بسم الله الرحمن الرحيم
Sudan University of Science and Technology
College of Graduate Studies

Identification of essential oil and piperine obtained from black pepper.

توصيف الزيت الاساسي و البايبرين المتحصل عليهما من الفلفل الاسود

A Thesis Submitted in Partial Fulfillment for the Requirements of the Master Degree. in Chemistry

By
Maysaa Mohammed Suliman

(B .Sc, P G diploma /chemistry)

Supervised by
Dr. Omer Adam M. Gibla

August 2016

إستهلال

قال تعالى:

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ
فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا
شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُورٌ عَلَىٰ نُورٍ ۗ
يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ
عَلِيم) صدق الله العظيم

سورة النور الآية 35

Dedication

To the soul of my father,

to my mother,

to my husband,

to my daughter Talia,

to my son Abd-Alrhman,

to my brothers and sister.

Acknowledgement

First of all my thanks are due to Almighty Allah the Most Gracious, the Most Merciful for giving me the strength and help to complete this work.

I would like to express my special appreciation to my supervisor Dr. Omer Adam Gibla for encouragement, close supervision and endless assistance.

My thanks and appreciation would extend to Allwia AL-emam, center for pharmaceutical development, university of medical sciences and technology for their help.

My thanks also extend to the staff of the chemistry department of Sudan University of Science and Technology for their continuous help and cooperation.

Abstract

The aim of this study was to identify the components that consist black pepper by extraction of it's essential oil and analyzing it, using GC-MS spectroscopic technique. The study also aimed to isolate piperine from the extracted oil and determinate it's percentage as a major component of black pepper oil.

The obtained results of GC-MS showed that the black pepper oil consist of 42 compounds. The highest percentages were mainly Oleic acid 23.07%, n-Hexadecanoic acid (16.62%) , Caryophyllene (13.34%) , Naphthalene,decahydro-2,2dimethyl (5.81%) , Gamma-Tocopherol 5.54% and Caryophyllene oxide (4.7%).

UV-Vis and IR spectroscopic techniques were used for piperine characterization and the obtained spectrum show all the characteristic peaks that confirm the piperine structure. λ-max was 344.5. The melting point determination technique was also used to confirm piperine purity, where melting point was found to be 130 ^{o}C.

المستخلص

هدفت هذة الدراسة لتحديد مكونات الفلفل الاسود عن طريق استخلاص الزيت الاساسي و تحليله بواسطة جهاز كورمتوغرافيا الغاز و مطيافية الكتلة.

هدفت الدراسة ايضا الي فصل البايبرين من الزيت الاساسي للفلفل الاسود كمكون رئيسي و تقدير نسبته .

النتيجة المتحصل عليها بواسطة كرومتوغرافيا الغاز و مطيافية الكتلة اظهرت وجود 42 مكون و كانت المركبات التاليه هي الاعلي نسبه

Oleic acid (23.07%) **,** n-Hexadecanoic acid (16.62%) **,** Caryophyllene (13.34%)

, Naphthalene,decahydro-2,2dimethyl (5.81%) **,**Gamma-Tocopherol(5.54%) and Caryophyllene oxide (4.7%).

استخدم كل من جهازي الاشعة فوق البنفسجية و المرئية ومطافية الاشعة تحت الحمراء لتحليل مركب البايبرين و قد اظهرت قمم الطيف المتحصل عليها وجود مركب البايبرين. الطول الموجي للبايبرين هو 344.5

استخدم ايضا جهاز تقدير نقطة الانصهار للتاكد من نقاء البايبرين وقد كانت نقطة الانصهارعند 130 درجه مئوية.

Table of Contents

Chapter one
Introduction

Chapter two
Materials and Methods

Chapter three
Results and Discussion

List of Tables

List of figures

1-Introduction

1.1 Definition of spices

The Geneva-based International Standards Organization (ISO) defines spices and condiments as vegetable products or mixtures thereof, free from extraneous matter, used for flavouring, seasoning and imparting aroma in foods.

Webster (1879) describes spices as any of various aromatic vegetable productions as pepper, cinnamon, nutmeg, mace, allspice, ginger, cloves, etc., used in cookery to season and to flavour sauces, pickles. Spices may in form of powder or in full form.

The spice author Rosengarten describes a spice as a product which enriches or alters the quality of a thing, for example altering the taste of a food to give it zest or pungency; a piquant or lasting flavouring; or a relish. The term 'spice' is thus used to cover the use of spices, herbs and certain aromatic vegetables to impart odour and flavour to foods **(K. V. Peter , 2001)**

1.2 Importance of spices

Spices and herbs have played a dramatic role in civilization and in the history of nations. The delightful flavour and pungency of spices make them indispensable in the preparation of palatable dishes. In addition, they are reputed to possess several medicinal and pharmacological properties and hence find position in the preparat`ion of a number of medicines **(Parthasarathy, et al , 2008).** Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavour **(Souza , Stamford T.L.M., Lima**

E.O.,Trajano V.N., Filho J.B. ,2005),(Priya N. C, P. Saravana Kumari,2016).

Herbs and spices play a pivotal role in the day-to-day life of mankind as important flavouring agents in foods, beverages and pharmaceuticals and also as ingredients in perfumes and cosmetics. The manufacturers of foods, beverages, cosmetics and pharmaceuticals are responding to the growing demand of consumer resistance and legislative limitations set for products containing chemical additives. Spices as sources of natural colours and flavours **(K. V. Peter, 2006).**

1.3 BLACK PEPPER

1.3.1 Description of the plant

Black pepper (*Piper nigrum*) belongs to the family Piperaceae. It is cultivated for its fruit, which is usually dried and used as a spice and seasoning. Black pepper is known as king of spices and extensively used all over the world. It is a perennial climber, climbing by means of ivy-like roots which adhere to the support tree. The sessile, small white flowers are borne in pendulous, dense, slender spikes of about 50 blossoms each. The berry-like fruits or peppercorns are round about 0.5 ~1.0 cm in diameter and contain a single seed. They become yellowish-red at maturity and bear a single seed. Spike length varies greatly based on cultivar. The young berries are green, whitish green or light purple, while mature ones are green, pale purple or pale yellow and change to red on ripening **(K. C. Saha,et al, 2013**) . The height of the tree about 3-4 meters and the roots distributed in the top 30-35 cm of soil. The leaves are alternate, entire, 5 to 10 cm long and 3 to 6 cm across **(Narong Chomchalow,1996).**

1.3.2 Distribution of the Plant

Geographically, the Western Ghats of the South Indian Peninsula is the primary center of the black pepper cultivation. Since then, black pepper cultivation has been introduced to other countries in South and Southeast Asia **(Ravindran et al. , 1994).** Black pepper is believed to have originated in India in the submontainous tracts of the Western Ghats **(Rahiman et al., 1979)**. It is chiefly cultivated in the tropical regions of the world such as India, Malaysia, Indonesia and Brazil and on a smaller scale in SriLanka and the West Indies **(Mathai et al., 1981).** The average total export from the different producing countries is about 138,000 t. India, Indonesia, Malaysia, Sri Lanka, Vietnam and Brazil are some of the major producing countries. Black pepper distributed in Africa from Guinea to Uganda **(Peter, 2000).**

1.3.3 Classification

Kingdom	Planate
Unranked	Angiosperms
Unranked	Magnolids
Order	Piperales
Family	Piperaceae
Genus	Piper
Species	P. nigrum
Binomial name	Piper nigrum

1.3.4 Chemical Constituents

The two main components of black and white pepper are volatile oil and pungent compounds. The volatile oil level in black pepper is usually higher than in white pepper. The hull of pepper contains fiber and some essential oil. Black pepper contains about 2.0–2.6% volatile oil and about 6–13% oleoresin. The nutritional composition of black pepper per 100 g. was given by **(Tainter and Grenis 1993)** in Table 1.1

Table 1.1 The nutritional composition of black pepper per 100 g.

Composition	Quantity	Composition	Quantity
Food energy	400.0(Kcal)	Sodium	10.0 mg
Water	8.0 g	Riboflavin	0.21 mg
Protein	10.0 g	Thiamine	0.07 mg
Fat	10.2 g	Phosphorus	160. mg
Carbohydrates	66.5 g	Potassium	1200.0 mg
Ash	4.6 g	Niacin	0.8 mg
Calcium	0.4 g	Iron	17.0 mg
Vitamin A activity	19.0	Ascorbic acid	Not detected

Chun *et al* (**2002**) found that 88% of the polysaccharide of black pepper berries was glucose, followed by galactose, arabinose, galacturonic acid and rhamnose in smaller proportions.

Pepper fruit contain 2% - 4% volatile oil (sabinene, areyophyllene, beta-pinene, alph-pinene camphene, limonene, myrcene, and piperonal), 5% - 9% alkaloids (piperine, piperidine, piperanine, pieretine and chavicine), fixed oil and chromium. The sharp taste is due to Piperiene, a nitrogenous substance **(Leug Albert Y. et al., 1996).**

1.3.5 Cultivation, Harvesting and storage conditions

Though pepper is essentially a tropical plant requiring a hot humid climate, it can be grown in a wide range of environmental conditions.

The characteristic and most suitable climate requirements are high rainfall(2000–4000 mm),temperature about(25–32ºC) and high relative humidity. Pepper grows in a wide range of soils with a pH of 4.5 to 6.9. The most favourable soil types are deep well drained brown red latosols or andosols, but the crop can grow well in deep sandy clay as well if provided with mineral nutrition and adequate drainage **(K. V. Peter, 2001).**

Purseglove et al **(1981)** suggested that the ideal soil for pepper growing is well distributed alluvium rich in humus with pH above 5.5 or 5.8.

Pepper fruits can be harvested either at the green immature or red mature stage.

Under favourable growing conditions, fruit production can continue for several months.Fruits are stored in a cool, shaded, dry place until they are sold. At typical tropical ambient temperature and humidity (28 °C and 60% RH), fruits may last unspoiled for 1–2 weeks. Anthracnose is the major cause of spoilage of dry fruits. Drying of fruits in the sun is a common practice, but this tends to bleach the fruits, and rainfall and dew promote fruit rot **(Berke et al., 2005).**

1.3.6 Uses and benefits

Piper nigrum L. (Piperaceae) is a medicinally and economically very important species as it yields the black pepper of commerce, known as King of Spices, profusely used as culinary spice and condiment all over the world, which is used as a food preservative and as an essential component in traditional medicines· The traditional uses include analgesic, antipyretic,CNS depressant, anti-inflammatory, antioxidant, anticonvulsant, anti-bacterial, anti-tumor and hepatoprotective activities, antihypertensive, antiplatelet, anti-asthmatics, anti-diarrheal, antispasmodic, antidepressants, immunomodulatory, anticonvulsant, anti-thyroids, antifungal, hepato-protective,insecticidal and larvicidal activities. Black pepper oil can be used to help in the treatment of pain relief, rheumatism, chills, flu, colds, exhaustion, muscular aches, physical and emotional coldness, fevers, as a nerve tonic and to increase circulation. Furthermore, it increases the flow of saliva, stimulates appetite, encourages peristalsis, tones the colon muscles and is a general digestive tonic **(Pruthi, 1993).** In various traditional systems of medicine, the fruits of the black pepper have been used in the treatment of cholera and dyspepsia, as well as a variety of gastric ailments and arthritic disorders **(Scott et al., 2008)**

1.3.7 Preparation of White pepper

White pepper is the white inner corn obtained after removing the outer skin or pericarp of the pepper berries. For white pepper the berries are harvested when ripe and prepared by retting, steaming, boiling and rolling or running water treatment **(Gopalan et al., 1990).** It is made from ripe pepper berries by keeping it in slow flowing water or dipping it in water for seven to nine days to soften the pericarp of the skin. The pericarp is then removed by scrubbing or macerated against a plastic wire mesh and the corns are washed and dried **(Madusoodanan et al., 1990).**

1.4 Essential oil of pepper

The essential oil of pepper is a mixture of a large number of volatile chemical compounds. More than 80 components have been reported by **Gopalakrishnan et al. (1993).** Major pepper oil constituents identified by various researchers are listed below.

