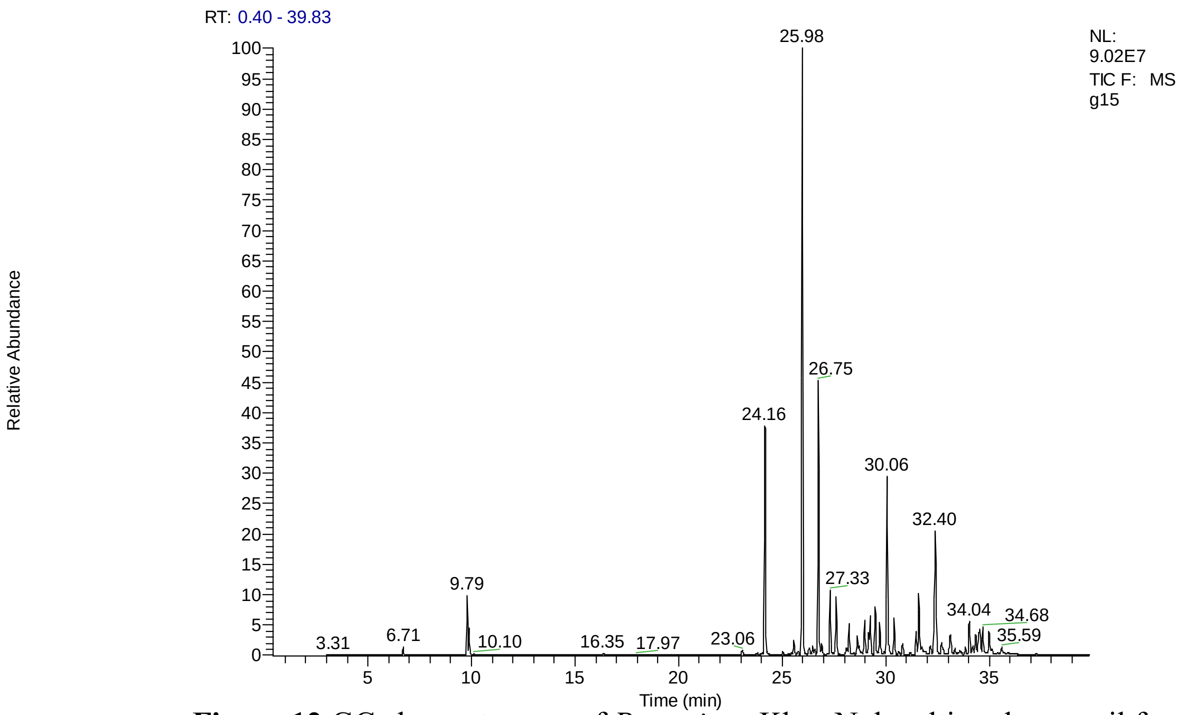


Figure 12 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Tak

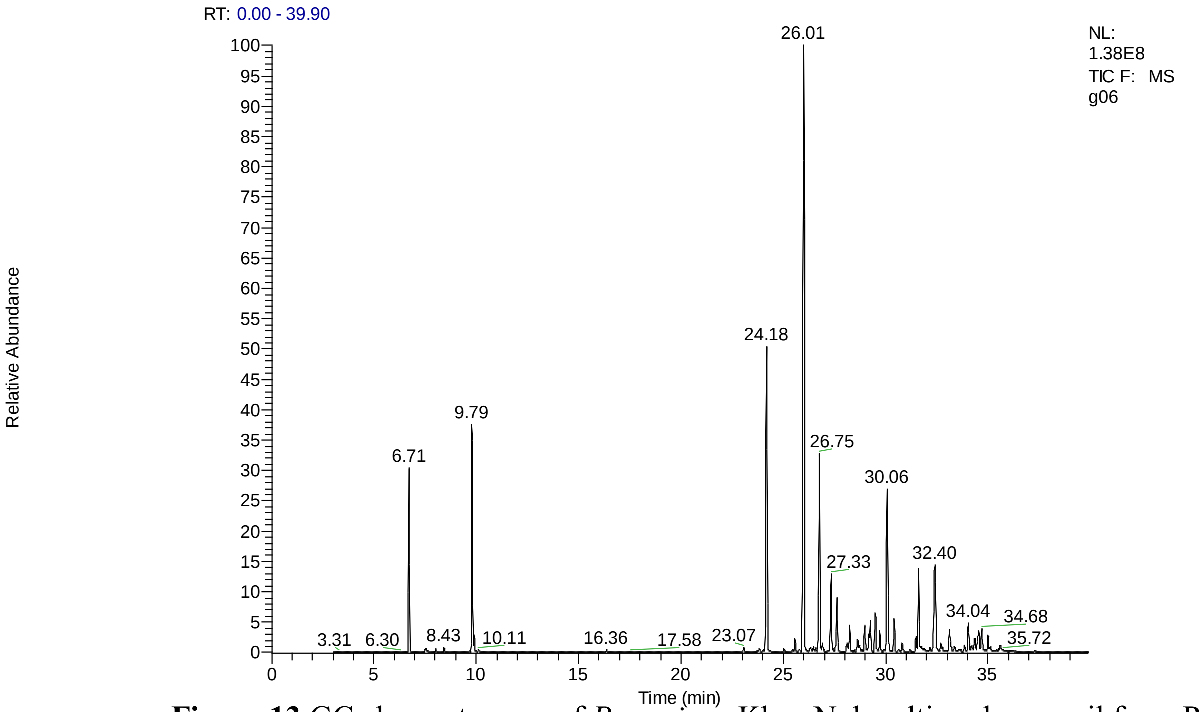


Figure 13 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Phichit

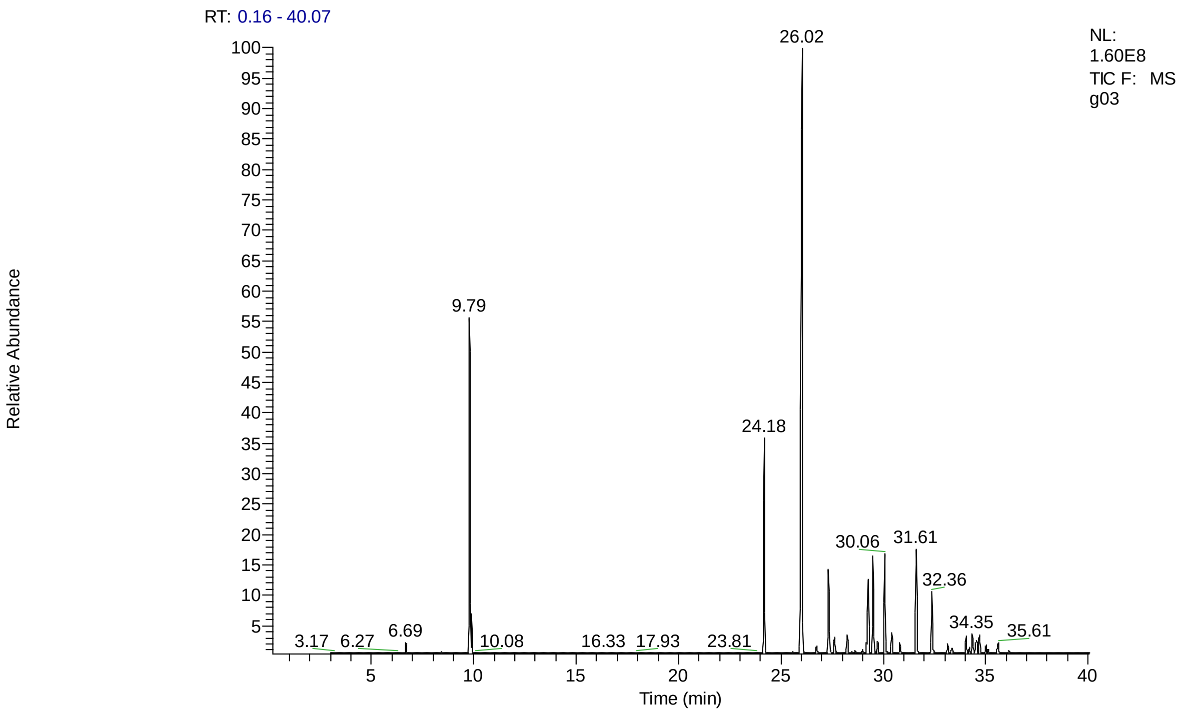


Figure 14 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Phitsanulok

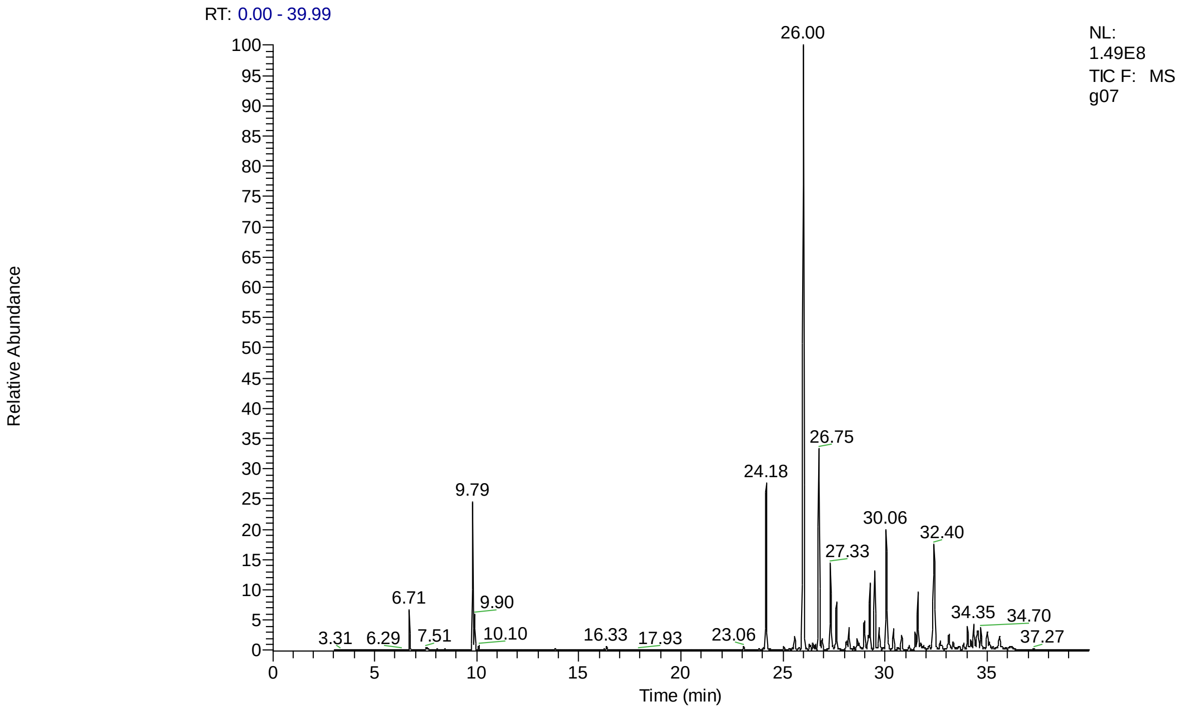


Figure 15 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Uthai Thani

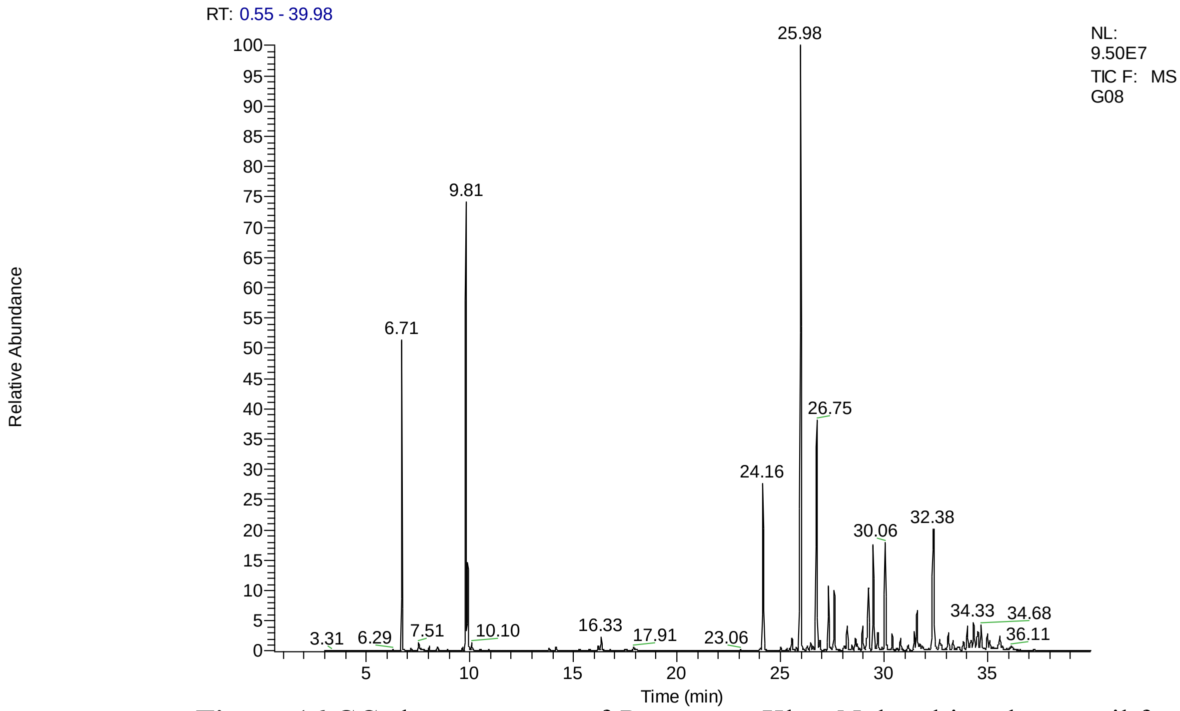


Figure 16 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Sukhothai

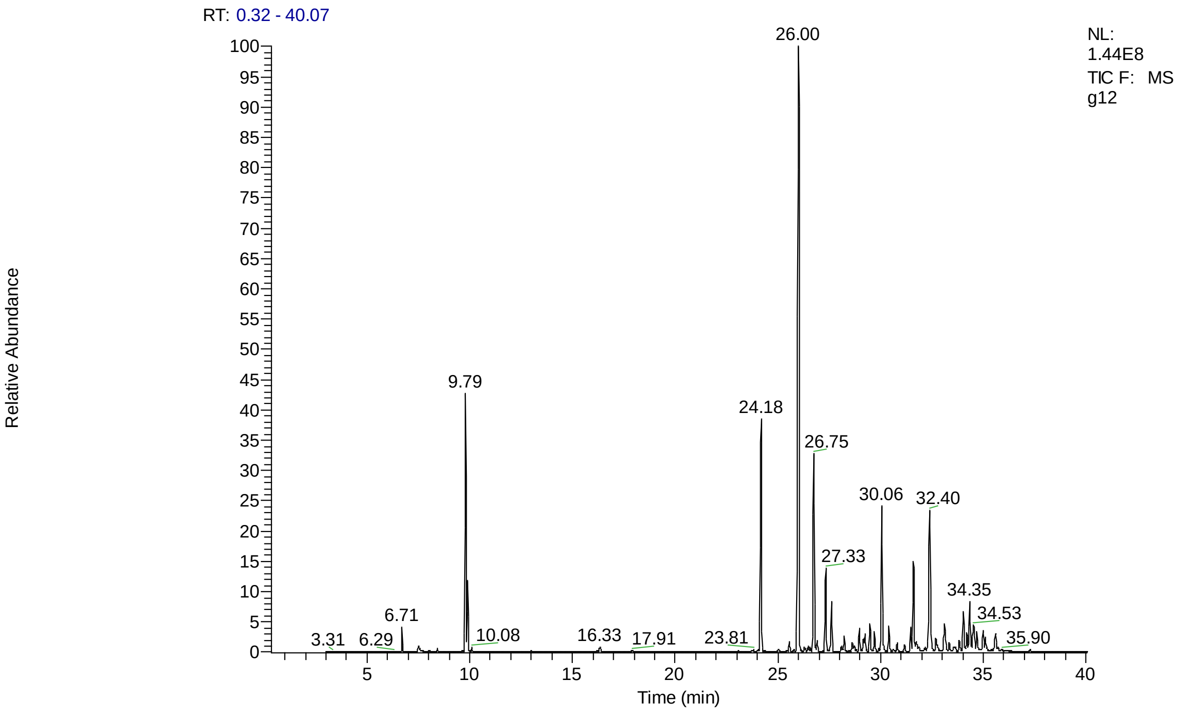


Figure 17 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Nakhon Si Thammarat

