Sudan University of Science and Technology

College of Postgraduate Studies

Chemical Constituents of *Sinapis alba* Oil and Its Antimicrobial Activity

المكونات الكيميائية والفعالية المضادة للميكروبات لزيت الخردل الأبيض

A Thesis Submitted in Partial Fulfillment of the Requirements of the M.Sc. Degree in Chemistry

By

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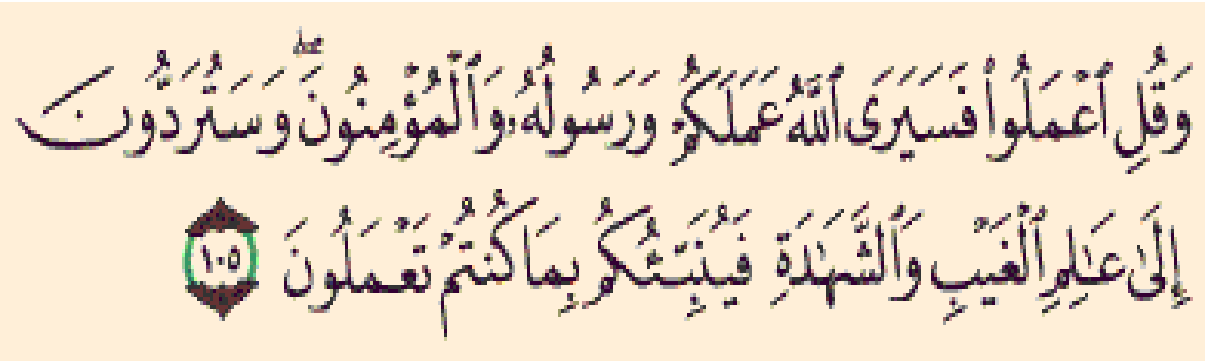

(التوبة-105)

صدق الله العظيم

Dedication

Dedicated to the soul my father.

My mother.

My sisters and brothers.

Acknowledgment

I am deeply grateful to my supervisor:Prof. Mohammed abdelkarim for his valuable information and advice throughout the duration of my research.

I am grateful to all friends and colleagues who supported me during the course of this study.

Thanks for the staff of chemisty Dept. sudan university of science and technology for all facilities.

Abstract

The oil from *Sniaps alba* was analyzed by GC-MS.The analysis showed 23 components. The major components are : 13-docosenoic acid, methyl ester (32.85%) , 9,12- octadecadienoic acid, methyl ester (16.20%), cis- 11-eicosenoic acid, methyl ester (12.58%) , 9,12,15- octadecatrienoic acid methyl ester (10.17%), hexadecanoic acid, methyl ester (5.78%) . Antimicrobial activity of *Sniaps alba* oil was conducted. The oil showed partial activity against *Bacillus subtilis* and *Staphylococcus aureus.*

مستخلص البحث

استخلصت بذور نبات الخردل بالهكسان حيث تم استخلاص الزيت الثابت، ثم حلل الزيت بتقنية كروماتوغرافيا الغاز- مطياف الكتلة والتي أوضحت وجود 23 مكونا اهمها:

13-docosenoic acid, methyl ester (32.85%).

9,12- octadecadienoic acid, methyl ester (16.20%).

cis- 11-eicosenoic acid, methyl ester (12.58%).

9,12,15- octadecatrienoic acid methyl ester (10.17%).
hexadecanoic acid, methyl ester (5.78%).

وفي اختبار مضاد الميكروبات اظهر الزيت فعالية متوسطة ضد:

(*Bacillus subtilis)* and (*Staphylococcus aureus).*

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Chapter One

Introduction

1-Introduction

1.1-Essential oils

Essential oils are compounds made up of several organic volatile substances. These may be alcohols, ketones, ethers, aldehydes, and are produced and stored in the secretion canals of plants. At room temperature they are usually liquid. Given their volatility, they can be extracted using steam distillation, though other methods exist. On the whole, they are responsible for the aromas of plants. They are defined as: products obtained from raw vegetable matter either by steam dragging or by mechanized processes (epicarpium of citrus fruits) or by dry distillation. The essential oil is later separated in the aqueous phase, using physical methods. They are able to undergo physical treatment without important changes in composition (re-distilling, airing…)[1].