1. Monoterpene hydrocarbons and oxygenated compounds. This group includes:

camphene, δ^3-carene, ρ-cymene, limonene, myrcene, cis-ocimene, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, terpinolene, α-thujene. Among them the major components are α-pinene, β-pinene, sabinene and limonene. There are many oxygenated monoterpenoid compounds present in pepper essential oil, about 43 are known. 2. Sesquiterpene hydrocarbons and oxygenated compounds. About 25 sesquiterpene hydrocarbons are present in pepper oil, the most important one being β-caryophyllene. The oxygenated sesquiterpenes identified in pepper essential oil about 19.

3. Miscellaneous compounds. In addition to the above groups of compounds many others were also identified in black pepper oil. They are: eugenol, methyl eugenol, benzaldehyde, trans-anethole, myristicin, safrole, piperonal, m-methylacetophenone,ρ-methylacetophenone,n-butyrophenone, methylheptanone, pinol, methyl heptanote, methyloctanoate, 2-undecanone, n-nonane, n-tridecane, and aromatic acids such as benzoic acid, phenyl acetic acid, cinnamic acid, piperonic acid, butyric acid, 3-methyl butyric acid, haxanoic acid and 2-methyl pentanoic acid **(K. V. Peter, 2001).**

1.5 Piperine

Pepper contains about 15 alkaloids whereas piperine is the main alkaloid in such fruits of black pepper. It is responsible for the hot taste and his name remains from the heterocyclic compound piperidine. It was first isolated in the 19th century **(S. Berger, D. Sicker,2009).**The Piperine (1-piperoylpiperidine), a nitrogenous pungent substance, is an alkaloid presents in the fruits of black pepper (Piper nigrum) and other piper species of family: Piperaceae **(Wood AB,et al,1988).**Piperine is the major constituent of pepper oleoresin **(Borges and Pino, 1993).**It is an alkaloid, is found in Piper longum obtained from botanical sources is about 98%pure. Since piperine has been recognized as a main alkaloid in these plants. Piperine it is the amide of 5-(2,4-dioxymethylene-phenyl)-hexa-2,4-dienoic acid (piperinicacid) with azinane (piperidine); only the trans, trans conformer contributes to pepper's pungency **(Srinivasan, 2007).** Piperine is the main compound leading to bioactivity of black and white pepper. His pungency has been estimated as 100000-200000 Scoville Unit **(F. Tausig, et al, 1956)** Piperine is an alkaloid and it is the carboxamide of piperic acid and piperidine. It shows low solubility in water, but ethanol and other organic solvent are suitable for solving this substance. In recent decades, Piperine came into the focus of pharmaceutical research **(J. S. Bang et al.; 2009)** .Piperine is a natural alkaloid which is used as Bio enhancer and responsible for the pungency of black pepper and long pepper, along with chavicine an isomer of piperine **(Dogra RK, et al , 2004)**. It has also been used in some forms of traditional medicine and as an insecticide **(Selvendiran K, et al, 2003).**Traxler **(1971)** several workers discovered that materials other than piperine also contributed to its pungency.Piperine was discovered in 1819 by Hans Christian Ørsted, who isolated it from the fruits of Piper nigrum, the source plant of both the black and white pepper.Piperine was isolated in good yield from ground black pepper by Oersted H Schweigers in 1821. Pepper’s pungency was found in 1821 to be due to piperine. Historically, it has been thought to cure many illnesses such as cancer, malaria and cholera **(Epstein et al., 1993).** Agrawal and Patwardhan **(1994)** reported that piperine present in black pepper fruit has antimicrobial activity. Dried, ground pepper, and its variants, is one of the most common spices in European cuisine, having been known and prized since antiquity for both its flavour and its use as a medicine. The spiciness of black pepper is due to the chemical, piperine **(Parthasarathy et al. ,2008).**The pungent dark oily resin obtained after removal of piperine from the oleoresin was named as chavicine **(Govindarajan, 1977).** Chavicine was claimed to possess a far greater bite on the tongue than crystalline piperine, but later workers demonstrated that piperine in solution was very pungent. The controversy over which compound, e.g. piperine, its *cis-cis* isomer chavicine or other possible isomers-isopiperine (*cis-trans*) and isochavicine (*trans-cis*), was more pungent lasted almost a century. However, later investigations demonstrated that piperine was the major pungent principle and chavicine was a mixture of piperine and several minor alkaloids.

Piperine is the major constituent of pepper oleoresin. Black pepper oil contributes towards the aroma, oleoresin contributes towards the overall taste and the alkaloid piperine imparts pungency. β-Carophyllene is the major sesquiterpene hydrocarbon present in pepper oil. Other important sesquiterpene hydrocarbons are β-bisabolene, δ- and γ-cadinenes, calamenene, α-copaene, α- and β-cubebenes, ar-curcumene, β- and δ-elemenes, β-farnesene, α-guaiene, α- and γ-humulenes, isocaryophyllene, γ-muurolene, α-santalene,α- and β-selinenes, ledene, sesquisabinene and zingiberene. Piperine is shown to possess bioavailability-enhancing activity with various structurally and therapeutically diverse drugs. The aroma and pungent constituents of pepper offer great future in pharmacology and industrial application. **(Parthasarathy, et al, 2008).**

1.5.1. Structure of piperine

Fig 1.1 below showed the acceptable structure proposed for piperine (Wood et al. 1988)

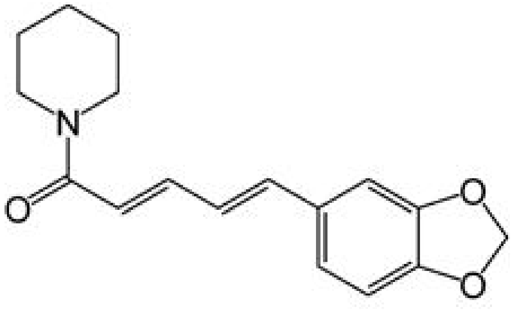


Figure 1.1 Piperine structure

Chemical name:-

Piperidine,1-[(2E,4E)-5-(1,3-benzodioxo1-5-y1)-1-oxo-2,4-pentadienyl];

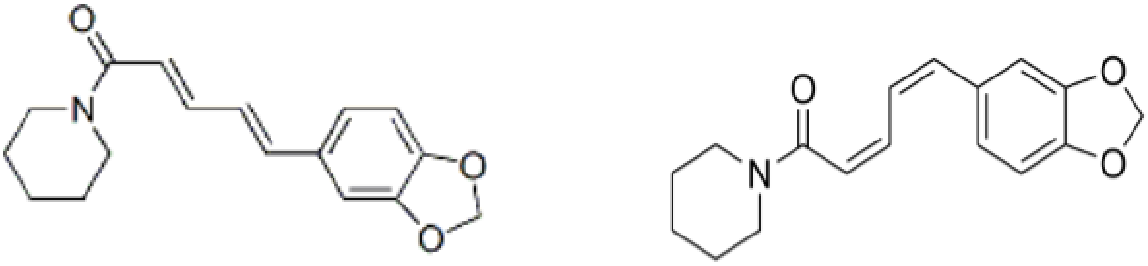
1-[5-(1,3-Benzodioxo1-5-y1)-1-oxo-2,4-pentadienyl]piperidine;

IUPAC name:
1-[5-(1, 3-benzodioxol- 5-yl)-1-oxo-2,4-pentadienyl]piperidine.
Formula: $C_{17}H_{19}NO_3$
Percent Composition by mass:
C= 71% O= 17% H=6% N=5%
Type of Bonding: Piperine has predominantly covalent bonds. However, with its large amount of hydrogen atoms it has been hydrogen bonds as well.
Molar Mass: 285.34 grams **(Majeed & Prakash, 2000)**

1.5.2 Stereochemistry

The stereochemistry of piperine at double bonds has been found to be trans-trans. The cis-cis stereoisomer of piperine is also present naturally in pepper as known as chavicine.
The isomers have different configuration at the double bonds as follows.
Piperine - Trans - Trans

Isopiperine - Cis-Trans
Iso chavicine - Trans-Cis
Chavicine — Cis-Cis.
(Mark index, 1976).



(a) Piperine (b) chavicine

Figure 1.2 Piperene & Chavicine

1.5.3 Extraction of piperine

H. Staudinger and H. Schnieider **(1923)** described a process for the extraction of piperine from Piper nigrum fruits, wherein the alkaloid was extracted using ethanol and the extract was treated With aqueous sodium hydroxide to remove all the resinous matter. Other solvents used for extraction are petroleum ether and dichloromethane. The extraction of piperine into these organic solvents is not selective as other compounds like gums, polysaccharides and resins are extracted reducing the purity of piperine. The post extraction processing to purify piperine is cumbersome and uneconomical. A liquid pepper composition containing pepper in a concentrated form has been prepared by extracting the pepper fruits with acetone and subsequent treatment with anhydrous lactic acid.

Piperine was obtained by Ikan R **(1991)** in a 5-7% yield and 98% purity, determined by GC-MS. The melting point (128-129 ºC). The pungency of black pepper has been the subject of chemical investigations since the early 19th century. In 1819, Oersted isolated piperine; the most abundant alkaloid in pepper, as a yellow crystalline substance and its structure was later identified as the trans form of piperoyl piperidine **(Narayanan, 2000).** Raman and Gaikar **(2002)** studied the extraction of piperine from powdered black pepper. The interest in piperine resides in its uses in medicine as a bioavailability enhancer in the production of certain drugs. Some groups of piperine may also be applied to use in insecticides. The conventional method for extracting piperine is via solvent extraction using aliphatic and chlorinated hydrocarbons. The extraction times can vary within 16 to 24 hours and the process is rather inefficient in its selectivity. This experiment used the P. nigrum species of pepper fruit. The acetone extracted of pepper showed the presence of 18 components, accounting for 75.59% of the total quantity. Piperine (33.53%), piperolein B (13.73%), piperamide (3.43%) and guineensine (3.23%) were the major components **(Singh et al., 2004).** Zachariah *et al***. (2005)** evaluated major black pepper cultivars for oil, oleoresin and piperine.