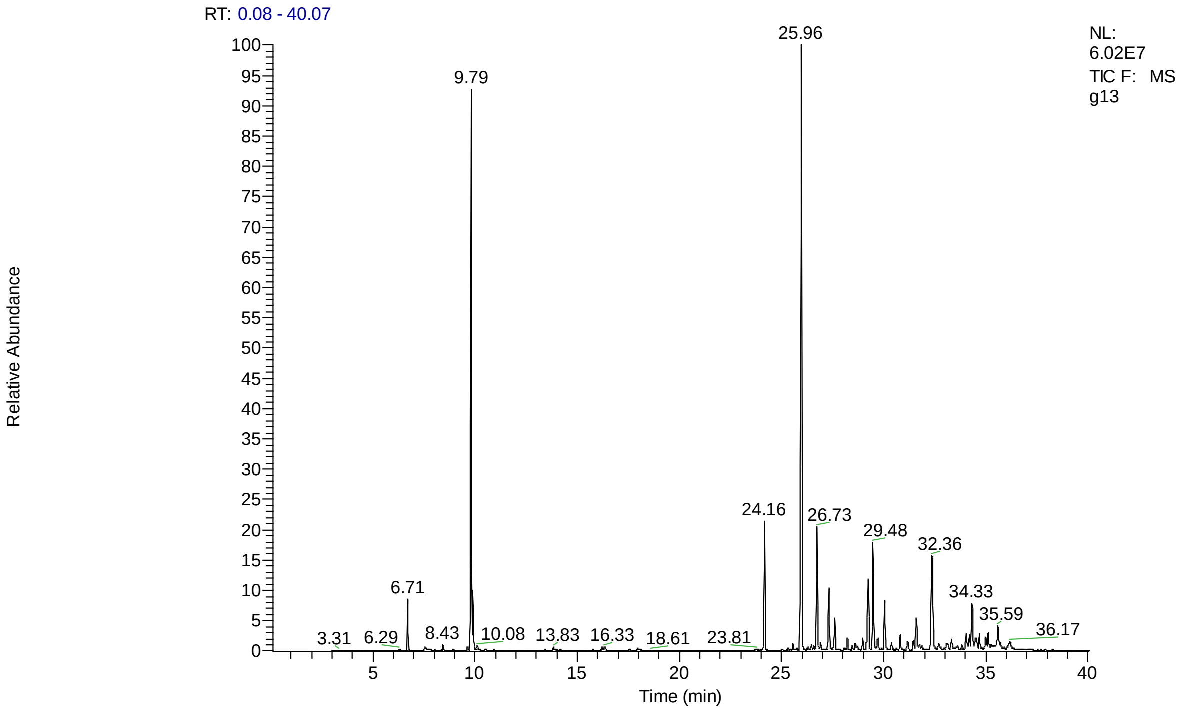


Figure 18 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Sa Kaeo

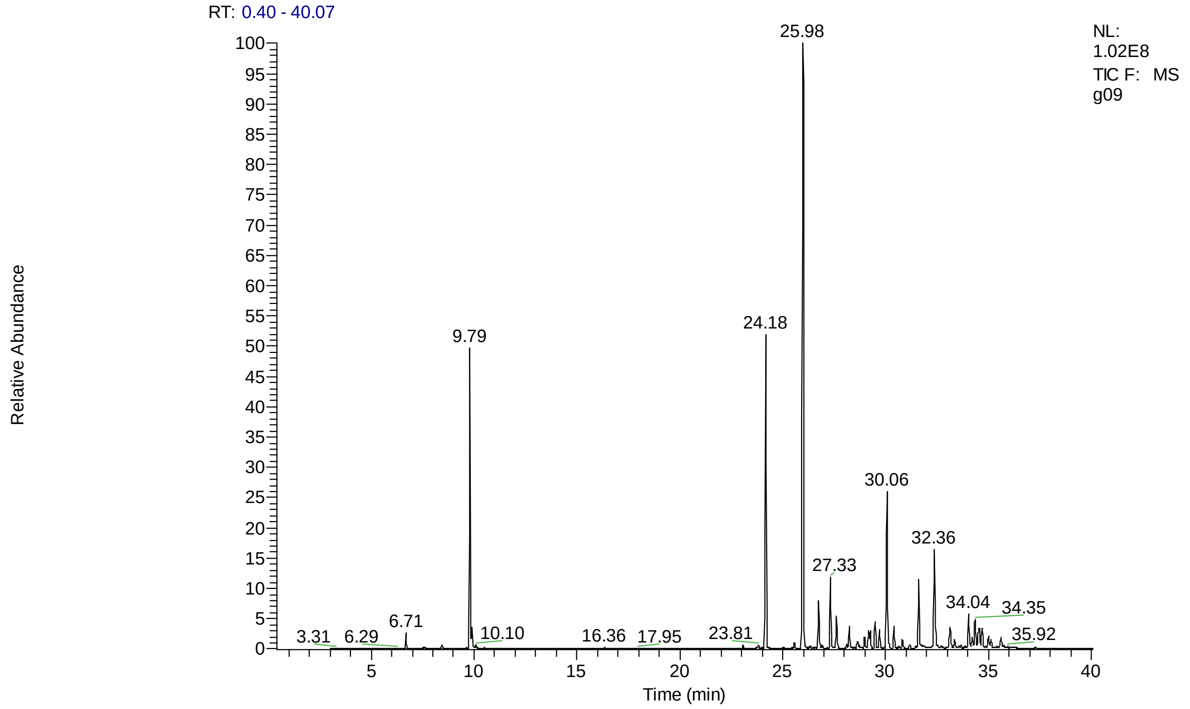


Figure 19 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Phrae

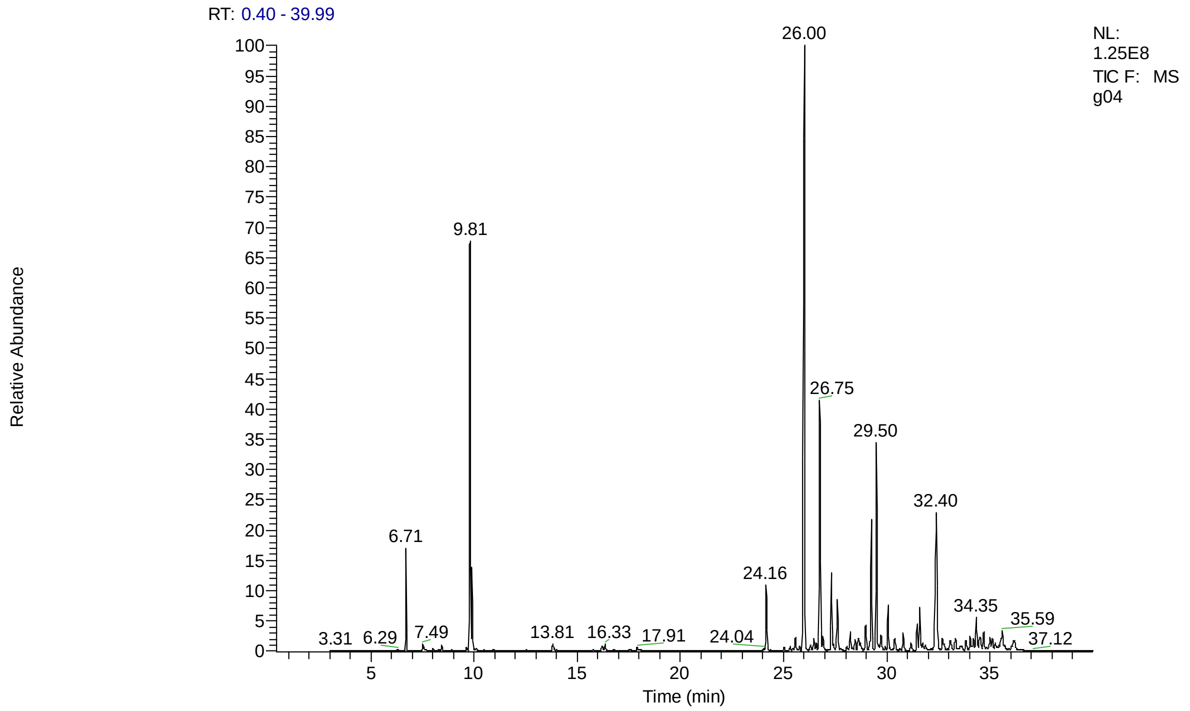


Figure 20 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Phetchabun

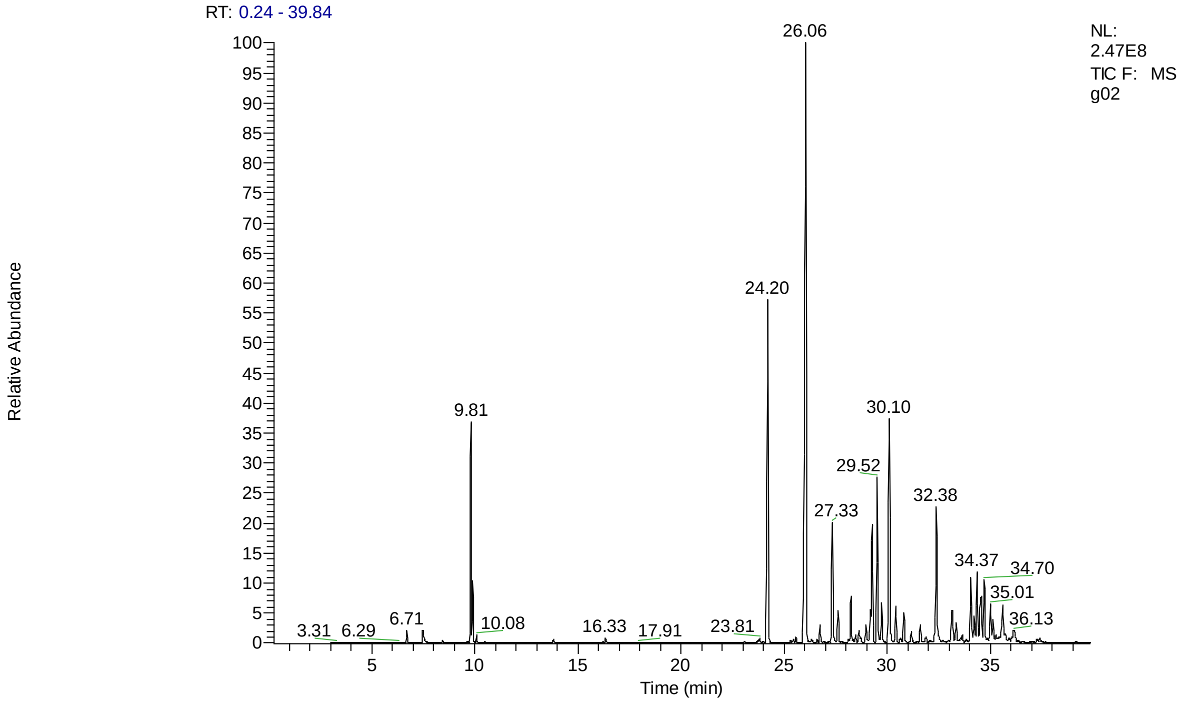


Figure 21 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Nakonsawan

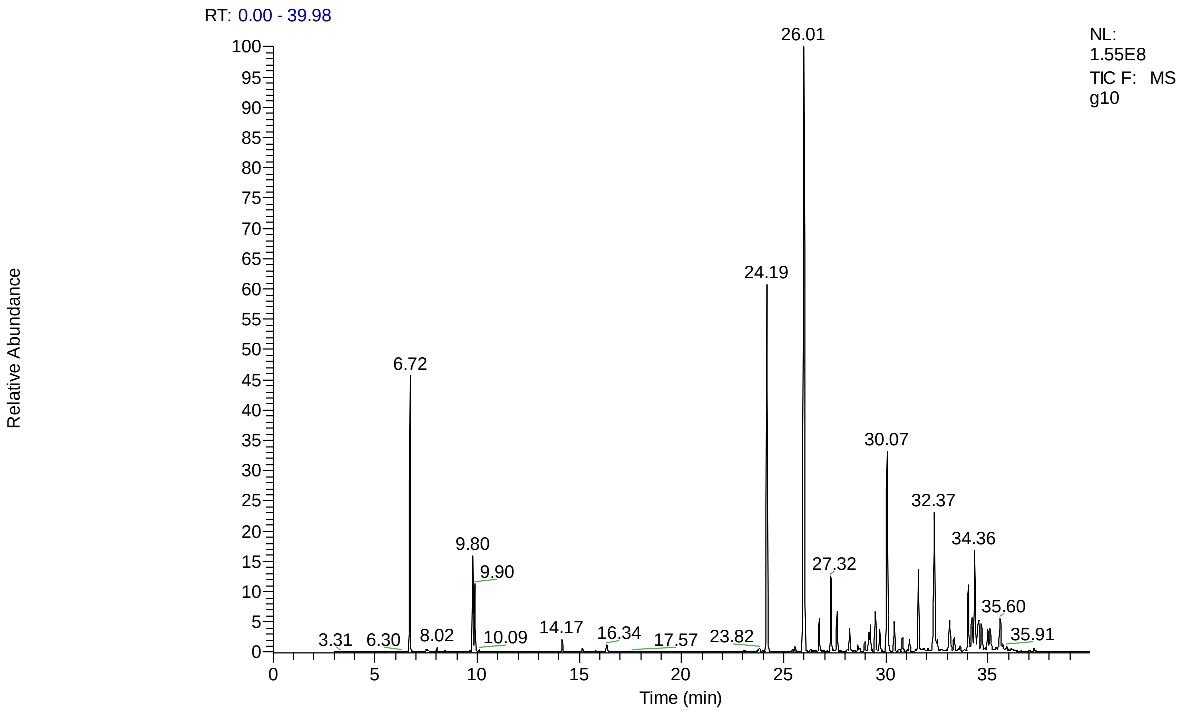


Figure 22 GC chromatogram of *P. guajava* Khee Nok cultivar leaves oil from Trang

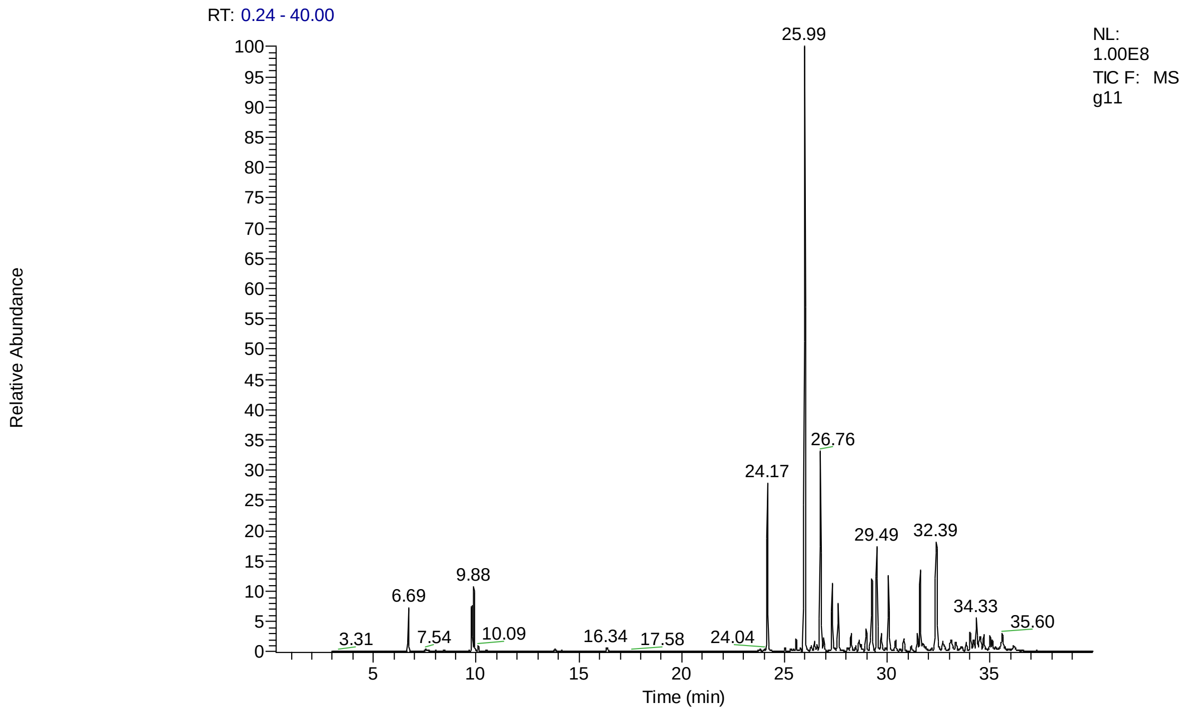


Figure 23GC chromatogram of Khee Nok cultivar *P. guajava* leaves oil from Lampang

APPENDIX D

The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves

Table 12 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Kamphaengphet

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.69	90178723	Pinene<alpha->	1.94	939
7.46	22264195	Benzaldehyde	0.48	960
9.80	137742559	Limonene	2.96	1029
9.90	59762916	1,8-cineole	1.28	1031
24.21	204624587	Copaene<alpha->	4.39	1376
25.59	25498159	Gurjunene<alpha->	0.55	1409
26.09	1233982313	Caryophyllene(E-)	26.49	1419
26.81	344738036	Aromadendrene	7.40	1441
26.96	18903355	Eudesma-6,11-diene<cis->	0.41	1489
27.37	147086744	Humulene<alpha->	3.16	1454
27.66	84326283	Caryophyllene<9-epi-(E)->	1.81	1466
28.16	33141855	Cadina-1(6),4-diene<trans->	0.71	1476
28.28	46349648	Muurolene<gamma->	0.99	1479
28.66	23661832	Selinene<beta->	0.51	1490
28.76	17241188	Longifolene-(V4)	0.37	-
29.03	74925123	Viridiflorene	1.61	1496
29.22	22923884	Muurolene<alpha->	0.49	1500
29.30	106903014	Bisabolene<(Z)-alpha->	2.29	1507
29.55	118547017	Bisabolene<beta->	2.54	1505
29.75	45573996	Cadinene<gamma->	0.98	1513
30.13	253043834	Cadinene<delta->	5.43	1523
30.46	72780994	Cadina-1,4-diene<trans->	1.56	1534
30.85	26572178	Calacorene<alpha->	0.57	1545
31.14	201930073	Elemol	4.33	1549
31.51	37702326	Epiglobulol	0.81	-
31.66	140398969	Nerolidol<E->	3.01	1563
31.78	17821388	Maaliol	0.38	1567
32.47	278647071	Globulol	5.98	1590
32.76	29616271	Viridiflorol	0.64	1592
33.17	57567154	Ledol	1.24	1602
33.61	19179815	Cubenol<1,10-di-epi->	0.41	1619
33.90	22494818	Rosifoliol	0.48	1600
34.08	49443225	Cubenol<1-epi->	1.06	1628