This definition clearly establishes the differences there are between medicinal essential oils and other aromatic substances used in pharmacy and perfumery which are commonly known as essences.

Essential oils are widely distributed in nature and are found in conifers , pine, fir (Myrtaceae) ; eucalyptus (Rutaceae) ;*citrus* spp) . However, the majority of plants with essential oils are

found in the family Labiateae (mint, lavender, thyme, rosemary) and family Uumbelliferous (aniseed) .They are found in different organs: roots, ryzomes (ginger), wood (camphor), leaf (eucalyptus), flowering parts (Labiateae family)[1].

The constituents depend on plant place of origin. The habitat where the plant grows (normally warm climates have more essential oils), also it depends on the moment of harvesting, extraction methods, etc….

Essential oils are widely used in ethnomedicine of many communities. Among the main therapeutic properties of essential oils antiseptic property stands out (for many years these spices have been added to foodstuffs not just for flavouring but to help preserve them). Other properties are: antispasmodic, expectorant, carminative, eupeptic…etc.

Certain essential oils, especially in high doses, may be toxic to the central nervous system in particular. Others, such as rue or juniper have abortive properties. Others may cause skin problems, rashes or allergic reactions. In addition to their therapeutic properties, essential oils are widely used in the pharmaceutical, food, and perfume industries[2].

1.2 – Different classes of essential oils

Essential oils may be classified in diverse ways based on : consistency, origin, and chemical nature of the main components.

1.2.1- Classification according to consistency

On the basis of their consistency, essential oils are classified into[3] :

-Essences

-Balsams

-Resins

Fluid essences are liquids which are volatile at room temperature , while balsams are natural extracts obtained from a a plant material. They usually have a high benzoic and cynnamic acid content along with their corresponding ethers. They are thicker, not very volatile, and less likely to react by polymerizing. Examples of balsams are copaiba balsam, Peruvian balsam, Banguy balsam, Tolu balsam, Liquid amber…

Within the resin class there are a number of possible combinations and mixes:

a-Resins.

Resins are semi-solid or amorphous solids of a complex chemical nature.. Colophony- a resin - is obtained by separating

turbentine which is an oleoresin. It contains abietic acid and its conjugates.

b-Oleoresins

Oleoresins are defined as homogeneous matrix of resins and essential oils. Trementine, for example, is obtained by making incisions in the trunk of different pine species. It contains resin (colophony) and essential oil (trementine essence) which are separated by steam drag distillation.

The term oleoresin is also used to refer to vegetable extracts obtained using solvents. They are frequently used instead of spices in foodstuffs and pharmacy because of their advantages (such as stability, microbiotic and chemical uniformity). They have the aroma of the plant in concentrated form and are highly viscous liquids or semi-solid substances (black pepper, paprika oleoresin, cloves…).

c-Gum-resins

Gum resins are natural plant or tree extracts. They are formed of a mix of gums and resins.

1.2.2-Classification according to origin

Depending on their origin, essential oils are categorized into[3]:

-natural oils

-artificial oils

-synthetic oils

a-Natural oils

Natural resins are obtained straight from the plant and are not modified physically or chemically afterwards. However, they are expensive because of their limited yield[3].

b-Artificial oils

Arteficial oils are obtained using processes of enriching the essence with one or several of its components. For example, essences of rose, geranium, and jasmine are enriched with linalool, and aniseed essence with athenol[3].

c- Synthetic oils

Synthetic oils are usually produced by combining their chemically synthesized components. These are the cheapest and are thus much more commonly used as fragrance and taste enhancers (vanilla, lemon and strawberry essences…)[3].

1.2.3-Classification according to chemical nature

The total essential oil content of a plant is generally low (less than 1%). However, by extraction we obtain a highly concentrated form which is used in industrial processes. Most of these are highly complex chemical compounds. The proportion of these substances varies depending on the oil, but also on season, time of day, growing conditions, and genetics[4].