Kanaki , et al,(2008) was developed a methods for the isolation of piperine from the fruit of piper nigum**.** It was isolated using column chromatography. Characterization of compound was done by spectroscopic technique.

Zainab, (2010) was extracted piperine from dry seeds of plant *Piper nigrum* L. with ethanol by using soxhlet extraction then isolation and purification by re-crystallization, the structure of Piperine was confirmed by the IR spectroscopy. P.B. Shamkuwar, S.R. Shahi, S.T Jadhav **(2012)** were obtained piperine form black pepper by ethanol extraction. Piperine was extracted from seeds of Piperaceae family plants, black pepper (3-9%) and long pepper (3-5%) **(A. Kumar, et al,2012)** P.N. Shingate, P.P. Dongre and D.M. Kannur **(2013)** were extracted piperine from black pepper by using three different solvents ethanol, dichloromethane, glacial acetic acid which gave piperine yield 3.2%, 5% and 4.6% respectively.The isolated piperine was characterized by carrying out NMR, UV and mass spectroscopy analyses **(Adosraku et al., 2013).**

Gopalakrishnan et al. **(1993)** studied four genotypes of pepper using GC-MS. The oil of these cultivars possessed ᾱ-pinene in the range of 5.07–6.18%, ß-pinene 9.16–11.68%, sabinene 8.5–17.16%, limonene 21.06–22.71% and ß-caryophyllene 21.52–27.70%. Zachariah **(1995)** studied 42 black pepper accessions and reported 3.8–16.6% pinene, 2.2–33% sabinene, 1.6–31.8% myrcene, 3.6–21.2% limonene, 0.2–1.8% linalool and 11.8–41.8% ß-caryophyllene. Murthy et al **(1999)** reported that pepper powder with an average particle size of 0.7 mm is essential to release the maximum concentration of monoterpenes and sesquiterpenes.White pepper as identified by Jagella and Grosch **(1999)** are limonene, linalool, ᾱ-pinene, 1,8-cineole, piperonal, butyric acid, 3-methyl butyric acid, methyl propanal and 2- and 3-methyl butanal.

Orav *et al*. **(2004)** determined the essential oil composition of black, green and white pepper using GC/mass spectrometry. Most abundant compounds in pepper oils were (E)- ß-caryophyllene (1.4–70.4%), limonene (2.9–38.4%), ß -pinene (0.7–25.6%), Δ-3-carene (1.7–19.0%), sabinene (0–12.2%), ᾱ -pinene (0.3–10.4%), eugenol (0.1–41.0%) terpinene-4-ol (0–13.2%) hedycaryol (0–9.1%), ß -eudesmol (0–9.7%) and caryophyllene oxide (0.1–7.2%). Green pepper oil (dried by sublimation method) had a higher content of monoterpenes (84.2%) than air-dried green pepper corns (26.8%). Aditi G, Monika G, Sudhakar G **(2013)** were extracted the oil of black pepper by petroleum ether extract was analyzed by GC-MS spectroscopy. Gas chromatography–mss spectrometry technique was used to analyze 40 different components constituting approximately 97.7 % of the volatile oil. Among them b-caryophyllene (16.0 %), sabinene (12.6 %), limonene (11.9 %) and torreyol (9.3 %) were the major components with many minor components. Both ethanol and n-hexane oleoresins comprise of 26 components having piperine, as the major component. **(Sunita Singh, et al, 2013)**.

TLC of the piperine crystals which were recrystallised using ethanol showed Rf value equals to 0.23 that is similar to Rf value of piperine observed in literature was 0.25 **(Madhavi, B.B et al., 2006).**

Piperine was successfully extracted and purified from black pepper. It was identified by running TLC. Rf was found to be 0.24**. (B. B, Madhavi, A. R Nath, 2009)**

A UV detector was employed to measure the absorbance up to 343nm (**Wood, 1988).** K. U. Sankar **(1989)** Piperine was determined by a spectrophotometric method, measuring absorption at 345 nm. UV spectrum, showing absorption at 342nm **(Berger, S., 2009)** .

The IR(neat) spectra showed the presence of absorption bands at 3421 cm^{-1} (intermolecular H-bonding), 3000cm^{-1} (aromatic C-H stretch), 1633 and1610 cm^{-1} (symmetric and asymmetric stretching of c=c of dienes), 1610,1585,1492 cm^{-1} (aromatic stretching of c=c of phenyl ring), 1633 cm^{-1} (stretching of-CO-N group), 2941 and 2859 cm^{-1} (CH_2 asymmetric and symmetric stretching), 1448 cm^{-1} (CH_2 bending), 1253 and 1193 cm^{-1} (asymmetric stretching of =C-O-C), 929 cm^{-1} (C-O stretching), 1134 cm^{-1} (in plane bending of phenyl CH), 997 cm^{-1} (CH bending for trans – CH=CH-), 848,830 and 804 cm^{-1}(out of plane C-H bending). It resembles to the IR spectra of piperine given in literature **(Berger, S., 2009).**

1.5.4 Properties

Piperine is a yellow crystalline substance having a melting point of 129°C.

Piperine, $C_{17}H_{19}NO_3$, was shown to be a weak base which, on hydrolysis with aqueous alkali or nitric acid, yielded a volatile base $C_5H_{11}N$, later identified as piperidine. The acidic product of hydrolysis, piperine acid ($C_{12}H_{19}O_4$, m.p. 216–217°C), was shown to be 5-(3,4-methylene dioxy phenyl)-2,4, pentadienoic acid. **(Narayanan, 2000).** Piperine yields salts only with strong acids. The solution of piperine in alcohol has a pepper-like taste **(Balsamo A, et al, 1977).** Tasteless at first, but burning after taste, Density 1.193 gm cm^{-3}. Solubility; Insoluble in water, soluble in benzene and acetic acid.

1.5.5 Pharmaceutical uses

Lee et al. **(1984)** found that piperine reduces inflammation in carragenin induced tests at an oral dose 50mg/kg body weight. Piperine also possesses strong potentiating effect on hexobarbital induced hypnosis in mice. A compound of great interest extracted from pepper is 1-(3-benzodioxol-5yl)-1-oxo-2-propenyl-piperidide, known as antiepilepsirine, which was shown to have strong antiepileptic properties. This is used in Chinese hospitals for the treatment of epilepsy **(Ebenhoech and Spadaro 1992)**. Piperine increases the bioavailability of medicaments including ampicillin and synthetic drugs as well as uptake of amino acids from food **(Johri et al.1992).**

Kaul and Kapil **(1993)** found that piperine reduces in vitro and in vivo lipid peroxidation and prevents depletion of GSH (Gastricsulfhydryls) and total thiols. This is a very significant property, as lipid peroxidation causes free radical production that causes tissue damage. piperine has been thought to cure many illnesses such as cancer, malaria and cholera **(Epstein et al., 1993).**

The anti-inflammatory effect was substantiated by Kapoor et al. **(1993).** It has been well documented that Black Pepper oil can help with pain relief, rheumatism, chills, flu, colds, muscular aches, fevers, increased circulation, saliva flow, appetite stimulation and other digestive benefits **(Pruthi, 1993).**

Agrawal and Patwardhan **(1994)** reported that piperine present in black pepper fruit has antimicrobial activity. . Jagella and Grosch **(1999)** reported that there are some compounds in black pepper fruit which are flavored and off flavored and inhibit the growth of Sclerotium rolfsi and Fusarium solani completely. Vijayan and Thampuran **(2000)** give a detailed account of the pharmacological and toxicological properties of pepper and piperine.

Piperine and its homologues get absorbed through skin, and hence are capable of acting on the subcutaneous tissues as well as on nerves and blood vessels. The effect of pepper on the nervous system and on sexual organs (priapism) indicates anticonvulsive and vasodilatoral properties. Pepper also has an effect on lactation by increasing milk production. Pepper oil warms the skin and brings blood to the surface, stimulating circulation. **(K. V. Peter, 2001).** Rajinder et al. **(2002)** studied the mode of action of piperine. Through human liver microsomal studies, they established that piperine, a major constituent of black pepper, inhibits human P-glycoprotein.

A considerable body of research has been conducted into assessing the effects of black pepper on human health. Piperine (1-piperoyl piperidine) in black pepper is shown to possess bioavailability enhancing activity with various structurally and therapeutically diverse drugs. This property of piperine may be attributed to increased absorption, which may be due to alteration in membrane lipid dynamics and a change in the conformation of enzymes in the intestine **(Khajuria et al., 2002).** Its antifungal and antimicrobial capabilities are also well documented **(Singh et al., 2004).** Piperine is used to loss weight by stimulating the fat cells breakdown, has antioxidant and antibacterial effects, useful in digestion process **(Srinivasan,**

K., 2007). Kuomoro *et al.* **(2009)** piperine helps to increase the absorption of vitamin B, β-carotene and selenium occasioning the rise of the generation of energy in the cell and consequently, it has been useful in the treatment of defective intestinal lining. Other application for this compound is as repellent for some insects; for instance, it has shown to be an effective insecticide against flies.

1.5.6 Sources of piperine

Piperine is an alkaloid found naturally in plants belonging to the Piperaceae family, such as Piper nigrum L, commonly known as black pepper, a household spice and Piper longum L, commonly known as long pepper. Piperine is the major pungent substance in these plants and is isolated from the fruit of the black pepper and long pepper plants. Piperine comprises 1 to 99% of these plants**.(Pei YQ.,1983)**

1.5.7 Characterization

1.5.7.1 Melting Point

Melting point was reported to be 129^0c **by Mark index (1976).**

1.5.7.2 Infra Red

Expected Absorption Bands for Piperine in KBr by **Mark index (1976).**

Table 1.2 Absorption Bands for Piperine.

Type of Phenomenon	Wave Number (cm^{-1})
C-H stretching	2941
C=O stretching	1633
C-C aromatic	800-600
C-H trans double bond out of plane deformation	997/929

1.5.7.3 TLC

B. B, Madhavi, A. R Nath**,(2009)** were identified piperine by TLC. The standard Rf- value of Piperine from the literature was 0.25.

1.5.7.4 UV

UV maxima of isolated Piperine was taken in 30 parts of ethanol and UV maxima obtained at 343nm presented in figure 1.3 **(Deepthi Swapna P R,et al,2012)**

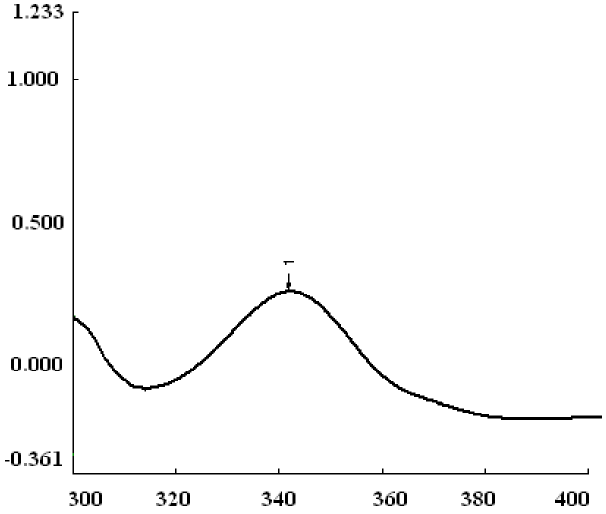


Figure 1.3 UV maxima of isolated Piperine

1.5.7.5 ^{1}HNMR

The ^{1}H NMR spectrum in CDCl3 was recorded at 400 MHz spectrophotometer and TMS (Tetramethylsilane) used as an internal standard. The 1H NMR data of piperine in CDCl3 in table 1.3

Table 1.3 ^{1}H NMR data of piperine.