34.23	126886040	Eudesmol<gamma->	2.72	1632
34.39	26131323	Caryophylla-4(12),8(13)-dien5-beta-ol	0.56	1640
34.60	102048585	Cadinol<epi-alpha-> (=tau-cadinol)	2.19	1640
34.74	57750350	Muurolol<alpha-> (=Torreyol)	1.24	1646
34.91	90589800	Eudesmol<beta->	1.94	1650
35.03	153787548	Eudesmol<alpha->	3.30	1653
35.65	35755915	Caryophyllene<14-hydroxy-9-epi-(E)->	0.77	1669

Table 13 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Kalasin

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	38366553	Pinene<alpha->	1.88	939
7.51	5625454	Benzaldehyde	0.28	960
9.79	56872664	Limonene	2.79	1029
9.90	54946445	1,8-cineole	2.70	1031
16.33	7861044	Terpineol<alpha->	0.39	1188
24.16	142821221	Copaene<alpha->	7.01	1376
25.55	11307873	Gurjunene<alpha->	0.56	1409
26.00	596308478	Caryophyllene(E-)	29.27	1419
26.29	5449291	Valencene	0.27	1496
26.46	7419646	Gurjunene<beta->	0.36	1433
26.75	179253886	Aromadendrene	8.80	1441
26.91	10217160	Eudesma-6,11-diene<cis->	0.50	1489
27.33	65230136	Humulene<alpha->	3.20	1454
27.60	43320923	Caryophyllene<9-epi-(E)->	2.13	1466
28.09	5973245	Cadina-1(6),4-diene<trans->	0.29	1476
28.22	18349302	Muurolene<gamma->	0.90	1479
28.61	16497069	Selinene<beta->	0.81	1490
28.96	33752862	Selinene<alpha->	1.66	1498
29.25	67558935	Bisabolene<(Z)-alpha->	3.32	1507
29.48	72203337	Bisabolene<beta->	3.54	1505
29.71	14487050	Cadinene<gamma->	0.71	1513
30.06	93468232	Cadinene<delta->	4.59	1523
30.39	20672842	Cadina-1,4-diene<trans->	1.01	1534
30.79	10875673	Calacorene<alpha->	0.53	1545
31.47	19214744	Epiglobulol	0.94	-
31.59	69155554	Nerolidol<E->	3.40	1563
31.74	13434432	Maaliol	0.66	1567
32.40	151145177	Globulol	7.42	1590
32.69	13448219	Viridiflorol	0.66	1592
33.11	22794333	Ledol	1.12	1590
33.33	6266212	Caryophyllene oxide	0.31	1583
33.83	8950979	Eudesmol<beta->	0.44	1650
34.04	22015393	Muurola-4,10(14)-dien-1-beta-ol	1.08	1631
34.20	9780210	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.48	1640
34.33	25610263	Caryophylla-4(12),8(13)-dien5-beta-ol	1.26	1640
34.53	26652112	Cadinol<epi-alpha-> (=tau-cadinol)	1.31	1640
34.68	22282404	Muurolol<alpha-> (=Torreyol)	1.09	1646
34.99	20270224	Muurolol <epi-alpha-> (=tau- Muurolol)	1.00	1642
35.09	6740350	Isoaromadendrene epoxide	0.33	-
35.59	20361859	Caryophyllene<14-hydroxy-9-epi-(E)->	1.00	1669