The term : chemotype refers to the variation in chemical composition of an essential oil, even of the same species. A

chemo-type is a distinct chemical entity, different from secondary metabolites. Certain small variations in the environment, geographical location, genes…) which have little or no effect on a morphological level can, however, produce big changes in chemical phenotypes.*Thymus vulgaris*-thyme- has 6 different chemo-types depending on which is the main component of its essence (timol, carvacrol, linalool, geraniol, tuyanol -4, or terpineol). When this is the case, the plant is named using the name of the species followed by the main component of its chemo-type. For example, *Thymus vulgaris* linalool, *Thymus vulgaris* timol[4].

1.3 Physical properties of essential oils

Essential oils are volatile and they are liquids at room temperature. When volatile oils are distilled they are at first colourless or slightly yellowish.They are less dense than water (sassafras essence and clove essence being exceptions).

They are nearly always rotational and have a high refractory index. They are soluble in alcohol and in the usual organic solvents, such as ether or chloroform, and also in high grade alcohol.Essential oils are lipo-soluble and sparingly soluble in water, but can be dragged using steam[4].

Essential oil components are divided into terpenoids and non-terpenoids[5].

a-Non-terpenoids

None terpenoids are less important than terpenoids in terms of applications.They comprise short-chain aliphatic substances, aromatic substances, nitrogenated substances, and substances containing sulphur.

b-Terpenoids

These are more important commercially phytochemicals and they find a wide array of applications. Terpenes are derived from isoprene units (C_5) units bonded in chain form. They are a type of chemical substance found in essential oils, resins, and other aromatic plant substances. They are usually found as sesquiterpenes(C_{15}) and diterpenes (C_{20}) . triterpenes(C_{30}) or polyterpenes(more than (C_{30}) . Terpenes could be: aliphatic, cyclic, or aromatic.

According to their function terpenoids are grouped into:

-Alcohols (menthol, bisabolol) and phenols (timol, carvacrol)

-Aldehydes (geranial, citral) and cetones (camphor, thuyone)

-Esthers (bornile acetate, linalile acetate, methyl salicilate, anti inflammatory compound similar to aspirin)

The functional groups for each category are outlined below[5]:

Table 1: Different classes of essential oils

Compound	Example	Properties
Alcohol	Menthol,geraniol	Anti--microbial, antiseptic,tonic, antispasmodic
Aldehyde	Citral, citronelal	antispasmodic, sedative,antiviral
Ketone	Camphor, tuyona	mucolitic, neurotoxic
Esther	Methyl salicilate	antispasmodic, sedative, antifungal
Ethers	Cineol, ascaridol	expectorant, stimulant
Phenolic ether	Safrol, anetol, miristicine	diuretic, carminative, stomach, expectorant
Phenol	Timol, eugenol, carvacrol	antimicrobial Irritant, stimulant
Hydrocarbons	Pinene, limonene	stimulant, decongestant antiviral,antitumoral

1.4-Monoterpenic hydrocarbons

Monoterpenes are common constituents in essential oils, and precursors of the more complex oxidized terpenes. Their names end in –ene. Limonene, for example, is the precursor to the main components of mint essences (*Mentha* spp, Lamiaceae

Family) such as carvone and menthol.Limonene is also found in citric plants and in dill (*Anethum graveolens*, Apiaceae family).

A- and β- Pinene are also widely distributed in nature, especially in trementine essence of the *Pinus* genus (Pinaceae family)[5].

1.4.1-Alcohols

Alcohols have the hydroxyl group (OH) bonded to a C10 skeleton. Their names end in –ol. They are highly sought after for their aroma. Linalool, for example, has two forms : R-linalool which is found in roses and lavender and is the main component of *Mentha arvensis*. S-linalool found in lavender oil. Linalool is the terpnoid which is responsible for the characteristic taste of tea, thyme, and cardamom leaves. Menthol is another component found in this group. It is responsible for the smell and taste of mint. Mint essence may contain up to 50% of this constituent. Other examples include: geraniol, from scented geraniums (*Pelargonium* spp**),** citronelol, from roses (*Rosa gallica*), borneol from rosemary, and santalol from sandalwood (*Santalum album*, Santalaceae family)[5].