Type of proton	Number of proton (1H)	chemical shift δ in ppm
Cycloaliphatic	10	1.54 ~ 1.62
Alkenic	4	5.7 ~ 6.7
Aromatic	3	6.65 ~ 6.93
Methylene attached To oxygen atoms	2	3.49 ~ 3.59

Cycloaliphatic proton (10H) The methylene hydrogens (–CH2–) of cycloaliphatic ring containing nitrogen atom show chemical shift at δ 1.54 ~ δ 1.62 where the reference data to those of are found δ 1.2 ~ δ 1.4 **(Pavia et al., 2006).** The methylene proton (–CH2–) shows resonance between methyl and methine hydrogen. Actually this shift is intermediate. The chemical shift of these protons is variable, depending not only on the chemical environment, but also on concentration, temperature and solvent.

Alkenic proton (4H)

The alkenic protons show experimental chemical shift at δ 5.7 ~ δ 6.7 which is shifted to a little bit downfield in comparison to that of standard values at δ 4.6 ~ δ 5.9. It may be happened for our sample due to the effect of carbonyl (C=O) group attached to the alkenic carbon.

Aromatic Proton (3H)

Phenyl rings are so common organic group that it is important to reveal a few facts about nmr absorptions in compounds that contain them. The ring protons of a benzenoid system have resonance, near 7.3 ppm. This value of chemical shift is diagnostic.

Methylene dioxy proton (-O-CH2-O-)

The chemical shift at δ 3.49 ~ δ 3.59 ppm was observed for the protons of methylne dioxy (-O-CH2-O-) group. The hydrogens on the carbon attached to the oxygen are deshielded due to electronegativity of the oxygen. It is cited from the text that in ethers (R-O-CH-), the hydrogen on the carbon next to oxygen are deshielded for the above mentioned reason and they appear in the range δ 3.2 ~ δ 3.8 ppm. The absorption of the methylene hydrogens next to the oxygen are seen at δ 3.4 ppm (Pavia et al., 2006).
In methylene dioxy group, there are two hydrogens on the carbon attached to the two electronegative oxygen atoms. Therefore, the greater the electronegativity, the more it deshields protons and hence the greater is the chemical shifts of those protons. **(K. C. Saha,et al, 2013**)

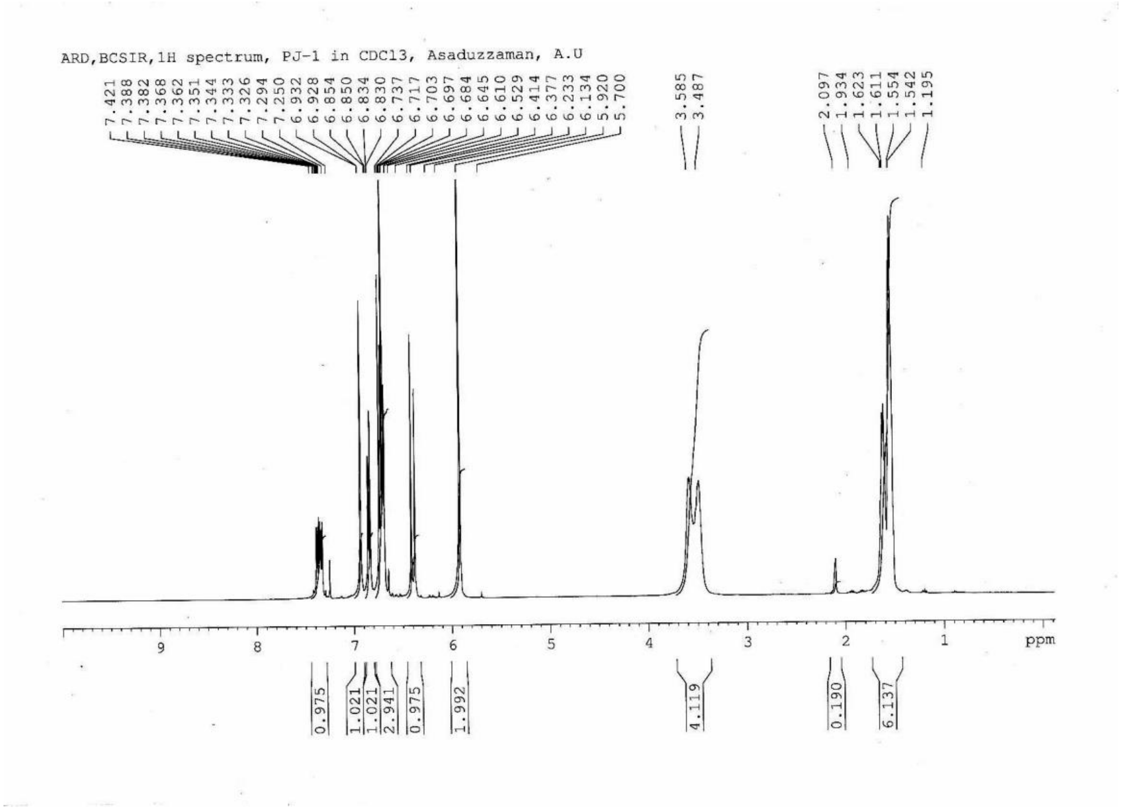


Figure 1.4 ^{1}H NMR spectrum of piperine

1.6 Objectives of the study

The aims of this study were:

I. To extract the essential oil from black pepper and to identify its chemical constituent using Gc-ms technique.

II. Isolation and characterization of piperine from black pepper oil.

2. Materials and Methods

2.1 Collection of Samples

Five samples were collected from local markets, cleaned from stones and other undesirable matters, then ground in an electrically operated grinder to fine particle size.

2.2 Chemicals

-Ethanol (assay 95%-wt.per/ml 0.789 g/cm^3- CDH)

-10% Alcoholic potassium hydroxide

-Acetone (assay 99%- wt.per/ml o.7905g/l –CDH)

- Hexane (assay 99%-wt.per/ml 0.779 g/cm^3-Loba chemie)

-Diethyl Ether (assay 98%-wt.per/ml 0.715 g/l –Alpha chemie)

-HCl (assay35.5%-wt. per /ml 1.18 g/l-Loba chemie)

-KBr (assay 99%- CDH)

2.3 Instruments

-Analytical balance

-Rotatory evaporator (Buchi R - 114 Water bath B – 480)

- GC-MS instrument (Shimadzu 2010)

- Melting point apparatus (Stuart)

- UV-VIS Spectrophotometer (Shimadzu UV-1800)

- Infra Red Spectrophotometer (Thermo nicolet- IR 300)

2.4 Methods

2.4.1 Extraction of oil procedure

Black pepper seeds were grained to a fine powder.25 g were then accurately weighed and transferred to a thimble in a Soxhlet. 100 ml of ethanol were used as extracting solvent for 90 minutes. The obtained extract was cooled, filtered, and concentrated by rotary evaporator at 60°C.The residue after evaporation was dissolved in 25 ml of 10% alcoholic potassium hydroxide. The solution was in an ice bath.30 ml of distilled water was added dropwise to precipitate the piperine. The piperine was colleted, dried and weighed. To re-crystallization the piperine was placed in a test tube and dissolved it in similarly 5ml of hot 3:2 acetone: hexane solution. It was allowed to sit for 15 min at room temperature and then 30 min in an ice bath. The crystals were filtered using a Hirsch funnel and washed with 4ml of cold ether.

2.4.2 GC-MS Analysis of oil

Volatile oil obtained from sample No. 5 was used for GC-MS analysis. Injections were performed by auto injector , A column (fused silica, 5%Phenyl), 30m × 0.25mm was used. Column oven temperature 70ºC, injection temperature 300ºC, injection mode splitless.The carrier gas used was helium with a flow rate of 8ml/min. While the detector was a mass spectrometer (MS) with EI ionization at 70eV in full scan mode.

2.4.3 UV-VIS analysis of piperine

Samples No. 1, 2, 3 and 4 used for piperine analysis.100mg of the isolated Piperine were placed in 100ml volumetric flask. The volume was made up to the mark with ethanolic HCl (1:1) (100µg/ml). From this solution 5ml is taken to 100ml volumetric flask and made up the volume (50µg/ml). The sample is scanned in UV Visible spectrophotometer.

2.4.4 Infra Red analysis of piperine

15mg of isolated Piperine and 300 mg of KBr (Potassium Bromide) were taken in a mortar and mixed. The mixture was placed into an evacuable die on a hydraulic laboratory press and compressed under 10-ton pressure to form a transparent pellet. KBr pellet was placed in a pellet holder and put it into the sample beam of an IR spectrophotometer. Spectrum of the pellet is taken from 4000 cm^{-1} to 400 cm^{-1}

3. Results and Discussion

3.1 Result of GC-MS analysis

The analysis of the extracted black pepper oil using GC-MS technique showed the presence of 42 components. More than 80 components have been reported by **Gopalakrishnan et al. (1993).** GC-MS analysis of essential oil showed the presence of 42 volatile compounds. Some monoterpene hydrocarbons and oxygenated compounds were present in GC-MS table below were : Alpha-pinene, 3-Carene, D-Limonene, L-alpha-Terpineol, and Myrtenol. Also some sesquiterpene hydrocarbons and oxygenated compounds were present in the oil like Alpha-Cubebene, Caryophyllene and Caryophyllene oxide. The analysis of oil showed highest percentage for Oleic acid 23.07%, n-Hexadecanoic acid 16.62% and Caryophyllene 13.34%, Naphthalene,decahydro-2,2dimethyl 5.81% ,Gamma-Tocopherol 5.54% and Caryophyllene oxide 4.7%. Gopalakrishnan et al. **(1993)** studied four genotypes of pepper using GC-MS. The oil of these cultivars possessed ᾱ-pinene in the range of 5.07–6.18%, limonene 21.06–22.71% and ß-caryophyllene 21.52–27.70%. Zachariah **(1995)** studied 42 black pepper accessions and reported 3.8–16.6% pinene, 3.6–21.2% limonene, and 11.8–41.8% ß-caryophyllene. Orav *et al.* **(2004)** determined the essential oil composition of black, green and white pepper using GC/mass spectrometry. Most abundant compounds in pepper oils were (E)-ß-caryophyllene (1.4–70.4%), limonene (2.9–38.4%), ,3-carene (1.7–19.0%), ᾱ -pinene (0.3–10.4%), and caryophyllene oxide (0.1–7.2%). By comparison this results by obtained results the percentage of some compounds as following ;Alpha-pinene 0.03% , Limonene 0.33%, ß-caryophyllene 13.34%, 3-carene 0.29% and caryophyllene oxide 4.72%.