Table 14 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Khonkaen

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	112030260	Pinene<alpha->	9.09	939
9.79	24129641	Limonene	1.96	1029
9.90	35477760	1,8-cineole	2.88	1031
16.33	4993388	Terpineol<alpha->	0.40	1188
24.16	39494968	Copaene<alpha->	3.20	1376
25.57	4080633	Gurjunene<alpha->	0.33	1409
25.98	329448395	Caryophyllene(E-)	26.72	1419
26.29	3886616	Valencene	0.32	1496
26.46	6241805	Gurjunene<beta->	0.51	1433
26.58	3989651	Gurjunene<gamma->	0.32	1477
26.75	155678138	Aromadendrene	12.62	1441
26.91	9192511	Eudesma-6,11-diene<cis->	0.75	1489
27.33	32806084	Humulene<alpha->	2.66	1454
27.62	31175703	Caryophyllene<9-epi-(E)->	2.53	1466
28.24	10649563	Muurolene<gamma->	0.86	1479
28.63	10097442	Selinene<beta->	0.82	1490
28.98	9841924	Selinene<alpha->	0.80	1498
29.25	25127712	Bisabolene<(Z)-alpha->	2.04	1507
29.48	57671868	Bisabolene<beta->	4.68	1505
29.71	8128974	Cadinene<gamma->	0.66	1513
30.06	22619168	Cadinene<delta->	1.83	1523
30.81	4092439	Calacorene<alpha->	0.33	1545
31.16	6964785	Elemol	0.56	1549
31.47	12843177	Epiglobulol	1.04	-
31.59	18166487	Nerolidol<E->	1.47	1563
31.74	8286873	Maaliol	0.67	1567
32.40	118200059	Globulol	9.59	1590
32.69	8542351	Viridiflorol	0.69	1592
33.08	8121964	Rosifoliol	0.66	1600
33.33	6515206	Humulene epoxide II	0.53	1608
33.85	5863144	Eudesmol<beta->	0.48	1650
34.04	9266106	Muurola-4,10(14)-dien-1-beta-ol	0.75	1631
34.20	9164983	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.74	1640
34.35	30116839	Caryophylla-4(12),8(13)-dien5-beta-ol	2.44	1640
34.51	9423984	Murrolol<epi-alpha-> (=tau-muurolol)	0.76	1642
34.70	9592942	Muurolol<alpha-> (=Torreyol)	0.78	1646
34.99	5501441	Cadinol<alpha->	0.45	1654
35.11	9297115	-	0.75	-
35.59	11014261	Caryophyllene<14-hydroxy-9-epi-(E)->	0.89	1583
36.17	5365129	Bisabolol<alpha->	0.44	1685

Table 15 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Tak

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	2715336	Pinene<alpha->	0.20	939
9.79	25371289	Limonene	1.83	1029
9.90	11714709	1,8-cineole	0.85	1031
23.06	2480095	Cubebene<alpha->	0.18	1348
24.16	133610891	Copaene<alpha->	9.65	1376
25.55	9311695	Gurjunene<alpha->	0.67	1409
25.98	375194690	Caryophyllene(E-)	27.10	1419
26.29	4351791	Valencene	0.31	1496
26.46	7315667	Gurjunene<beta->	0.53	1433
26.75	157221170	Aromadendrene	11.36	1441
26.91	6941822	Eudesma-6,11-diene<cis->	0.50	1489
27.33	38848528	Humulene<alpha->	2.81	1454
27.62	36361736	Caryophyllene<9-epi-(E)->	2.63	1466
28.09	5647452	Cadina-1(6),4-diene<trans->	0.41	1476
28.22	20009302	Muurolene<gamma->	1.45	1479
28.61	15563108	Selinene<beta->	1.12	1490
28.96	21600497	Selinene<alpha->	1.56	1498
29.17	9837334	Muurolene<alpha->	0.71	1500
29.25	23174102	Bisabolene<(Z)-alpha->	1.67	1507
29.48	27663056	Bisabolene<beta->	2.00	1505
29.71	18673166	Cadinene<gamma->	1.35	1513
30.06	108494146	Cadinene<delta->	7.84	1523
30.41	21934693	Cadina-1,4-diene<trans->	1.58	1534
30.81	6731323	Calacorene<alpha->	0.49	1545
31.47	12935551	Epiglobulol	0.93	-
31.59	38763464	Nerolidol<E->	2.80	1563
32.15	5171827	Spathulenol	0.37	1578
32.40	102208017	Globulol	7.38	1590
32.69	8777569	Viridiflorol	0.63	1592
33.13	16692033	Ledol	1.21	1602
33.33	2914939	Humulene epoxide II	0.21	1608
33.56	3974368	Cubenol<1,10-di-epi->	0.29	1619
33.83	4010308	Eudesmol<beta->	0.29	1650
34.04	18962972	Muurola-4,10(14)-dien-1-beta-ol	1.37	1631
34.20	4457315	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.32	1640
34.33	12002464	Caryophylla-4(12),8(13)-dien5-beta-ol	0.87	1640
34.53	24827042	Murrolol<epi-alpha-> (=tau-muurolol)	1.79	1642
34.68	16251091	Muurolol<alpha-> (=Torreyol)	1.17	1646
34.99	16334528	Cadinol<alpha->	1.18	1654
35.59	5383315	Caryophyllene<14-hydroxy-9-epi-(E)->	0.39	1669

Table 16 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Phichit

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	97152072	Pinene<alpha->	4.32	939
7.52	4065499	Benzaldehyde	0.18	960
9.79	163296590	Limonene	7.26	1029
9.90	13623402	1,8-cineole	0.61	1031
23.07	3971761	à-Cubebene	0.18	1348
24.18	255810866	Copaene<alpha->	11.37	1376
25.57	11959823	Gurjunene<alpha->	0.53	1409
26.01	622861943	Caryophyllene(E-)	27.69	1419
26.30	5945269	Guaiene<alpha->	0.26	1439
26.46	4295747	Gurjunene<beta->	0.19	1433
26.75	177414939	Aromadendrene	7.89	1441
26.92	7946309	Eudesma-6,11-diene<cis->	0.35	1489
27.33	67056203	Humulene<alpha->	2.98	1454
27.62	49663383	Caryophyllene<9-epi-(E)->	2.21	1466
28.12	9571587	Cadina-1(6),4-diene<trans->	0.43	1476
28.24	24625214	Muurolene<gamma->	1.09	1479
28.62	16101061	Selinene<beta->	0.72	1490
28.99	26381641	Viridiflorene	1.17	1496
29.17	12695687	Muurolene<alpha->	0.56	1500
29.26	28963867	Bisabolene<(Z)-alpha->	1.29	1507
29.48	34990468	Bisabolene<beta->	1.56	1505
29.71	18945941	Cadinene<gamma->	0.84	1513
30.06	156459567	Cadinene<delta->	6.96	1523
30.42	30845285	Cadina-1,4-diene<trans->	1.37	1534
30.81	8750937	Calacorene<alpha->	0.39	1545
31.47	13036599	Epiglobulol	0.58	-
31.60	80478300	Nerolidol<E->	3.58	1563
32.40	118449218	Globulol	5.27	1590
32.69	10606054	Viridiflorol	0.47	1592
33.13	26816876	Ledol	1.19	1602
33.34	5571077	Humulene epoxide II	0.25	1608
33.56	4837174	Cubenol<1,10-di-epi->	0.22	1619
33.83	5334298	Eudesmol<beta->	0.24	1650
34.04	26265438	Muurola-4,10(14)-dien-1-beta-ol	1.17	1631
34.21	6337872	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.28	1640
34.33	14312503	Caryophylla-4(12),8(13)-dien5-beta-ol	0.64	1640
34.56	30386943	Cadinol<epi-alpha-> (=tau-cadinol)	1.35	1640
34.68	24047195	Muurolol<alpha-> (=Torreyol)	1.07	1646
34.99	19012611	Cadinol<alpha->	0.85	1654
35.61	10521095	Caryophyllene<14-hydroxy-9-epi-(E)->	0.47	1669

Table 17 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Phitsanulok

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.69	9437801	Pinene<alpha->	0.42	939
7.49	3893824	Benzaldehyde	0.18	960
8.42	3075248	Pinene<beta->	0.14	979
9.79	290075805	Limonene	13.05	1029
9.90	32469435	1,8-cineole	1.46	1031
10.08	3078036	Ocimene<(Z)-beta->	0.14	1037
24.18	219136989	Copaene<alpha->	9.86	1376
25.32	3604198	Italicene	0.16	1405
25.57	3266767	Gurjunene<alpha->	0.15	1409
26.02	771945821	Caryophyllene(E-)	34.72	1419
26.75	10713683	Aromadendrene	0.48	1441
27.33	88151359	Humulene<alpha->	3.97	1454
27.62	18846975	Caryophyllene<9-epi-(E)->	0.85	1466
28.24	21973020	Muurolene<gamma->	0.99	1479
28.47	3247704	Curcumene<ar->	0.15	1480
28.63	4135113	Selinene<beta->	0.19	1490
28.98	7912334	Selinene<alpha->	0.36	1498
29.17	9866578	Muurolene<alpha->	0.44	1500
29.25	80886860	Bisabolene<(Z)-alpha->	3.64	1507
29.50	99136294	Bisabolene<beta->	4.46	1505
29.71	15948109	Cadinene<gamma->	0.72	1513
30.06	116119534	Cadinene<delta->	5.22	1523
30.41	25159000	Cadina-1,4-diene<trans->	1.13	1534
30.81	13286860	Calacorene<alpha->	0.60	1545
31.18	3688435	Isoaromadendrene epoxide	0.17	-
31.61	111807061	Nerolidol<E->	5.03	1563
32.36	79208202	Caryophyllene oxide	3.56	1583
33.13	14617259	Globulol	0.66	1590
33.35	8207632	Caryophyllene oxide	0.37	1583
33.64	2779664	Murolan-3,9(11)-diene-10-peroxy	0.13	-
34.04	21814618	Muurola-4,10(14)-dien-1-beta-ol	0.98	1631
34.20	9075677	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.41	1640
34.35	25442407	Caryophylla-4(12),8(13)-dien5-beta-ol	1.14	1640
34.55	25719669	Cadinol<epi-alpha-> (=tau-cadinol)	1.16	1640
34.70	22206079	Muurolol<alpha-> (=Torreyol)	1.00	1646
35.01	10564465	Murrolol<epi-alpha-> (=tau-muurolol)	0.48	1642
35.11	5429138	Isoaromadendrene epoxide	0.24	-
35.61	19205358	Caryophyllene<14-hydroxy-9-epi-(E)->	0.86	1669
36.04	4914782	Bisabolol<alpha->	0.22	1685
40.29	3175533	Calamenene<5-hydroxy-cis->	0.14	1713