1.4.2-Aldehydes

It is known that aldehydes are highly reactive compounds. Many aldehydes, such as those found in citrus fruits, match their respective alcohols. For example: geraniol – **geranial**, and

citronelol – citronelal. Aldehydes are abundant in citrus plants, and are responsible for their characteristic odor, particularly the isomers geranial (α citral) and neral (β citral) known as citral in combination .

Citral has anti-viral, antimicrobiotic, and sedative properties. But many aldehydes, including citral, cause irritation to the skin.Another important group are the aromatic aldehydes, such as:

-benzaldehyde**,** main ingredient of bitter almond oil and cause of their typical aroma[5].

1.4.3-Phenols

Phenols are only found in a few species but are very powerful and irritating. Important examples are: timol and carvacrol, which are found in thyme (*Thymus*) and oregano (*Origanus*), both belong to the family Labiatae. Eugenol is another important example found in many species, for example, clove essence. Both are powerful bactericidal agents. They also possess anaesthetic properties[5].

1.4.4-Phenolic ethers

Phenolic ethers are the main components of species such as celery and parsley (apiol), aniseed (anetol), basil (metilchavicol), and estragon (estragol).

Another component – safrol- is another component which is used extensively in the perfume industry and is found in the bark of the sassafras tree (*Sassafras albidum* , Lauraceae family).

1.4.5-Ketones

Ketones are usually formed by oxidation of alcohols and are fairly stable molecules. They end in –one. The ketone -carvone - is a constituent of *Mentha spicata*.

Another ketone is tuyone -first isolated from *Thuja occidentalis* in the Cupressaceae family. The ketone pulegone is fairly toxic and should never be used during pregnancy.Tuyone is found in plants of the Artemisia genus (*Artemisia absinthium*) and in Salvia species (*Salvia officinalis*). The ketone pulegona was first isolated from *Mentha pulegium*[6].

1.4.6-Ethers

Ethers or monoterpenic oxides. They are unstable ane very reactive. One example is bisabolol oxide a constituent of *Matricaria chamomilla*. Another common ether is 1.8 –cineol which is the main constituent of eucalyptus oil. This ether has been used expectorant and mucolitic. 1,8-cineol is a major component of cough syrops.

The aroma of eucalyptus oil largely depends on the content of 1.8 –cineol : oils with a high content (*Eucalptus globulus*) are

used for medicinal purposes, whereas those with a lower content are usually used in aromatherapy[6].

1.4.7-Esters

Esters are obtained via the reaction of alcohols with carboxylic acids. Their aroma is characteristic of the oils in which they are found.Lavender oil, for example, contains linalool in its ester- linalile acetate. The relative abundance of both these components is an indicater of high quality. The ester :Methyl salicilate, a derived from salicylic acid and methanol . It possesses anti-inflammatory properties and is found in a certain type of heather (*Gaultheria procumbens*, Ericaceae family)[6].

1.5- Extraction of essential oils

1.5.1-Steam drag distillation

During steam distillation the plant material is placed on a perforated base or sieve at a certain distance and is heated using an in-built heater. The steam flows at a low pressure and penetrates the vegetable matter. The volatile constituents are then condensed in a refrigeration tube and collected in a Florentine flask where water and oil are separated because of difference in density[6].

1.5.2-Pericarpium squeezing

The process of pericarpium squeezing consists of a tray with spikes on it and a channel underneath to collect essential oils. It is usually used for citrus fruits.

1.5.3-Solution in fats (enfleurage)

Oils are soluble in fats and higher alcohols. A thin film of fat is placed on a glass plate and flower petals are then spread over it. The essence passes into the fat until saturated. Then the essential oil is extracted using 70% alcohol. It is used for flowers with a low but prized essential oil content (roses, violets, jasmine, orange blossom)[6].