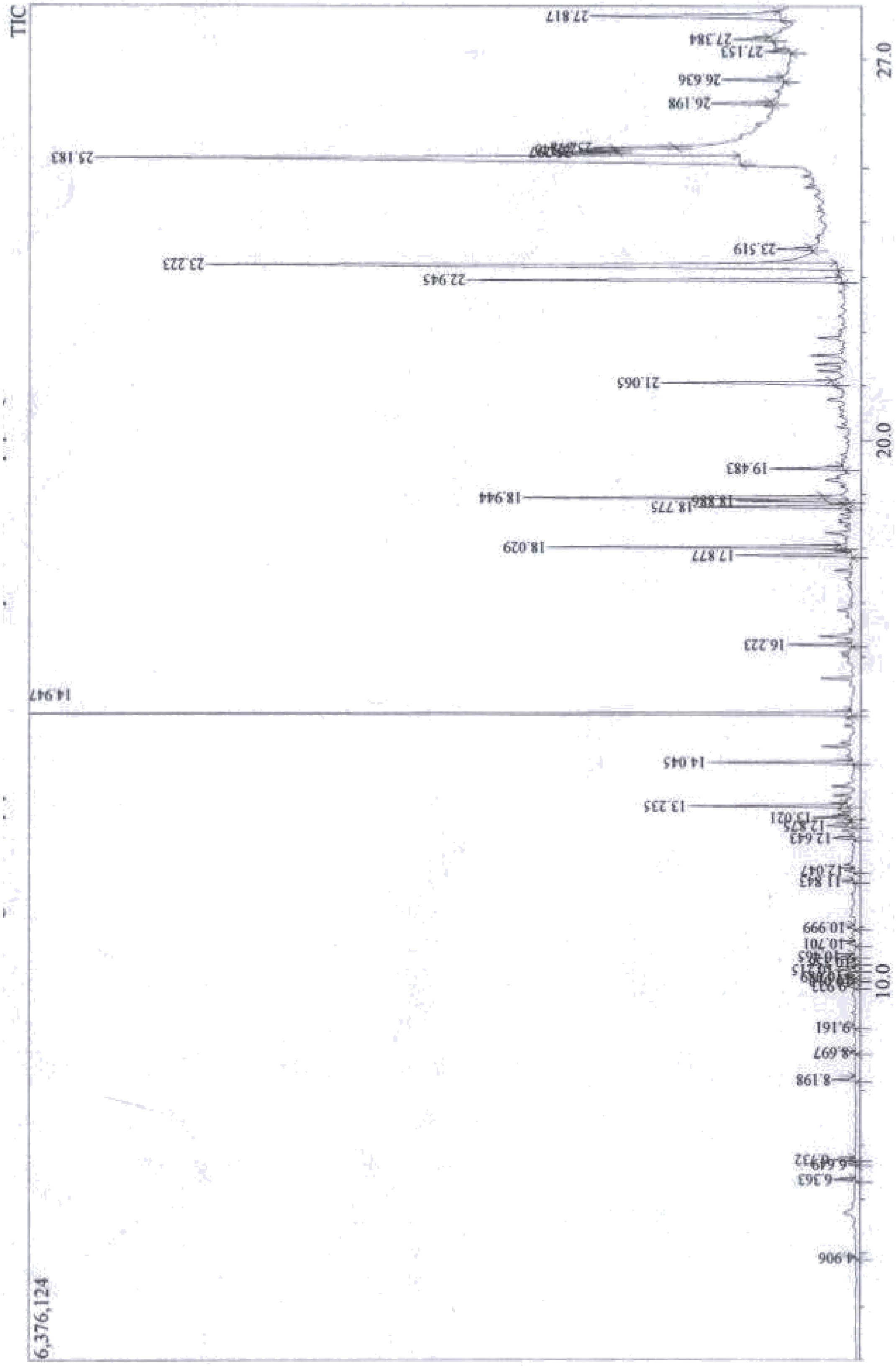


Figure 3.1 The spectrum GC-MS of the essential of black pepper oil.

Table 3.1 GC-MS data for essential oil.

Peak No.	**Name of compound**	**R.Time**	**Percentage ٪**
1	Alpha-pinene	4.906	0.03
2	3-Carene	6.363	0.29
3	Benzene,1-methyl-3-(1-methyethyl)	6.649	0.12
4	D-Limonene	6.732	0.33
5	1,6-Octadien-3-ol,3,7-dimethyl	8.198	0.39
6	1,3-Cyclohexadiene-1-methanol,4-(1-methylethyl)	8.697	0.09
7	1-piperidinecarboxaldehyde	9.161	0.06
8	Ethanol,2-(3,3-dimethylcyclohexadiene	9.923	0.16
9	Phenol,2-methyl-5-(1-methylethyl)	10.010	0.03
10	Thymol	10.089	0.15
11	L-alpha-Terpineol	10.215	0.35
12	(-)-Myrtenol	10.356	0.05
13	Bicyclo[3.1.0]hexan-3-ol,4-methylene-1-(methylethyl)-,acetate	10.465	0.22
14	Linayl acetate	10.701	0.18
15	Naphth[2,3-b]oxirene,decahydro	10.999	0.16
16	Cis-p-mentha-2,8-dine-1-ol	11.843	0.16
17	4-Oxatricyclo[5.2.1.0(2,6)]decan-8-ol	12.047	0.21
18	3-Heptadcen-5-yne,(z)	12.643	0.34
19	Bicyclo(3.1.1)heptan-2,3-diol,2,6,6-trimethyl	12.875	0.46
20	Cyclobutane,3-hexyl-1,1,2-trimethyl	13.021	0.55
21	Cyclohexane,4-ethenyl-4-methyl-3-(1methylethyl)-,(3R-trans)	13.235	2.58
22	Alpha-Cubebene	14.045	2.30
23	Caryophyllene	14.947	13.34
24	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1methylethyl)-,[4aR-(4a.alpha.,7alpha.,8a.beta)]	16.223	1.11
25	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-ethylene	17.877	2.12
26	Caryophyllene oxide	18.029	4.72
27	Gamma-Himachalene	18.775	2.53
28	Acetic acid,4a-methyl-octahydro-1-oxacyclopropa[d]naphthlen-7-yl ester	18.886	2.12

29	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol,4,4-dimethyl	18.944	4.64
30	Androstan-17-one,3-ethyl-3-hydroxy-,(5.alpha.)	19.483	1.22
31	Aromadendrene oxide-(2)	21.065	3.37
32	Naphthalene,decahydro-2,2dimethyl	22.945	5.81
33	n-Hexadecanoic acid	23.223	16.62
34	Hexadecanoic acid, ethyl ester	23.519	0.47
35	Oleic acid	25.183	23.07
36	Pyrimidin-4-ol,6-methyl-2-(1piperiryl)	25.297	0.44
37	Methyl 9-cis,11-trans-octadecadienoate	25.340	0.50
38	Ethyl Oleate	25.378	0.93
39	Palmitoyl chloride	26.198	0.86
40	1-(+)-Ascorbic acid 2,6-dihexadecanoate	26.636	0.94
41	N-(1cyclohexen-1-yl)piperidine	13.235	0.43
42	Gamma-Tocopherol	13.235	5.54
			100.00

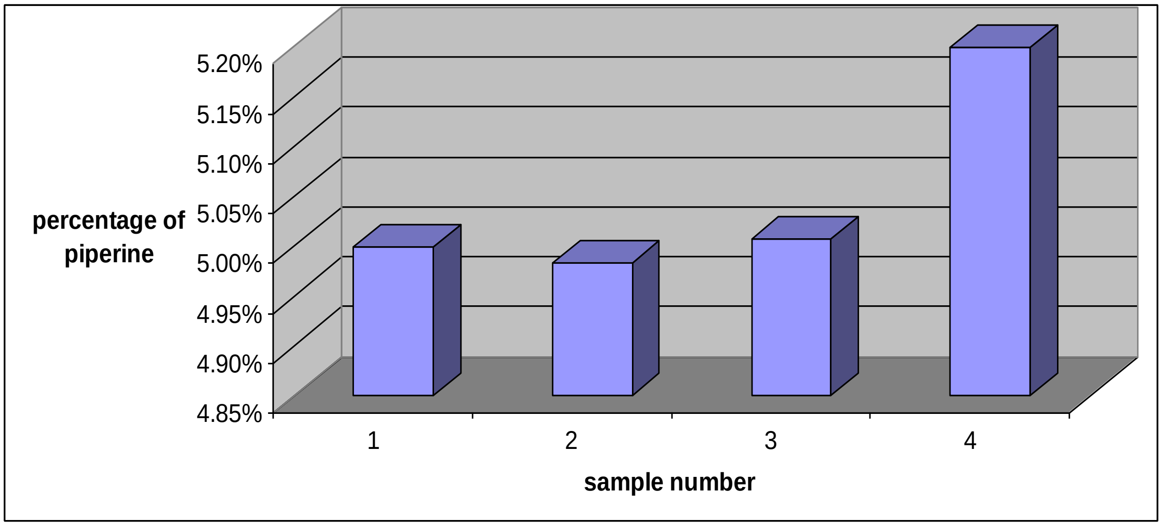
.

3.2 Piperine

The isolation of piperine from black pepper fruits using 95% of ethanol which is found to be a suitable solvent for this procedure and the mean percentage yield obtained was found to be 5.048% w/w.piperine was obtained by Ikan R **(1991)** in 5-7% percentage so the piperine obtained in the range with reference.

Table 3.2 weight and percentage of piperine.

Sample No.	Weight (g)	Percentage
1	1.250	5.00 %
2	1.246	4.984 %
3	1.252	5.008 %
4	1.300	5.200 %



(g)

Figure 3.2 The percentage of piperine

The melting point range of 131^{o}C-134^{o}C . After re-crystallization the melting point decrease and became 130^{o}C.

Table 3.3 melting point of piperine.

Samples	**Melting point**	**Reference**
Piperine	131-134 ^{o}C	128-129 ^{o}C
Piperine after purification	130 ^{o}C	

3.2.1 U V-Vis analysis of piperine

The spectrophotometer analysis of piperine samples shows different absorption peaks (fig 3.2) and different wave lengths (table 3.3). Deepthi Swapna P R, et al (**2012**) reported that UV maxima obtained at 343nm.