Table 18 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Uthai Thani

RT	**Peak Area**	**Chemical Composition**	**Area %**	**Kovat's index**
6.71	22594581	Pinene<alpha->	0.99	939
9.79	105123997	Limonene	4.60	1029
9.90	25768410	1,8-cineole	1.13	1031
24.18	164068691	Copaene<alpha->	7.18	1376
25.57	15631167	Gurjunene<alpha->	0.68	1409
26.00	735965181	Caryophyllene(E-)	32.22	1419
26.29	6754598	Valencene	0.30	1496
26.46	6222331	Gurjunene<beta->	0.27	1433
26.58	5049136	Gurjunene<gamma->	0.22	1477
26.75	193879122	Aromadendrene	8.49	1441
26.91	10512474	Eudesma-6,11-diene<cis->	0.46	1489
27.33	80006443	Humulene<alpha->	3.50	1454
27.62	49638622	Caryophyllene<9-epi-(E)->	2.17	1466
28.12	9385755	Cadina-1(6),4-diene<trans->	0.41	1476
28.24	22666206	Muurolene<gamma->	0.99	1479
28.63	16424960	Selinene<beta->	0.72	1490
28.98	28469576	Viridiflorene	1.25	1496
29.17	10799639	Muurolene<alpha->	0.47	1500
29.25	60612634	Bisabolene<(Z)-alpha->	2.65	1507
29.50	78198807	Bisabolene<beta->	3.42	1505
29.71	21296259	Cadinene<gamma->	0.93	1513
30.06	123100603	Cadinene<delta->	5.39	1523
30.41	22879914	Cadina-1,4-diene<trans->	1.00	1534
30.81	14334832	Calacorene<alpha->	0.63	1545
31.16	4989542	Elemol	0.22	1549
31.47	16889583	Epiglobulol	0.74	-
31.59	65004365	Nerolidol<E->	2.85	1563
32.40	152850003	Globulol	6.69	1590
32.69	11415596	Viridiflorol	0.50	1592
33.13	20630225	Ledol	0.90	1602
33.33	7401018	Humulene epoxide II	0.32	1608
33.83	7364359	Eudesmol<beta->	0.32	1650
34.04	23032179	Muurola-4,10(14)-dien-1-beta-ol	1.01	1631
34.20	10663579	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.47	1640
34.35	26951643	Caryophylla-4(12),8(13)-dien5-beta-ol	1.18	1640
34.53	31758315	Murrolol<epi-alpha-> (=tau-muurolol)	1.39	1642
34.70	23598404	Muurolol<alpha-> (=Torreyol)	1.03	1646
34.99	25932094	Cadinol<alpha->	1.14	1654
35.59	19976352	Caryophyllene<14-hydroxy-9-epi-(E)->	0.87	1669
36.13	6330972	Bisabolol<alpha->	0.28	1685

Table 19 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Sukhothai

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	139350620	Pinene<alpha->	8.22	939
7.51	6371792	Benzaldehyde	0.38	960
9.81	208522879	Limonene	12.30	1029
9.90	50243784	1,8-cineole	2.96	1031
16.33	8503709	Terpineol<alpha->	0.50	1188
17.91	3483907	Mentha-1(7),8-dien-2-ol<cis-p->	0.21	1230
24.16	95019299	Copaene<alpha->	5.61	1376
25.55	6991936	Gurjunene<alpha->	0.41	1409
25.98	391644067	Caryophyllene(E-)	23.11	1419
26.27	3795361	Longifolene	0.22	1407
26.46	8217509	Gurjunene<beta->	0.48	1433
26.75	144908201	Aromadendrene	8.55	1441
26.89	6453199	Eudesma-6,11-diene<cis->	0.38	1489
27.31	40468295	Humulene<alpha->	2.39	1454
27.60	38491237	Caryophyllene<9-epi-(E)->	2.27	1466
28.22	15212336	Muurolene<gamma->	0.90	1479
28.61	10859197	Selinene<beta->	0.64	1490
28.96	15595283	Viridiflorene	0.92	1496
29.23	46707625	Bisabolene<(Z)-alpha->	2.76	1507
29.48	61019443	Bisabolene<beta->	3.60	1505
29.69	12421526	Cadinene<gamma->	0.73	1513
30.06	72841807	Cadinene<delta->	4.30	1523
30.39	10345489	Cadina-1,4-diene<trans->	0.61	1534
30.79	7581512	Calacorene<alpha->	0.45	1545
31.16	4218143	Elemol	0.25	1549
31.47	10449247	Epiglobulol	0.62	-
31.59	27876898	Nerolidol<E->	1.64	1563
32.38	113664729	Globulol	6.71	1590
32.69	3941804	Viridiflorol	0.23	1592
33.10	13077354	Ledol	0.77	1602
33.33	5679798	Humulene epoxide II	0.34	1608
33.83	5661775	Eudesmol<beta->	0.33	1650
34.02	16510517	Muurola-4,10(14)-dien-1-beta-ol	0.97	1631
34.18	6931137	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.41	1640
34.33	18746106	Caryophylla-4(12),8(13)-dien5-beta-ol	1.11	1640
34.53	19327827	Murrolol<epi-alpha-> (=tau-muurolol)	1.14	1642
34.68	15487167	Muurolol<alpha-> (=Torreyol)	0.91	1646
34.99	9357749	Cadinol<alpha->	0.55	1654
35.09	5615470	-	0.33	-
35.59	13435272	Caryophyllene<14-hydroxy-9-epi-(E)->	0.79	1669

Table 20 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Nakhon Si Thammarat

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	14497138	Pinene<alpha->	0.59	939
7.49	7180524	Benzaldehyde	0.29	960
9.79	180805862	Limonene	7.42	1029
9.90	47956335	1,8-cineole	1.97	1031
24.18	212272563	Copaene<alpha->	8.71	1376
25.57	10532122	Gurjunene<alpha->	0.43	1409
26.00	703615818	Caryophyllene(E-)	28.86	1419
26.46	9612993	Gurjunene<beta->	0.39	1433
26.75	185366759	Aromadendrene	7.60	1441
26.91	11251799	Eudesma-6,11-diene<cis->	0.46	1489
27.33	75952790	Humulene<alpha->	3.11	1454
27.62	48875788	Caryophyllene<9-epi-(E)->	2.00	1466
28.22	15338562	Muurolene<gamma->	0.63	1479
28.61	13610069	Selinene<beta->	0.56	1490
28.96	22794403	Viridiflorene	0.93	1496
29.17	10740338	Muurolene<alpha->	0.44	1500
29.25	18438661	Bisabolene<(Z)-alpha->	0.76	1507
29.48	24768166	Bisabolene<beta->	1.02	1505
29.71	17689352	Cadinene<gamma->	0.73	1513
30.06	142869983	Cadinene<delta->	5.86	1523
30.41	24583128	Cadina-1,4-diene<trans->	1.01	1534
30.81	8345143	Calacorene<alpha->	0.34	1545
31.16	6890435	Elemol	0.28	1549
31.47	22352957	Epiglobulol	0.92	-
31.59	83437666	Nerolidol<E->	3.42	1563
31.74	16927560	Maaliol	0.69	1567
32.40	206615648	Globulol	8.47	1590
32.69	16530118	Viridiflorol	0.68	1592
33.13	38602651	Ledol	1.58	1602
33.33	7771265	Humulene epoxide II	0.32	1608
33.56	7610843	Cubenol<1,10-di-epi->	0.31	1619
33.83	9872463	Eudesmol<beta->	0.40	1650
34.04	35960218	Muurola-4,10(14)-dien-1-beta-ol	1.47	1631
34.20	17753247	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.73	1640
34.35	50608855	Caryophylla-4(12),8(13)-dien5-beta-ol	2.08	1640
34.53	38199576	Murrolol<epi-alpha-> (=tau-muurolol)	1.57	1642
34.68	18090246	Muurolol<alpha-> (=Torreyol)	0.74	1646
34.99	18126479	Cadinol<alpha->	0.74	1654
35.11	13112448	-	0.54	-
35.59	22770750	Caryophyllene<14-hydroxy-9-epi-(E)->	0.93	1669

Table 21 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Sa Kaeo

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	11670919	Pinene<alpha->	1.34	939
8.43	1559762	Myrcene	0.18	990
9.79	170926269	Limonene	19.58	1029
9.90	18248289	1,8-cineole	2.09	1031
24.16	46952809	Copaene<alpha->	5.38	1376
25.55	2199596	Gurjunene<alpha->	0.25	1409
25.96	240972745	Caryophyllene(E-)	27.61	1419
26.46	3074042	Gurjunene<beta->	0.35	1433
26.73	49756179	Aromadendrene	5.70	1441
27.31	24856408	Humulene<alpha->	2.85	1454
27.60	13419958	Caryophyllene<9-epi-(E)->	1.54	1466
28.22	4302107	Muurolene<gamma->	0.49	1479
28.47	1357988	Curcumene<ar->	0.16	1480
28.61	4260952	Selinene<beta->	0.49	1490
28.96	4554505	Viridiflorene	0.52	1496
29.25	30532127	Bisabolene<(Z)-alpha->	3.50	1507
29.48	40484683	Bisabolene<beta->	4.64	1505
29.71	5531894	Cadinene<gamma->	0.63	1513
30.06	19627362	Cadinene<delta->	2.25	1523
30.39	3318534	Cadina-1,4-diene<trans->	0.38	1534
30.81	6281063	Calacorene<alpha->	0.72	1545
31.16	3668625	Elemol	0.42	1549
31.47	3340096	Epiglobulol	0.38	-
31.59	13936337	Nerolidol<E->	1.60	1563
32.36	56963274	Caryophyllene oxide	6.53	1583
32.69	1430761	Viridiflorol	0.16	1592
33.10	4038006	Ledol	0.46	-
33.33	4446222	Humulene epoxide II	0.51	1602
33.83	2202995	Eudesmol<beta->	0.25	1650
34.04	7433866	Muurola-4,10(14)-dien-1-beta-ol	0.85	1631
34.20	6816655	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.78	1640
34.33	19799257	Caryophylla-4(12),8(13)-dien5-beta-ol	2.27	1640
34.51	7558617	Murrolol<epi-alpha-> (=tau-muurolol)	0.87	1642
34.68	5901050	Muurolol<alpha-> (=Torreyol)	0.68	1646
34.99	4737335	Cadinol<alpha->	0.54	1654
35.11	6628757	-	0.76	-
35.59	13580867	Caryophyllene<14-hydroxy-9-epi-(E)->	1.56	1669
36.17	6465829	Bisabolol<alpha->	0.74	1685
30.06	19627362	Cadinene<delta->	2.25	1523
30.39	3318534	Cadina-1,4-diene<trans->	0.38	1534

Table 22 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Phrae

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	5990888	Pinene<alpha->	0.42	939
8.43	1552940	Myrcene	0.11	990
9.79	159612725	Limonene	11.18	1029
9.90	12714681	1,8-cineole	0.89	1031
10.10	2213443	Ocimene<(Z)-beta->	0.15	1037
23.06	1883969	Cubebene<alpha->	0.13	1348
23.81	2901324	Longicyclene	0.20	1371
24.18	198150977	Copaene<alpha->	13.87	1376
25.57	3495387	Gurjunene<alpha->	0.24	1409
25.98	444853666	Caryophyllene(E-)	31.15	1419
26.31	2421437	Gurjunene<beta->	0.17	1433
26.75	35548262	Aromadendrene	2.49	1441
27.33	46498680	Humulene<alpha->	3.26	1454
27.62	22152377	Caryophyllene<9-epi-(E)->	1.55	1466
28.12	2949751	Cadina-1(6),4-diene<trans->	0.21	1476
28.24	15328938	Muurolene<gamma->	1.07	1479
28.61	6917791	Selinene<beta->	0.48	1490
28.96	8107121	Selinene<alpha->	0.57	1498
29.17	11629784	Muurolene<alpha->	0.81	1500
29.25	10631363	Bisabolene<(Z)-alpha->	0.74	1507
29.48	17915858	Bisabolene<beta->	1.25	1505
29.71	13341447	Cadinene<gamma->	0.93	1513
30.06	107583563	Cadinene<delta->	7.53	1523
30.41	15247635	Cadina-1,4-diene<trans->	1.07	1534
30.81	6020946	Calacorene<alpha->	0.42	1545
31.16	2938749	Elemol	0.21	1549
31.47	1599452	Epiglobulol	0.11	-
31.59	50822678	Nerolidol<E->	3.56	1563
32.36	79838439	Caryophyllene oxide	5.59	1583
33.13	16490397	Ledol	1.15	1602
33.33	5500179	Humulene epoxide II	0.39	1608
33.64	2401248	Cubenol<1,10-di-epi->	0.17	1619
34.04	23974983	Muurola-4,10(14)-dien-1-beta-ol	1.68	1631
34.20	7766604	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.54	1640
34.35	21722646	Caryophylla-4(12),8(13)-dien5-beta-ol	1.52	1640
34.55	21614948	Murrolol<epi-alpha-> (=tau-muurolol)	1.51	1642
34.68	13929629	Muurolol<alpha-> (=Torreyol)	0.98	1646
34.99	13539527	Cadinol<alpha->	0.95	1654
35.59	8155054	Caryophyllene<14-hydroxy-9-epi-(E)->	0.57	1669
40.29	2210692	Calamenene<5-hydroxy-cis->	0.15	1713