1.5.4-Extraction using organic solvents

Organic solvents penetrate the vegetable matter and dissolve substances, which are then evaporated and concentrated at low temperature. Then the solvent is eliminated, leaving only the desired fraction.Solvents should have boiling point so that they can quickly be eliminated, though this should not cause ingredient losses. It should be chemically inert, so as not to react with the components in the oils, non-flammable, and cheap[6].

This ideal solvent does not exist, and petroleum ether (boiling point between 30 and 70 degrees, flammable, easy to evaporate), benzene (which also dissolves waxes and pigments),

and alcohol (soluble in water) are commonly used. Alcohol is used when there are components with a high molecular weight but which are not volatile enough.

1.5.5-Extraction using gases in super-critical conditions

Gases (usually CO_2) at a temperature and pressure above their critical points) are used to extract essential oils. nde these conditions, yield is good and changes in the components of the essential oils are avoided. The necessary infrastructure is expensive, put has its advantages, such as the rapid elimination of the extractor gas by decompression, the absence of solvent residue, and the fact that gases are not expensive[6].

1.6-Rectification

Rectification is a common process. It consists in fractioning in a rectification column so as to obtain portions which are then analyzed separately. Those of the same quality are mixed together. On the whole, essential oils are fractioned into three parts:

-Top or light part.

-Heart or middle part.

-Heavy fraction.

1.7-Fractionation

Fractioning is similar to rectification but the split is more specific. Essential oils with a 60-70% citral content are

fractioned to eliminate other components so as to obtain a 90-97% purity.

1.8-Deterpenation

When terpenes are eliminated(if they do not have the wanted organo-leptic properties) the essential oil becomes more soluble in water, and smell and colour are concentrated.

1.9-Dewaxing

When an essential oil is extracted by squeezing rather than steam distilling, it contains compounds such as the wax from the epicarp of the fruit (as well as volatile terpenic fraction).

1.10-Filteration

Raw essential oils are filtered using filtering soils or other materials which retain residual water (anhydrous sodium sulphate, magnesium carbonate…). This agents eliminate impurities.

1.11-Chemical reactions

To obtain new aromatic products of a better quality or value, with pleasanter sensations, we can use[6]:

-Estherification (cedar, vetiver, and mint).

-Hydrogenation (citronella).

-Hydration (trementine)

1.12-Discolouration

This is done for essences with bright colours as in the cases of:

-Patchouli.

-Bursera graveolens.

-Clove.

1.13-Washing

Oil is washed with a 1% sodium hydroxide or 10% sodium carbonate solution. This eliminates the unpleasant smell caused by the presence of acids and phenols[6].

1.14-Standardization

Standardization is not an industrial process in itself. It arises from the need to homogenize or normalize the quality of a product, because of the many variables which modify its characteristics. It is carried out to comply with industrial requirements: same characteristics whatever the origin, time of year, time of harvest[6].

1.15-Isolation of specific products

Some essences are commercialized to isolate some of their main components, such as eugenol (essence of clove) or cedrol (essence of cedar).

1.16-Uses of essential oils

a-Food industry

Essential oils are used to season or condiment meats, dried and cured meats, soups, ice-cream, cheese… the most commonly used essential oils are cilantrum, orange, and mint. They are

also used in the elaboration of alcoholic and soft drinks, especially the latter. Specific mention here is made for the essences of orange, lemon, mint and fennel, which are also used in making of sweets and chocolates[6].

b-Pharmaceutical industry

Essential oils are used in toothpastes (mint and fennel essences), analgesics, and decongestant inhalers (eucalyptus). Eucalyptol is also widely used in dentistry. Essential oils are also used in many medicines to neutralize unpleasant tastes (essence of orange or mint, for example)[6].

c-Cosmetic industry

Cosmetic industry uses essential oils to make cosmetics, soaps, scents, perfumes, and make-up. Mention should be made for geranium, lavender, roses and patchouli essences as common examples[7].