A UV maximum of isolated Piperine was taken in ethanol and UV maxima obtained at 344.5 nm and this value agreed with the reference values

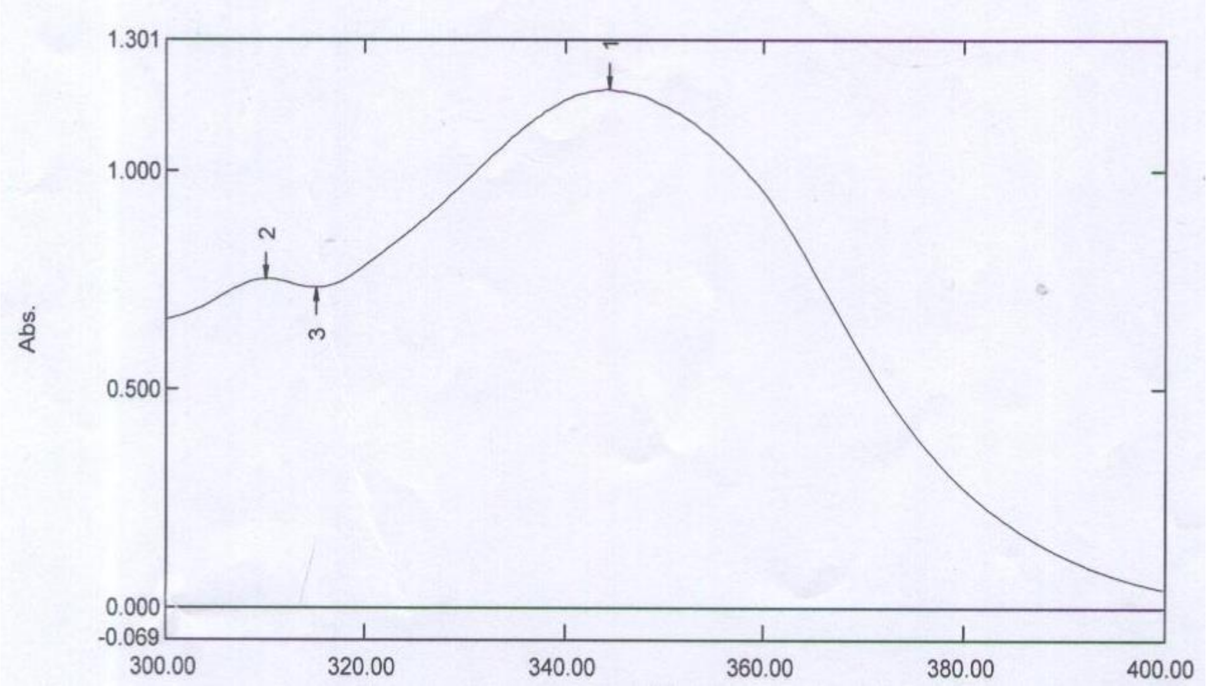


Figure 3.3 The uv-vis spectrum of piperine.

3.2.2 IR analysis of piperine

Infra red spectrometric analysis showed eleven peaks (fig 3.4).The peaks show the presence of aromatic C–H stretching showed absorption 2927.71 cm $^{-1}$ and around 2855.26cm^{-1} for aliphatic C–H stretching agree with the reference data in table(3.5).

Absorption band in the region around 1633 cm^{-1} is due to the C=O stretching and the presence of this band indicates that the ketonic group (C=O) may be present in the compound. The compound showed absorption at 1630.92 cm^{-1} which indicating the presence of ketonic (C=O) group.

An ether group, aromatic methylenedioxy is distinctly clear from the strong bands at 1250 and 1190 for asymmetrical stretching =C-O-C and 1030 for symmetrical stretching =C-O-C. in the experimental data found at 1248.57 and 1124.53cm^{-1} asymmetric stretching of the ether group and 1027.84 cm^{-1} for symmetrical stretching of the same group.

The symmetric and asymmetric stretching absorption bands at 1630.92 cm^{-1} and, 1587.26 cm^{-1} respectively were observed for conjugated diene and the reference value were1635 cm-1 and1608 cm^{-1} which indicate the presence of conjugated diene in the compound.

.

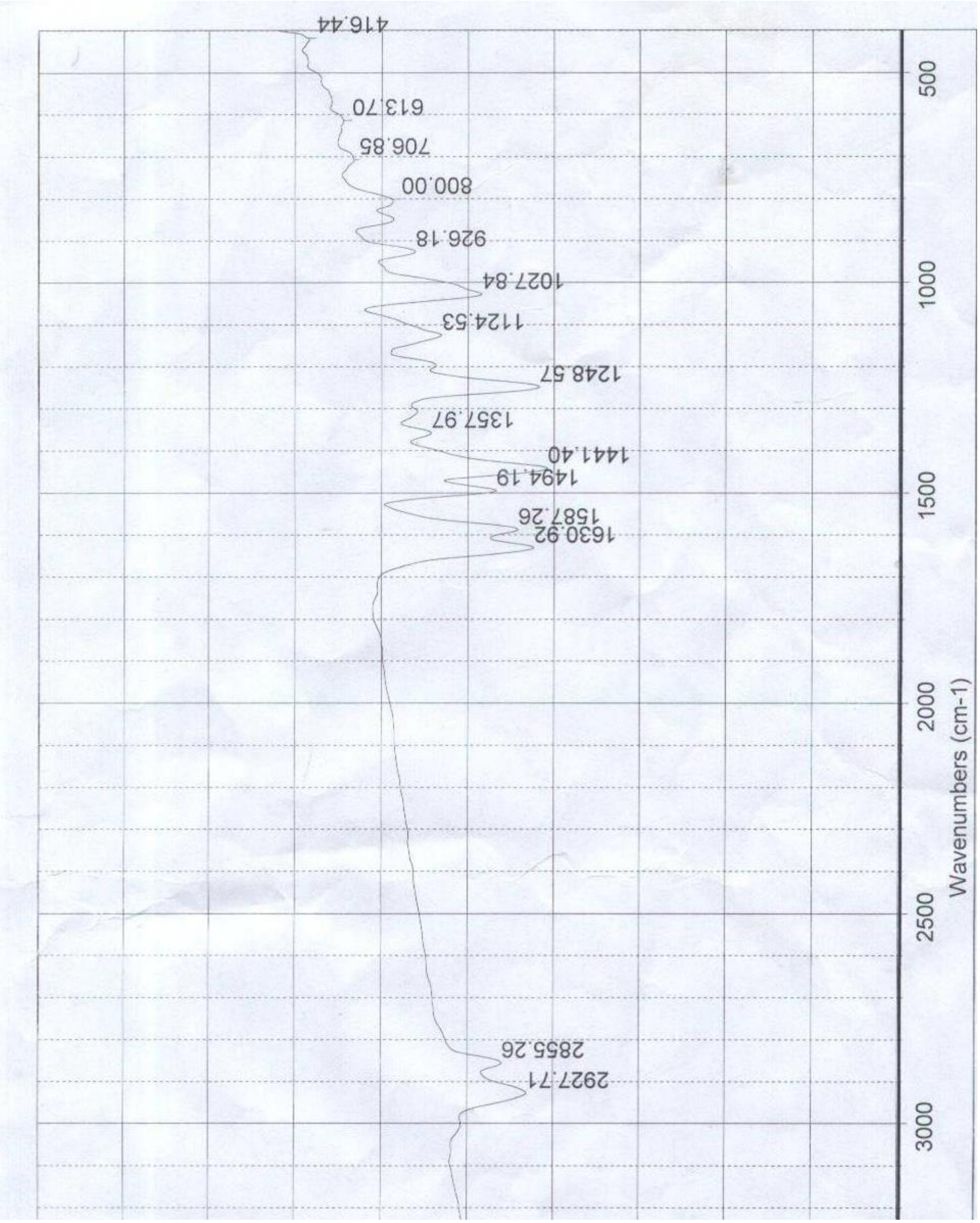


Figure 3.4 The spectrum of the infra red of Piperine

Table 3.4 infra red data of piperine

Peak No	**Type of phenomena**	**Wave Number (cm^{-1})**	**Reference Pungor, E. A 1995**
1	Aromatic C-H stretching	2927.71	3000
2	Aliphatic C-H stretching	2855.26	2840
3	C=O stretching	1630.92	1633
4	Symmetric and asymetric stretching of C=C (diene)	1630.92 1587.26	1635 1608
5	Aromatic stretching of C=C (benzene ring)	1494.19	1495
6	CH2 bending	1441.40	1450
7	C-N stretching	1357.97	1364
8	asymmetrical stretching =C-O-C (Aromatic methylenedioxy compounds)	1248.57 1124.53	1250 1190
9	symmetrical stretching =C-O-C (Aromatic methylenedioxy compounds)	1027.84	1030
10	C-O stretching (most characteristic)	926.18	930
11	Out-of-plane C-H bending 1,2,4-trisubstituted phenyl (two adjacent hydrogen atoms)	800 706.85 613.70	850; 830; 805

Conclusion

Analysis using Gas Chromatography-Mass Spectrometer was found to be the best method to identify even the minor components of particular oil along with major components.

Alkaloid Piperine was successfully isolated from extracted oil using 95% ethanol. Isolated Piperine was identified by various methods such as UV-Visible spectroscopy. The purity of isolated piperine was confirmed by FTIR.

References

- Aditi G. , Monika G., Sudhakar G.(2013) Isolation of Piperine and Few Sesquiterpenes from the Cold Petroleum Ether Extract of Piper nigrum (Black Pepper) and its Antibacterial Activity. *International Journal of Pharmacognosy and Phytochemical Research*, **5**, 101-105
- Adosraku RK, Kyekyeku JO and Attah IY (2013) Characterization and HPLC quantification of piperine isolated from Piper guineense, *International Journal. of pharmacy and pharmaceutical Science*, **5**,252-256.
- Agrawal, R. and Patwardhan, M.V. (1994) Formation of piperine from in vitro culture of Piper nigrum L, *Indian Journal of Plant Physiology*, vol. **37(3),** p-171~173.
- B. B, Madhavi, A. R Nath, (2009) Extraction, Identification, Formulation and Evaluation of piperine, *International Journal of Pharm*acology vol**1**.
- Berke T, Black L, Talekar Ns, Wang J, Gniffke P, Green SK, Wang TC and Morris R. (2005). *Suggested Cultural Practices for Chili Pepper*. AVRDC 05-620.
- Berger S, Sicker D. (2009), *Classics in Spectroscopy*, Wiley-vch Verlag Gmbh and co. KczA, Weinhein, 53-56.
- Borges, P. and Pino, J. (1993) Preparation of black pepper oleoresin by alcohol extraction. *Nahrung* **37(2**), 127–130.
- Chun, H., Shin, D.H., Hong, B.S., Cho, W.D., Cho, H.Y. and Yang, H.C. (2002) Biochemical properties of polysaccharides from black pepper. *Biological and Pharmaceutical Bulletin* **25(9),** 1203–1208.

- Deepthi Swapna P R, Junise V, Shibin P, Senthila S, Rajesh R S,(2012),Isolation, identification and antimycobacterial evaluation of piperine from Piper longum, *Scholar Research Library* **4(3)** :863-868
- Dogra RK, Khanna S, Shanker R.(2004), Immunotoxicological effects of piperine in mice, *Toxicology* **196(3):**229-236.
- Ebenhoech, A. and Spadaro, O. (1992) Antiepilepsirine: a new Chinese anticonvulsant herb drug. *J. Eco. Tax. Bot*., **16**, 99–102.
- Epstein WW ,Netz, D. F.;Seidel, J. L. *J*,(1993) Isolation of piperine from black pepper, *Journal of Chemical Education,* **70**: 598-599.
- F. Tausig, JI Suzuki and RE Morse, (1956)*, Food Technology*, Volume **10**: pp. 151–154.
- Gopalan, A. et al. (1990), *Indian Perfumer*, **34**: 152–156
- Gopalakrishnan, M., Menon, N., Padmakumari, K.P., Jayalakshmi, A. and Narayanan, C.S. (1993). G C analysis and odor profiles of four new Indian genotypes of Piper nigrum L. *Journal of Essential Oil Research*, **5**: 247–253.
- Govindarajan, V.S. (1977) Pepper chemistry, technology and quality evaluation. *Critical Reviews in Food Science and Nutrition* **9**, 115–225.
- H. Staudinger and H. Schnieider (1923) *Wealth of India, Raw Materials*, Vol. **3**, pg. 111).
- Ikan, R. (1991), *In natural products. A laboratory guide*. Academic Press. New York, USA.
- J. S. Bang et al.(2009) *Arthritis Research & Therapy*, Volume **11**: pp. 19
- Jagella, T. and Grossch, W. (1999), Flavour and off-flavour compounds of black and white pepper (*Piper nigrum* L.). III. Desirable and

undesirable odorants of white pepper. *European Food of Research* and *Technology*, **209**: 27–31.