Table 23 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Phetchabun

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	58090766	Pinene<alpha->	2.64	939
9.81	287634618	Limonene	13.05	1029
9.90	51812300	1,8-cineole	2.35	1031
24.16	51567555	Copaene<alpha->	2.34	1376
25.57	10617720	Gurjunene<alpha->	0.48	1409
26.00	545975146	Caryophyllene(E-)	24.77	1419
26.46	9187437	Gurjunene<beta->	0.42	1433
26.75	212377293	Aromadendrene	9.64	1441
26.91	10950007	Eudesma-6,11-diene<cis->	0.50	1489
27.33	64476262	Humulene<alpha->	2.93	1454
27.62	46878201	Caryophyllene<9-epi-(E)->	2.13	1466
28.24	17383705	Muurolene<gamma->	0.79	1479
28.47	7599884	Curcumene<ar->	0.34	1480
28.63	14616035	Selinene<beta->	0.66	1490
28.98	22186193	Viridiflorene	1.01	1496
29.25	113383380	Bisabolene<(Z)-alpha->	5.14	1507
29.50	160546542	Bisabolene<beta->	7.28	1505
29.71	14185436	Cadinene<gamma->	0.64	1513
30.06	35294055	Cadinene<delta->	1.60	1523
30.39	10467513	Bisabolene<(E)-gamma->	0.47	1531
30.81	15581608	Calacorene<alpha->	0.71	1545
31.16	7578685	Isoaromadendrene epoxide	0.34	-
31.47	20877735	Epiglobulol	0.95	-
31.59	34641065	Nerolidol<E->	1.57	1563
31.74	12808599	Maaliol	0.58	1567
32.40	169862537	Globulol	7.71	1590
32.69	14205125	Viridiflorol	0.64	1592
33.08	11485889	Ledol	0.52	1602
33.33	9831854	Caryophyllene oxide	0.45	1583
33.83	8246574	Eudesmol<beta->	0.37	1650
34.04	11146566	Muurola-4,10(14)-dien-1-beta-ol	0.51	1631
34.20	8733862	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.40	1640
34.35	28977291	Caryophylla-4(12),8(13)-dien5-beta-ol	1.31	1640
34.51	15989424	Cadinol<epi-alpha-> (=tau-cadinol)	0.73	1640
34.70	15676633	Muurolol<alpha-> (=Torreyol)	0.71	1646
34.99	11085530	Muurolol <epi-alpha-> (=tau- Muurolol)	0.50	1642
35.11	10071613	Isoaromadendrene epoxide	0.46	-
35.26	6450024	-	0.29	-
35.59	29623024	Caryophyllene<14-hydroxy-9-epi-(E)->	1.34	1669
36.17	15700523	Bisabolol<alpha->	0.71	1685

Table 24 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Nakonsawan

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.71	12105476	Pinene<alpha->	0.22	939
7.47	23138255	Benzaldehyde	0.43	960
9.81	281962439	Limonene	5.20	1029
9.89	74611285	1,8-cineole	1.38	1031
24.20	633146119	Copaene<alpha->	11.69	1376
26.06	1469602543	Caryophyllene(E-)	27.13	1419
26.75	31812589	Aromadendrene	0.59	1441
27.33	209105312	Humulene<alpha->	3.86	1454
27.62	51897130	Caryophyllene<9-epi-(E)->	0.96	1466
28.24	79454648	Muurolene<gamma->	1.47	1479
28.47	13078905	Curcumene<ar->	0.24	1480
28.63	30667456	Selinene<beta->	0.57	1490
28.96	34133371	Selinene<alpha->	0.63	1498
29.17	41945672	Muurolene<alpha->	0.77	1500
29.27	206853356	Bisabolene<(Z)-alpha->	3.82	1507
29.52	262916412	Bisabolene<beta->	4.85	1505
29.73	74436903	Cadinene<gamma->	1.37	1513
30.10	425636878	Cadinene<delta->	7.86	1523
30.41	63008994	Cadina-1,4-diene<trans->	1.16	1534
30.81	51145528	Calacorene<alpha->	0.94	1545
31.16	20997420	Isoaromadendrene epoxide	0.39	-
31.59	27454213	Nerolidol<E->	0.51	1563
31.86	12278530	Caryophyllenyl alcohol	0.23	1572
32.38	278162195	Caryophyllene oxide	5.13	1583
33.12	80417180	Globulol	1.48	1590
33.33	40587996	Caryophyllene oxide	0.75	1583
33.64	14358137	Murolan-3,9(11)-diene-10-peroxy	0.27	-
34.06	125494616	Muurola-4,10(14)-dien-1-beta-ol	2.32	1631
34.22	52569530	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.97	1640
34.37	125288154	Caryophylla-4(12),8(13)-dien5-beta-ol	2.31	1640
34.55	134461489	Murrolol<epi-alpha-> (=tau-muurolol)	2.48	1642
34.70	105397709	Muurolol<alpha-> (=Torreyol)	1.95	1646
35.01	70070927	Cadinol<alpha->	1.29	1654
35.11	43164152	Isoaromadendrene epoxide	0.80	-
35.26	14186462	-	0.26	-
35.44	17407522	Ledene oxide	0.32	-
35.61	116304306	Caryophyllene<14-hydroxy-9-epi-(E)->	2.15	1669
35.92	11129795	-	0.21	-
36.13	42060766	Bisabolol<alpha->	0.78	1685
40.27	14869299	Calamenene<5-hydroxy-cis->	0.27	1713

Table 25 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Trang

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.72	169491789	Pinene<alpha->	6.25	939
7.50	4129888	Benzaldehyde	0.15	960
9.80	70471209	Limonene	2.60	1029
9.90	54546741	1,8-cineole	2.01	1031
14.17	11286500	Pinocarveol<trans->	0.42	1139
16.34	7654517	Terpineol<alpha->	0.28	1188
23.73	6445852	Longicyclene	0.24	1374
24.19	366983487	Copaene<alpha->	13.54	1376
25.56	4675007	Gurjunene<alpha->	0.17	1409
26.01	726848181	Caryophyllene(E-)	26.82	1419
26.32	4845323	Copaene<beta->	0.18	1432
26.74	34215293	Aromadendrene	1.26	1441
27.32	77518305	Humulene<alpha->	2.86	1454
27.61	40845621	Caryophyllene<9-epi-(E)->	1.51	1466
28.23	24260236	Muurolene<gamma->	0.90	1479
28.62	10970172	Selinene<beta->	0.40	1490
28.97	10942170	Selinene<alpha->	0.40	1498
29.16	18746063	Muurolene<alpha->	0.69	1500
29.24	27724223	Bisabolene<(Z)-alpha->	1.02	1597
29.49	37458963	Bisabolene<beta->	1.38	1505
29.70	24847824	Cadinene<gamma->	0.92	1513
30.07	208268186	Cadinene<delta->	7.68	1523
30.40	30261360	Cadina-1,4-diene<trans->	1.12	1534
30.82	15493271	Calacorene<alpha->	0.57	1545
31.17	12921055	Elemol	0.48	1549
31.60	78933578	Nerolidol<E->	2.91	1563
32.37	174288774	Caryophyllene oxide	6.43	1583
33.13	38979042	Globulol	1.44	1590
33.34	13574305	Humulene epoxide II	0.50	1608
34.05	68216414	Muurola-4,10(14)-dien-1-beta-ol	2.52	1631
34.21	35963105	Caryophylla-4(12),8(13)-dien-5-alpha-ol	1.33	1640
34.36	105555560	Caryophylla-4(12),8(13)-dien5-beta-ol	3.89	1640
34.54	49101385	Murrolol<epi-alpha-> (=tau-muurolol)	1.81	1642
34.69	28048525	Muurolol<alpha-> (=Torreyol)	1.03	1646
35.00	19510057	Cadinol<alpha->	0.72	1654
35.12	24051038	Isoaromadendrene epoxide	0.89	-
35.60	47831540	Caryophyllene oxide	1.76	1583
35.91	6198574	-	0.23	-
36.16	6987414	Bisabolol<alpha->	0.26	1685
40.27	11078163	Calamenene<5-hydroxy-cis->	0.41	1713

Table 26 The chemical constituents of the volatile oil of *P. guajava* Khee Nok cultivar leaves from Lampang

RT	Peak Area	Chemical Composition	Area %	Kovat's index
6.69	19316530	Pinene<alpha->	1.37	939
9.80	24284043	Limonene	1.72	1029
9.88	35047757	1,8-cineole	2.48	1031
10.09	2888884	Ocimene<(Z)-beta->	0.20	1037
16.34	2824305	Terpineol<alpha->	0.20	1188
24.17	100790468	Copaene<alpha->	7.12	1376
25.56	7025962	Gurjunene<alpha->	0.50	1409
25.99	417198036	Caryophyllene(E-)	29.48	1419
26.28	3739371	Longifolene	0.26	1407
26.47	10415464	Gurjunene<beta->	0.74	1433
26.76	136815582	Aromadendrene	9.67	1441
26.90	8453452	Eudesma-6,11-diene<cis->	0.60	1489
27.32	44880098	Humulene<alpha->	3.17	1454
27.60	33455553	Caryophyllene<9-epi-(E)->	2.36	1466
28.23	11604981	Muurolene<gamma->	0.82	1479
28.62	9888987	Selinene<beta->	0.70	1490
28.97	14671447	Viridiflorene	1.04	1496
29.24	53867744	Bisabolene<(Z)-alpha->	3.81	1507
29.49	65496032	Bisabolene<beta->	4.63	1505
29.72	13266467	Cadinene<gamma->	0.94	1513
30.07	49945639	Cadinene<delta->	3.53	1523
30.40	7386012	Cadina-1,4-diene<trans->	0.52	1534
30.79	8460611	Calacorene<alpha->	0.60	1545
31.17	4280622	Elemol	0.30	1549
31.48	10885748	Epiglobulol	0.77	-
31.60	55883625	Nerolidol<E->	3.95	1563
32.39	111637140	Globulol	7.89	1590
32.70	9250275	Viridiflorol	0.65	1592
33.11	12664904	Ledol	0.90	1602
33.34	6958994	Humulene epoxide II	0.49	1608
33.57	4567187	Cubenol<1,10-di-epi->	0.32	1619
33.84	5120726	Eudesmol<beta->	0.36	1650
34.02	13308364	Muurola-4,10(14)-dien-1-beta-ol	0.94	1631
34.21	8457367	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.60	1640
34.33	23394237	Caryophylla-4(12),8(13)-dien5-beta-ol	1.65	1640
34.50	15772128	Murrolol<epi-alpha-> (=tau-muurolol)	1.11	1642
34.69	11119706	Muurolol<alpha-> (=Torreyol)	0.79	1646
35.00	10161788	Cadinol<alpha->	0.72	1654
35.10	9421720	-	0.67	-
35.60	20353536	Caryophyllene<14-hydroxy-9-epi-(E)->	1.44	1669

APPENDIX E

Inhibition zone by agar disc diffusion

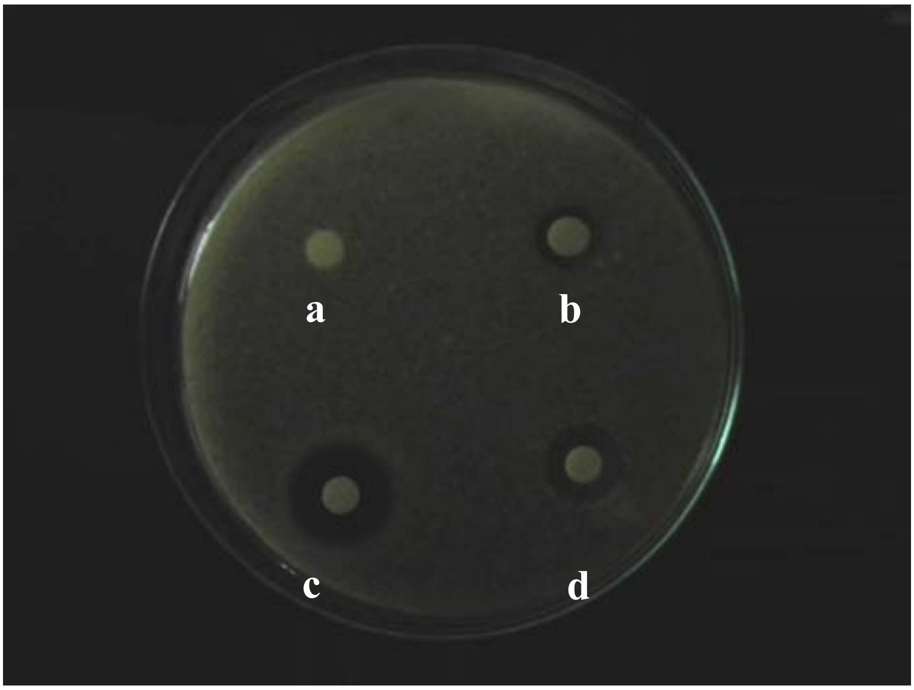


Figure 24 The inhibition zone of *Bacillus subtilis* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