d-Veterinary products

Veterinary industry uses the essential oil of the *Chenopodium ambrosoides*, which is highly prized for its worm-killing properties. Limonene and menthol are also used to make insecticides[7].

e-Industrial deodorants

The use of essences to disguise the unpleasant smell of industrial products like rubber, plastic and paint is being

developed. Paint manufacturers use limonene as a biodegradable solvent. Toys are also scented. In the textile industry they are used to mask unpleasant smells before and after dyeing. Some essential oils are also utilized for their masking properties in paper products- such as notebooks, toilet paper, and face wipes[7] .

f-Tobacco industry

Menthol for mentholated cigarettes are manufactured.

1.17-Chromatography

Chromatography from Greek chroma which means "color" and graphein "to write" is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of

purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture.

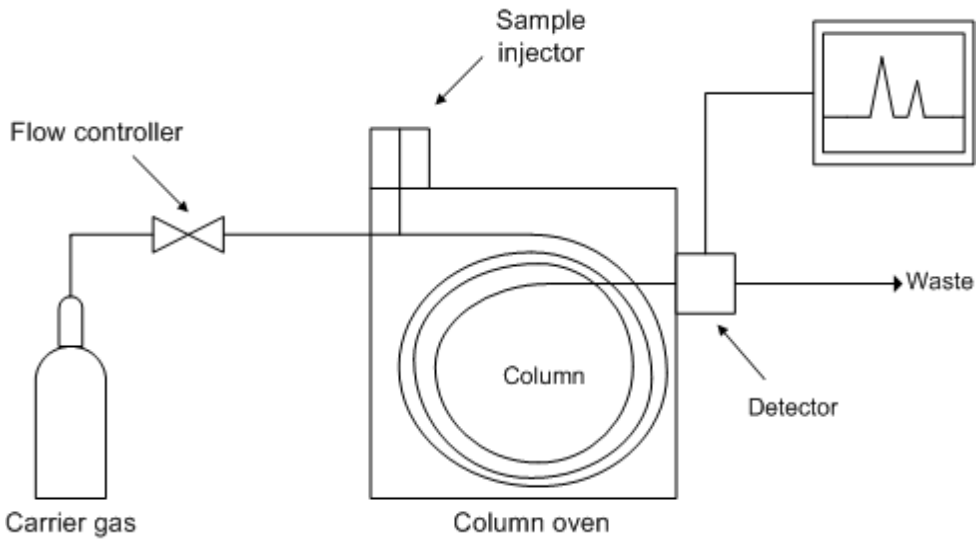
The chromatographic technique is used for the separation of amino acids, proteins and carbohydrates. It is also used for the analysis of drugs, hormones, vitamins. It is helpful for the qualitative and quantitative analysis of complex mixtures. The technique is also useful for the determination of molecular weight of proteins. Types of Chromatography include; Paper Chromatography ,Thin Layer Chromatography(TLC) , Gel Chromatography, Column Chromatography, Ion Exchange Chromatography, Gel Filtration Chromatography, Gas Liquid Chromatography, Affinity Chromatography.

a-Gas chromatography (GC)

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition.

Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a

compound in preparative chromatography; GC can be used to prepare pure compounds from a mixture.



GC system

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a

different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature[7].

In general, substances that vaporize below 300 °C (and therefore are stable up to that temperature) can be measured quantitatively. The samples are also required to be salt-free; they not contain ions. Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.

Some gas chromatographs are connected to a mass spectrometer which acts as the detector. The combination is known as GC-MS.