- JB Harborne. (2005) phytochemical methods. A guide to modern techniques of plant analysis, 3rd ed., Springer (India) Private Limited, Delhi, **2005**; 1-32.
- Johri, R.K., Thusa, N., Khajura, A. and Zutshi, U. (1992) Piperine mediated changes in permeability of rat intestinal epithelial cells. *Biochem. Pharmacol*, **43**, 1401–7.
- K.C. Saha, H. P. Seal and M. A. Noor, (2013),Isolation and characterization of piperine from the fruits of black pepper (Piper nigrum) , *Journal of Bangladesh* , **11(1):**11–16.
- K. U. Sankar, (1989), "Studies on the Physicochemical Characteristics of Volatile Oil from Pepper (Piper nigrum) Extracted by Supercritical Carbon Dioxide", *Journal of the Science of Food and Agriculture,* **48** pp. 483–493
- K.V. Peter, (2001), *Handbook of herbs and spices, vol* **1**, Woodhead Publishing Ltd, Cambridge England.
- K.V. Peter, (2006), *Handbook of herbs and spices, vol* **3,** Woodhead Publishing Ltd, Cambridge England.
- Kanki; M Dave; H Padh; M Rajani. (2008**)** *Journal of Medicine*, **62**, 281-283.
- Kapoor, V.K., Chawla, A.S., Kumar, M. and Kumar P. (1993) Search for anti-inflammatory agents. *Indian Drugs*, **30**, 481–93.
- Kaul, I.B. and Kapil, A. (1993) Evaluation of liver protective potential of piperine – an active principle of black pepper. *Planta Medica*, **59**, 413–17.

- Khajuria, A., Thusu, N. and Zutshi, U. (2002) Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: influence on brush border membrane fluidity, ultra structure and enzyme kinetics. *Phytomedicine* **9(3),** 224–231.
- Kumoro, A. C., Singh, H. y Hasan, M. (2009). Solubility of Piperine in Supercritical and Near Critical Carbon Dioxide. *Chinese Journal of Chemistry and Engineering*, **17(6),** 1014 – 1020.
- Lee, E.B., Shin, K.H. and Woo, W.S. (1984) Pharmacological study of piperine. *Arch. Pharm. Res.*, **7**, 127–32.
- Leug Albert Y. et al., (1996): Encyclopedia of Common Natural Ingredients (use in food drugs and cosmetic), A Wiley- Interscience Publication, Johan Wiley & Sons, Inc.
- Madhavi BB, Nath AR, Banji D, Madhu M,Ramalingam R. (2009). Extraction, Identification, Formulation and Evaluation of Piperine in Alginate beads. *International Journal. of pharmacy and pharmaceutical Science*; **1(2)** :156-161.
- Madhusoodanan,K.J. et al.(1990). *Spices India*, **3**:5-7
- Majeed and Prakash L, (2000). The Chemical Constituents of Pepper, *In Pepper News*, **25(1)**123-131.
- Mark index (1976) General information on piperine ,9th Edition.
- Mathai, C.K., Kumaran, P.M. and Chandy, K.C. (1981). Evaluation of commercially important chemical constituents in wild black pepper types. *Qual. Plant Food Hum. Nutr* **30**:199-202.
- Murthy, C.T., Meenakshi Rani and Srinivasa Rao, P.N. (1999). Optimal grinding characteristics of black pepper for essential oil yield. *Journal of Food Process Engineering*, **22**: 161–173.

- Narong Chomchalow (1996) *Spice Production in Asia* , **96**: 27-28
- Orav, A., Stulova, I., Kailas, T. and Muurisepp, M. (2004). Effect of storage on the essential oil composition of Piper nigrum L. fruits of different ripening status. *Journal of Agricultural Food Chemistry*, **52**: 2582–1586.
- P.B. Shamkuwar; SR Shahi; S.T. Jadhav. (2012) *Asian Journal of Plant Science and Research*, **2 (1)**, 48-53.
- P.N. Shingate, P.P. Dongre and D.M. Kannur (2013) new method development for extraction and isolation of piperine from black pepper. *International Journal of Pharmaceutical Sciences and Research*, **4(8)**: 3165-3170.
- Parthasarathy VA, Chempakam, B, Zachariah, TJ (2008) *Chemistry of spices*,pg 21-38
- Pavia, D.L., Lampman, G.M. and Kriz, G.S. (2006). *Introduction to spectroscopy. A guide for students of Organic Chemistry*. (New Edition), Harcourt College Publishers, India. p-122~256.
- Pei YQ(1983) *Epilepsia* ; **24**:177-182.
- Peter, K.V. (2000) Futurology of black pepper In: Ravindran, P.N. (ed.) *Black Pepper* (*Piper nigrum* L.). Harwood Academic, Amsterdam, The Netherlands, pp. 481–487
- Priya N. C, P. Saravana Kumari,(2016), identification of classes of compounds and estimation of flavanoid and phenol From Seeds of piper longum and Piper nigrum, *International Journal of Pharma and Bio Sciences* , **7(1):** (B) 593 – 601.
- Pruthi, JS (1993) '*Major spices of India*: crop management and post-harvest technology.' Indian Council of Agricultural Research.

- Pungor, E. A (1995) *Practical Guide to Instrumental Analysis*; CRC: Boca Raton, pp 74–80.
- Purseglove, J.W. Brown, E.G., Green C.L. and Robbins, S.R.J. (1981) *Spices,* Vol. **1**,Longman, London.
- Rajinder, K.B., Glaeser, H., Becquemont, L., Klotz, U., Gupta, K.S. and Fromm, F.M. (2002) Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. *The Journal of Pharmacology and Experimental Therapeutics* **302(2),** 645–65.
- Rahiman, B.A., Murthy, K.N., Nair, M.K. and Nair, N.M. (1979). Distribution, Morphology and Ecology of Piper species in Karnataka. *Indian J. Plant. Crops*. **7**:93-100.
- Raman, G., Gaikar, V.G. (2002) Microwave-assisted extraction of piperine from Piper nigrum. *Ind. Eng. Chem. Res*. **41**:2521-2528.
- Ravindran P N, Nirmal Babu K. 1994. Genetic resources of black pepper. In: Chadha K L, Rethinum P, eds., *Advances in Horticulture*. vol. **9**. Malhotra Publishing House, New Delhi. pp. 99-120.
- S. Berger, D. Sicker, (2009) Classics in Spectroscopy. Isolation and Structure elucidation of natural products 1st edition, 53.
- Scott I M, Jensen H R, Philogene B J R, Arnason J T. (2008) A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. *Phytochemistry Reviews*, **7**, 65-75.
- Selvendiran, K., Singh, J.P.V., Krishnan, K.B., Sakthisekaran, D., (2003), Cytoprotective effect of piperine against benzo(a)pyrene induced lung cancer with reference to lipid peroxidation and antioxidant system in swiss albino mice" *Fitoterapia* **74**: 109-115.

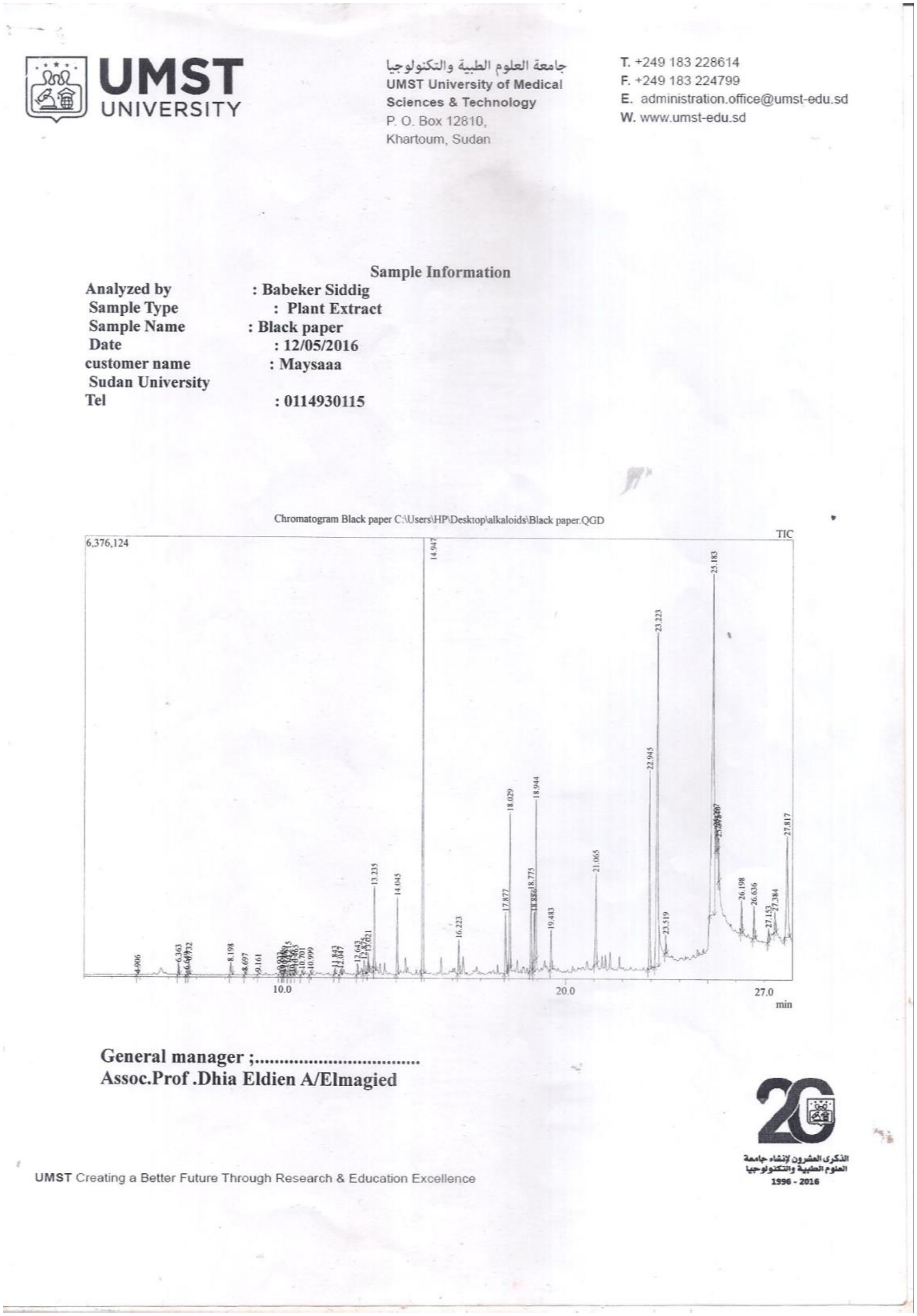
- Singh, G, Marimuthu, P, Catalan, C, de Lampasona, MP (2004) Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. *Journal of the Science of Food and Agriculture* **84**, 1878-1884.
- Souza , Stamford T.L.M., Lima E.O.,Trajano V.N., Filho J.B. , (2005), Antimicrobial effectiveness of spices, **48(4)** : 549-558.
- Srinivasan, K., (2007). Black pepper and its pungent principle - piperine, a review of diverse physiological effects. *Crit Rev Food Sci Nutr,* **47**: 735-748.
- Sunita Singh, I. P. S. Kapoor, Gurdip Singh, Carola Schuff, M. P. De Lampasona & Cesar A. N. Catalan (2013), Chemistry, Antioxidant and Antimicrobial Potentials of White Pepper (Piper nigrum L.) Essential Oil and Oleoresins, *ResearchGate* **83(3):**357–366
- Traxler, J.T. (1971) Piperanine, a pungent component of black pepper. *Journal of Agricultural and Food Chemistry* **19(6),** 1135.
- Tainter, R.D. and Grenis, A.T. (1993) Spices and Seasonings: a Food Technology Handbook. VCH Publishers,Inc., New York, pp.104–112.
- Vijayan, K.K and Thampuran, A. (2000) Pharmacology, toxicology and clinical applications of black pepper. In: Ravindran, P.N. (Ed.) *Black Pepper*, Harwood Academic, pp. 455–66.
- Wood AB, Barrow ML, James DJ.(1988) Piperine determination in pepper