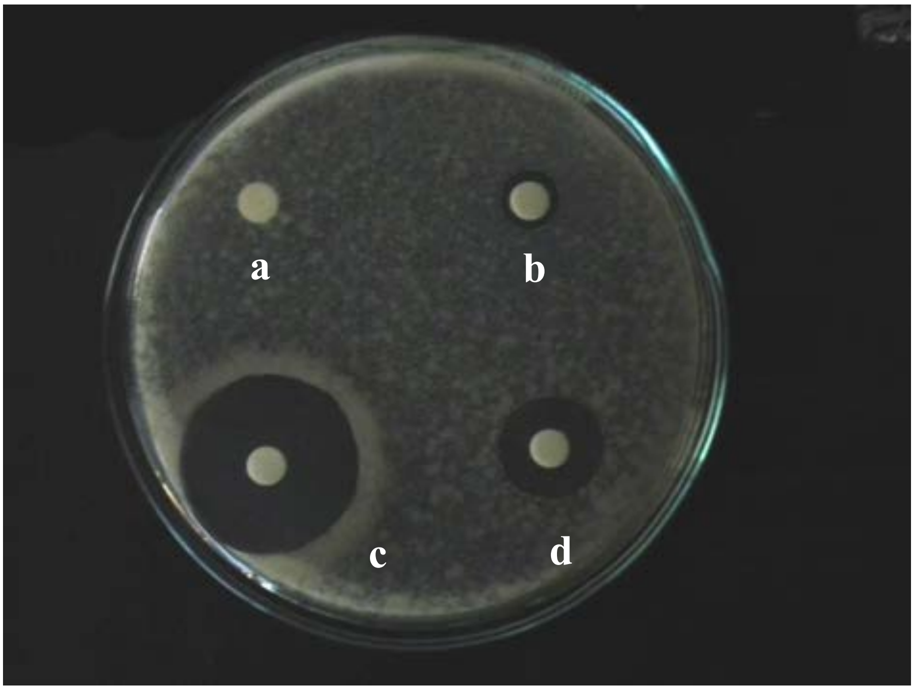


Figure 25 The inhibition zone of *Bacillus cereus* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

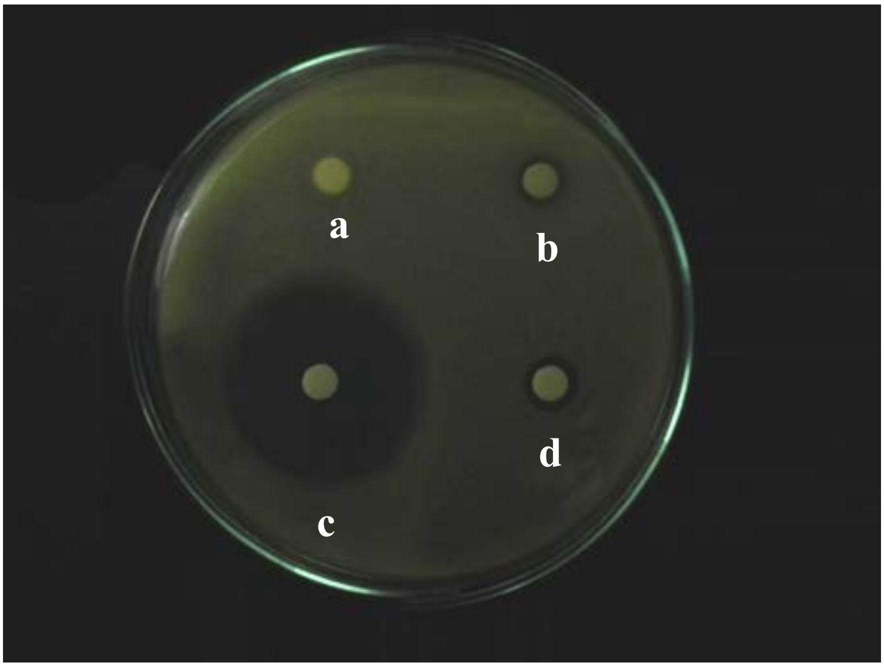


Figure 26 The inhibition zone of *Staphylococcus aureus* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

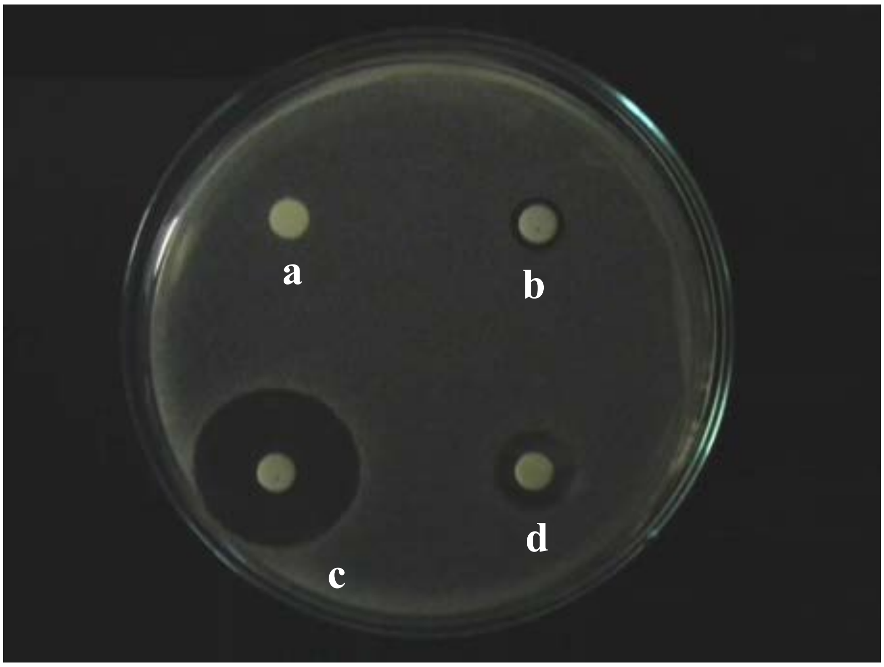


Figure 27 The inhibition zone of *Straphylococcus epidermidis* (Isolates) from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

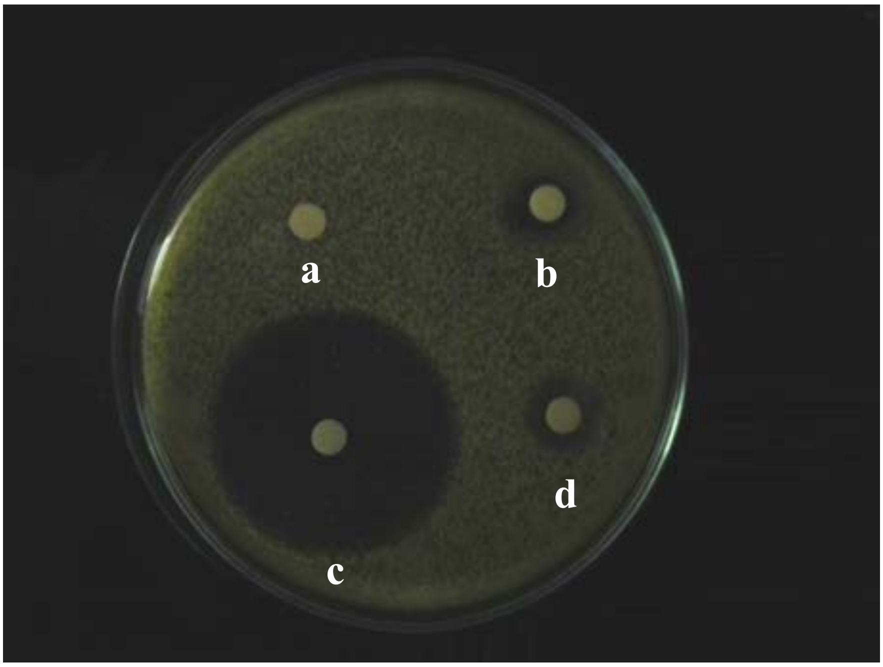


Figure 28 The inhibition zone of *Micrococcus luteus* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

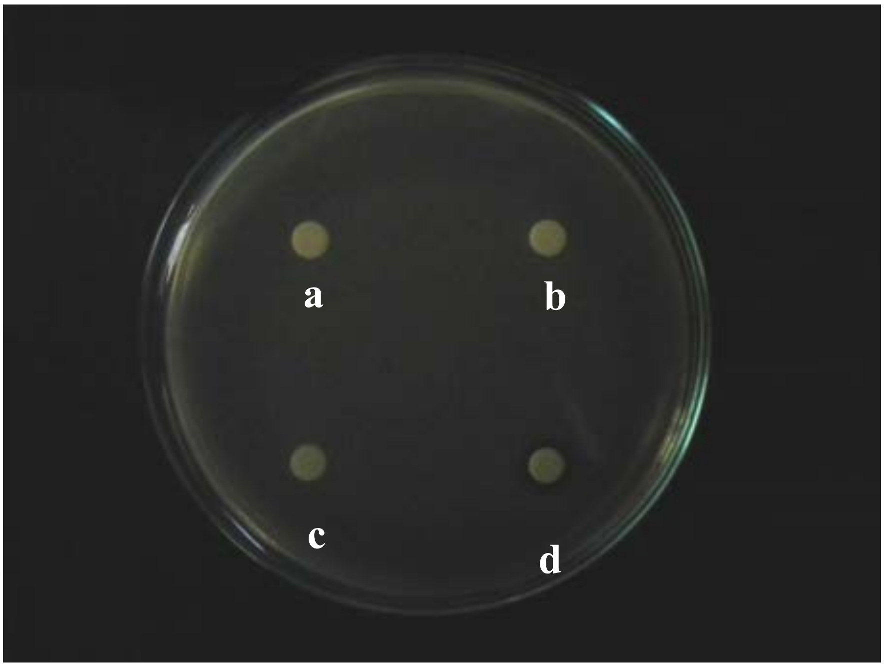


Figure 29 The inhibition zone of *Pseudomonas aeruginosa* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

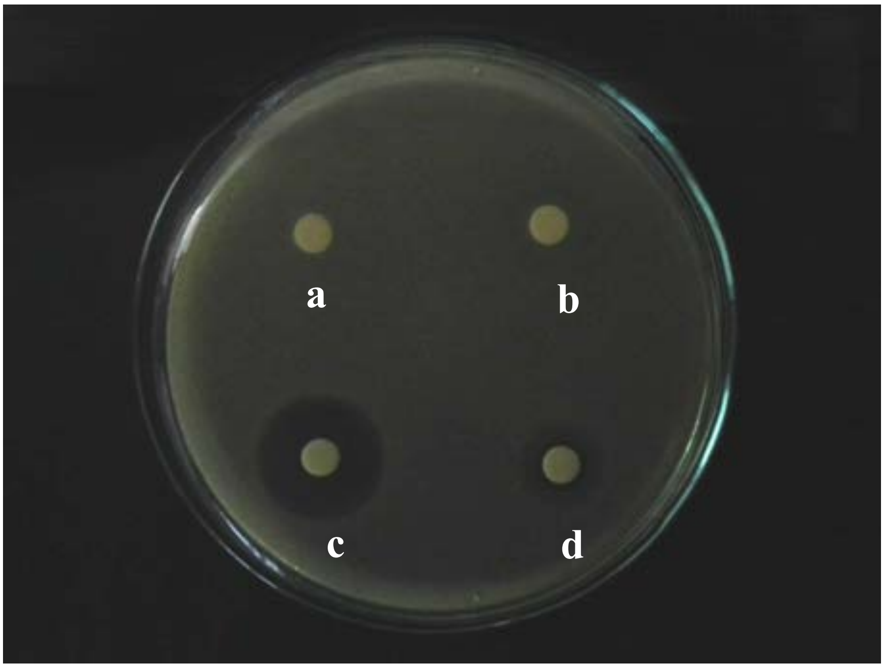


Figure 30 The inhibition zone of *Escherichia coli* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