(Piper nigrum L.) and its oleoresins-a reversed-phase high performance liquid chromatographic method;**3**:55-64.

- Zachariah. J. (1995). Essential oil and its major constituents in selected black pepper accessions. *Plant Physiology and Biochemistry*, New Delhi, **22(2)**: 151–153.
- Zainab, T.K. A., (2010) Antimicrobial Activity of Piperine purified from piper nigrum .*J. Basrah Res.* **36**: pp 15-18.

Appendix

1.GC-MS analysis of oil



UMST
UNIVERSITY

جامعة العلوم الطبية والتكنولوجيا
UMST University of Medical
Sciences & Technology
P. O. Box 12810,
Khartoum, Sudan

T. +249 183 228614
F. +249 183 224799
E. administration.office@umst-edu.sd
W. www.umst-edu.sd

Sample Information

Analyzed by	**: Babeker Siddig**
Sample Type	**: Plant Extract**
Sample Name	**: Black paper**
Date	**: 12/05/2016**
customer name	**: Maysaaa**
Sudan University	
Tel	**: 0114930115**

Chromatogram Black paper C:\Users\HP\Desktop\alkaloids\Black paper.QGD

General manager ;.................................
Assoc.Prof .Dhia Eldien A/Elmagied

الذكرى العشرون لإنشاء جامعة العلوم الطبية والتكنولوجيا
1996 - 2016

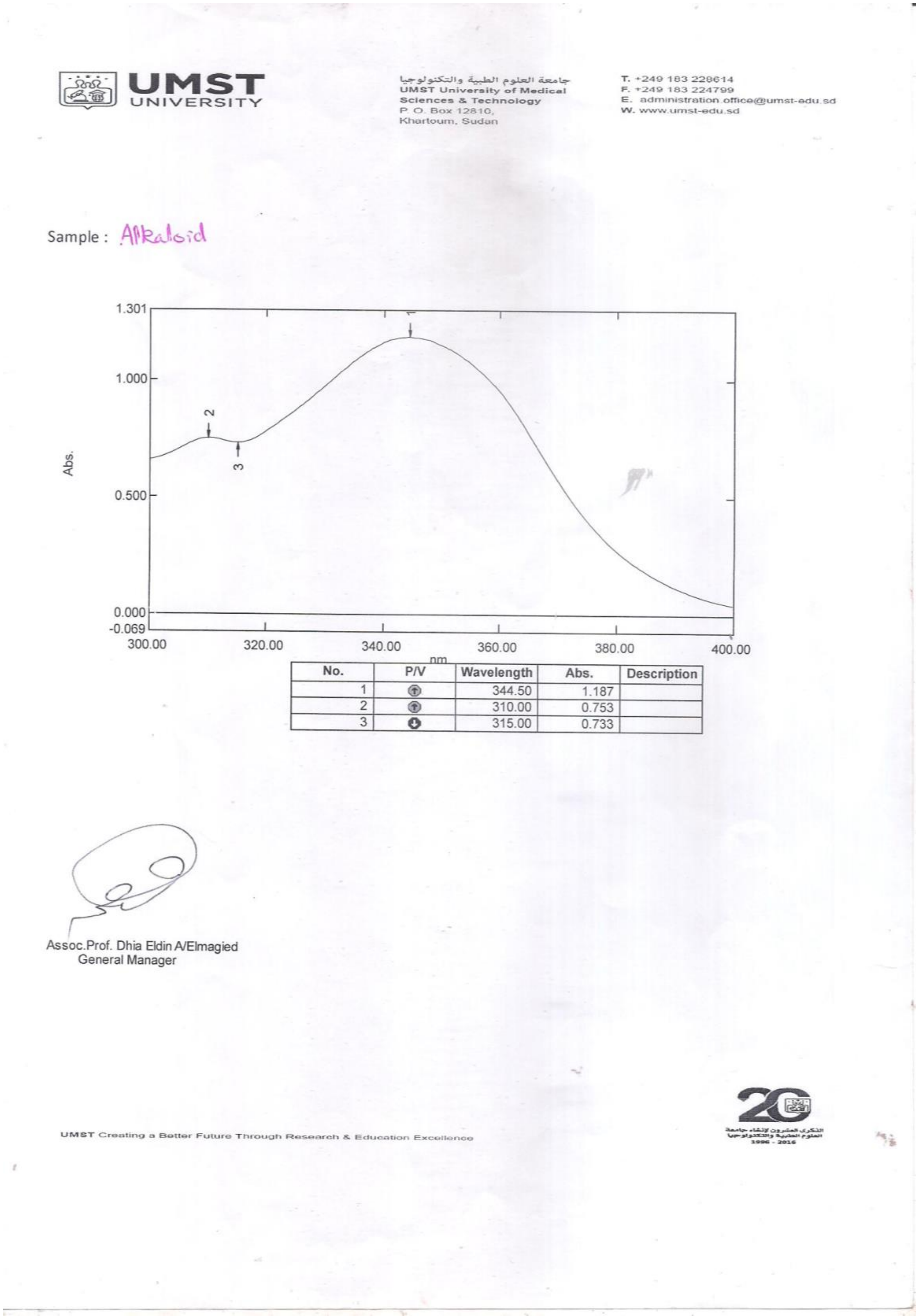
UMST Creating a Better Future Through Research & Education Excellence

5/14/2016 08:45:11

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	4.906	32621	0.03	.alpha.-Pinene
2	6.363	295399	0.29	3-Carene
3	6.649	123237	0.12	Benzene, 1-methyl-3-(1-methylethyl)-
4	6.732	338717	0.33	D-Limonene
5	8.198	394056	0.39	1,6-Octadien-3-ol, 3,7-dimethyl-
6	8.697	94053	0.09	1,3-Cyclohexadiene-1-methanol, 4-(1-meth
7	9.161	63924	0.06	1-Piperidinecarboxaldehyde
8	9.923	163250	0.16	Ethanol, 2-(3,3-dimethylcyclohexylidene)-,
9	10.010	29180	0.03	Phenol, 2-methyl-5-(1-methylethyl)-
10	10.089	150738	0.15	Thymol
11	10.215	350762	0.35	L-.alpha.-Terpineol
12	10.356	52832	0.05	(-)-Myrtenol
13	10.465	219599	0.22	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1
14	10.701	182345	0.18	Linayl acetate
15	10.999	160452	0.16	Naphth[2,3-b]oxirene, decahydro-
16	11.843	165365	0.16	cis-p-Mentha-2,8-dien-1-ol
17	12.047	210258	0.21	4-Oxatricyclo[5.2.1.0(2,6)]decan-8-ol
18	12.643	340993	0.34	3-Heptadecen-5-yne, (Z)-
19	12.875	470288	0.46	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trime
20	13.021	555608	0.55	Cyclobutane, 3-hexyl-1,1,2-trimethyl-
21	13.235	2614682	2.58	Cyclohexene, 4-ethenyl-4-methyl-3-(1-met
22	14.045	2329872	2.30	.alpha.-Cubebene
23	14.947	13500849	13.34	Caryophyllene
24	16.223	1119597	1.11	Naphthalene, decanydro-4a-methyl-1-met
25	17.877	2142797	2.12	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimeth
26	18.029	4780025	4.72	Caryophyllene oxide
27	18.775	2564300	2.53	.gamma.-HIMACHALENE
28	18.886	2149313	2.12	Acetic acid, 4a-methyl-octahydro-1-oxacy
29	18.944	4693745	4.64	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol
30	19.483	1230117	1.22	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.
31	21.065	3410942	3.37	Aromadendrene oxide-(2)
32	22.945	5884799	5.81	Naphthalene, decahydro-2,2-dimethyl-
33	23.223	16819346	16.62	n-Hexadecanoic acid
34	23.519	480471	0.47	Hexadecanoic acid, ethyl ester
35	25.183	23356960	23.07	Oleic Acid
36	25.297	448394	0.44	Pyrimidin-4-ol, 6-methyl-2-(1-piperidyl)-
37	25.340	506879	0.50	Methyl 9-cis,11-trans-octadecadienoate
38	25.378	939207	0.93	Ethyl Oleate
39	26.198	869935	0.86	Palmitoyl chloride
40	26.636	954882	0.94	l-(+)-Ascorbic acid 2,6-dihexadecanoate
41	27.153	431030	0.43	N-(1-Cyclohexen-1-yl)piperidine
42	27.817	5602967	5.54	.gamma.-Tocopherol
		101224786	100.00	

2.UV-Vis of piperine



UMST
UNIVERSITY

جامعة العلوم الطبية والتكنولوجيا
UMST University of Medical
Sciences & Technology
P. O. Box 12810,
Khartoum, Sudan

T. +249 183 228614
F. +249 183 224799
E. administration.office@umst-edu.sd
W. www.umst-edu.sd

Sample : Alkaloid

No.	P/V	Wavelength	Abs.	Description
1	⬆	344.50	1.187	
2	⬆	310.00	0.753	
3	⬇	315.00	0.733	

Assoc.Prof. Dhia Eldin A/Elmagied
General Manager

UMST Creating a Better Future Through Research & Education Excellence

الذكرى العشرون لإنشاء جامعة
العلوم الطبية والتكنولوجيا
1996 - 2016

3.Infra Red of piperine