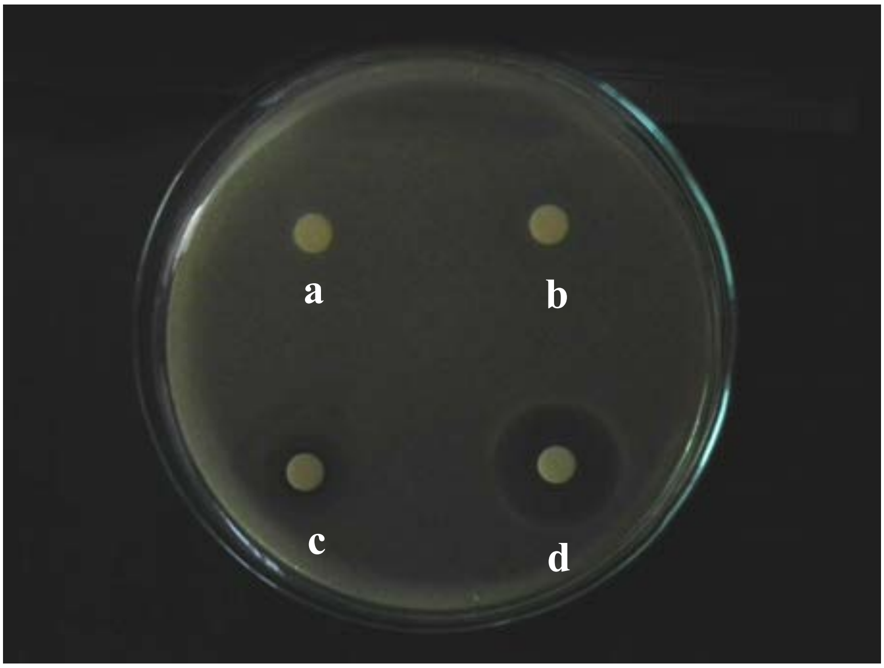


Figure 31 The inhibition zone of *Enterobacter aerogenes* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

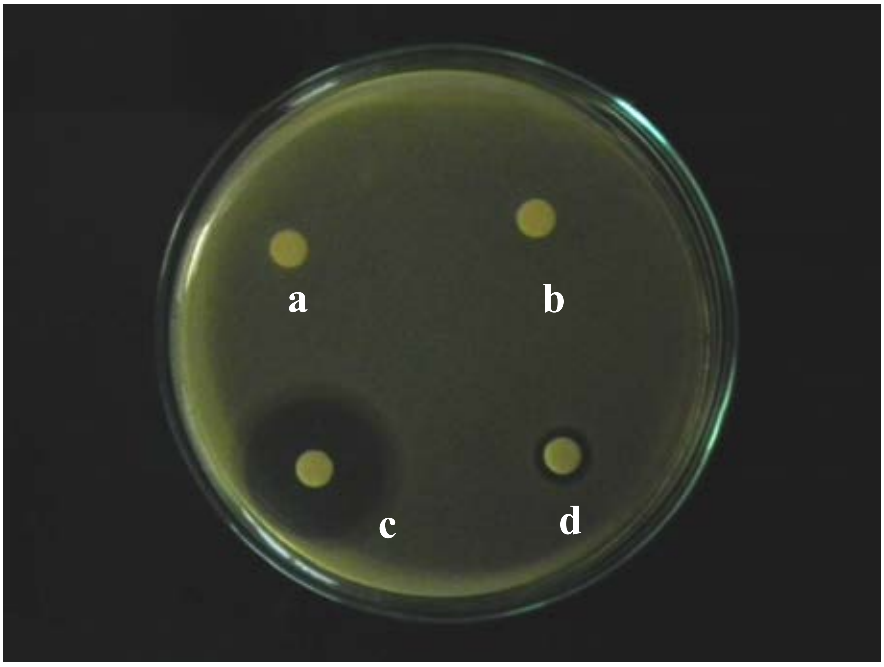


Figure 32 The inhibition zone of *Salmonella typhi* (Isolates) from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

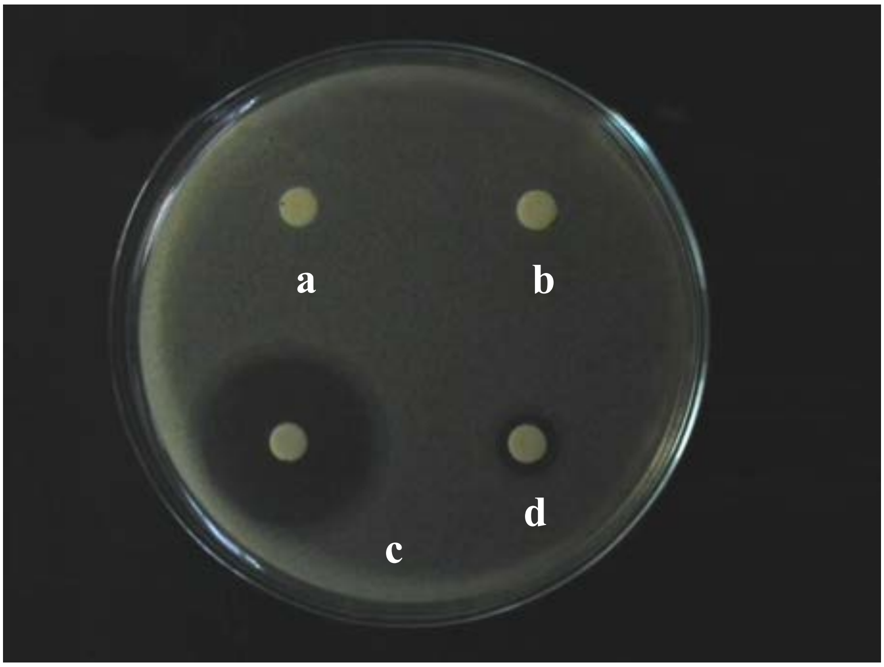


Figure 33 The inhibition zone of *Salmonella typhimurium* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

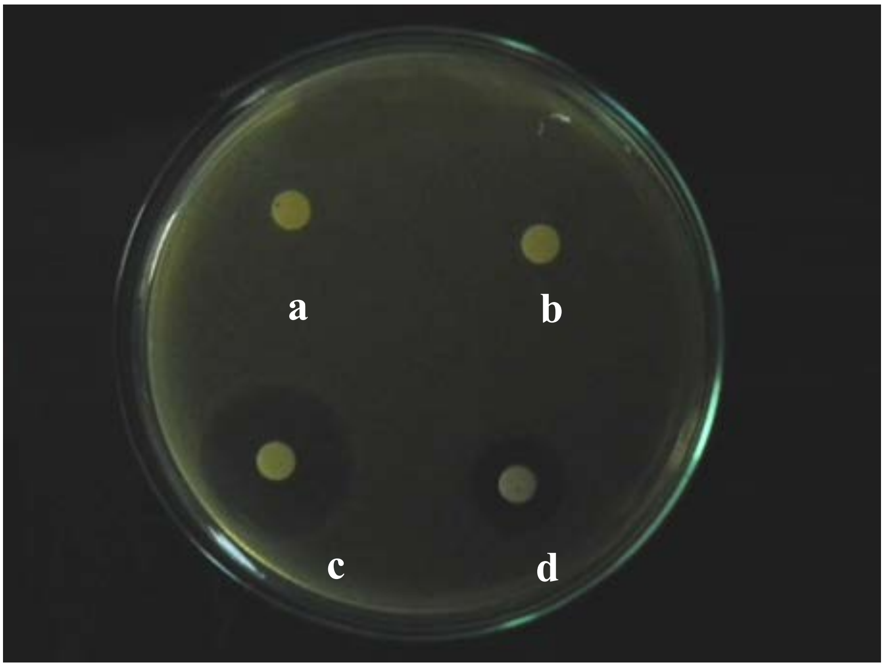


Figure 34 The inhibition zone of *Shigella* sp. (Isolates) from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

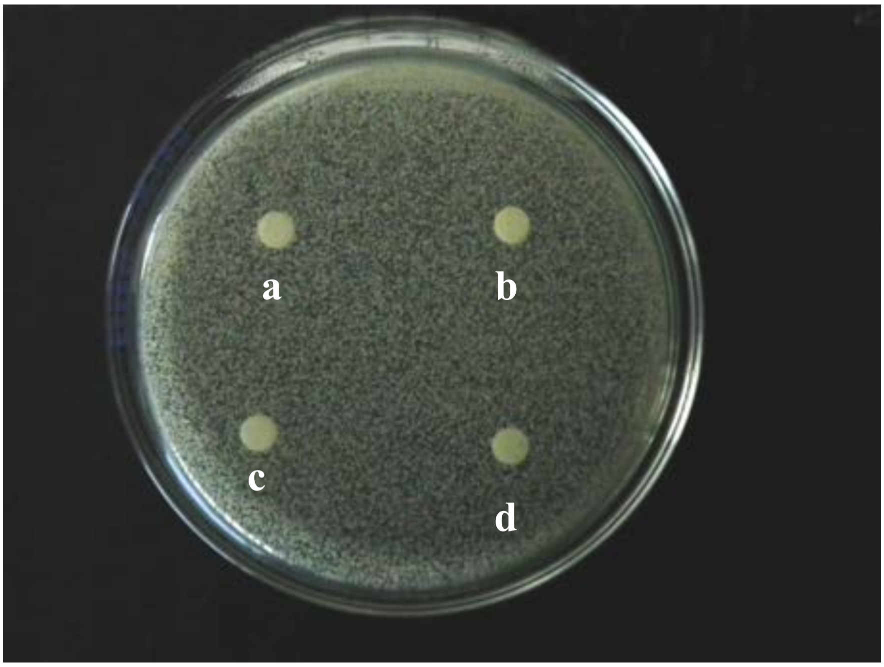


Figure 35 The inhibition zone of *Candida albicans* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

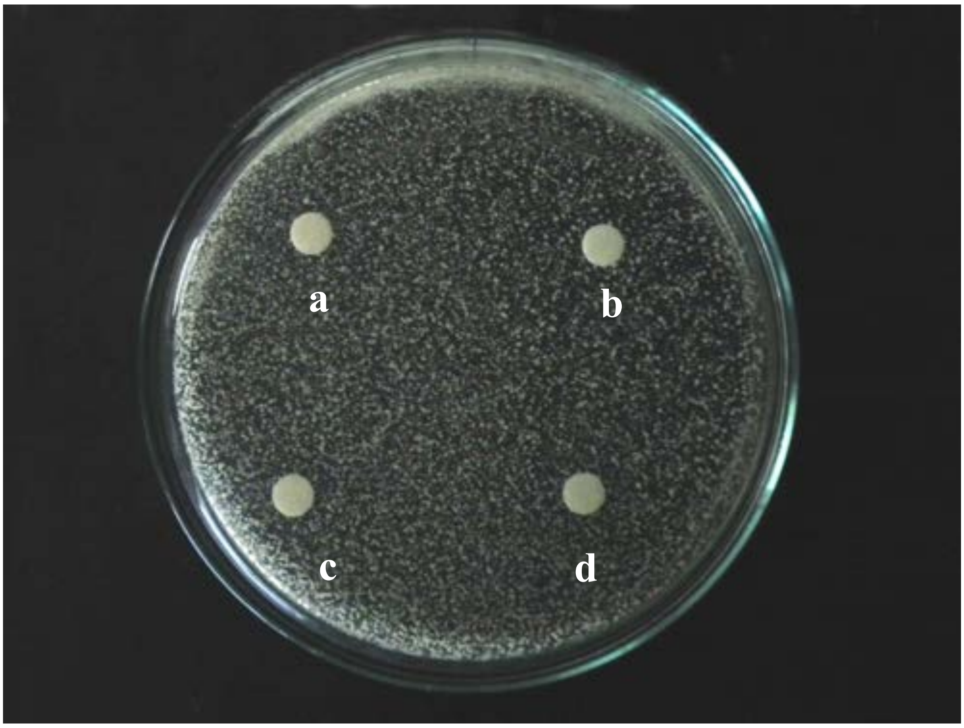


Figure 36 The inhibition zone of *Saccharomyces cerevisiae* from: a. DMSO, b. *P.guajava* Khee Nok cultivar leaves oil, c. Amikacin sodium, d. Ampicillin sulfate

VITAE

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Proceedings:

Tongkamkaew S., Palanuvej C. and Raungrungsi N. Pharmacognostic specification, antibacterial activity and chemical constituents of volatile oil of *Psidium guajava* (Khee nok cultivar) leaves. Proceedings of the 39th Congress on Science and Technology of Thailand. Bangkok, October 21 - 23, 2013.