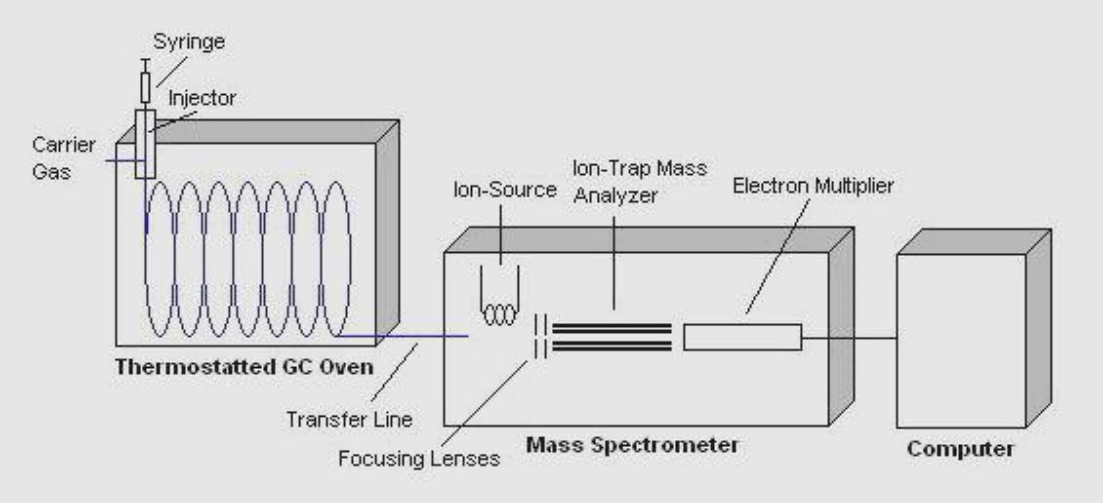
Some GC-MS are connected to an NMR spectrometer which acts as a backup detector. This combination is known as GC-MS-NMR. Some GC-MS-NMR is connected to an infrared spectrophotometer which acts as a backup detector. This combination is known as GC-MS-NMR-IR. It must, however, be stressed this is very rare as most analyses needed can be concluded via purely GC-MS.

b-Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas

chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples.

GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.



GC/MS system

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s after being originated by James and Martin in 1952.

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample. For the analysis of volatile compounds, a purge and trap (PT) concentrator system may be used to introduce samples.

1.18-Mustard

Mustard is one of the oldest recorded spices according to records dating back to 3000 BC. It was recognized both for its therapeutic value and condiment value, historically being used to treat scorpion bites, entomb kings and as a flavouring agent to disguise degraded food[8,9]

Globally, three types of mustard seeds are used as condiments: pale yellow or white mustard (*Sinapis alba* syn. *Brassica hirta* or *B. alba*); brown or oriental mustard (*B. juncea*) and black or dark brown mustard (*B. nigra*). Mustard is also widely used as a salad crop (its green leaves), as an oilseed crop in India, for green manure or as a fodder crop or for industrial oil purposes. In Canada, *Sinapis alba* is also listed as a traditional Chinese medicine in the Natural and Non-Prescription Health Products Directorate database[9].

Canada is one of the major contributors to the world trade market of mustard, supplying both yellow (*Sinapis alba)* and brown/oriental (*B. juncea*) mustards. Canada produced 123,000 tonnes of mustard seed in 2015. According to the most current statistics from FAO, Canada was the highest producer of mustard seed in the world in 2013 with 154,500 tonnes[9] .

Aim of this study

This study was designed to:

-Extract mustard oil.

-Conducting a GC-MS analysis.

-Evaluating the target oil for antimicrobial activity.

Chapter Two
Materials and Methods

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

Sinapis alba seeds were purchased from the local market - Khartoum state(Sudan) and authenticated by direct comparison with a herbarium sample.

2.1.2-Solvents

Solvents of analytical purity were used in this study.

2.1.2- Instruments

For GC-MS studies a Shimadzo GC-MS-QP2010 Ultra instrument(column: 30m,length ; 0.25mm diameter ; 0.25 μm, thickness) was used **.**

2.1.3-Test organisms

The oil from *Sinapis alba* was evaluated for antimicrobial potency using the following microbial isolates :G+ve : *Bacillus subtilis, Staphylococcus aureus* ; (G-ve), *Pseudomonas aeroginosa*, *Escherichia coli* (G-ve) and the fungal species *Candida albicans*.

2.2- Methods

2.2.1-Extraction of oil

Powdered *Sinapis alba* seeds (500g) were percolated with n-hexane .Evaporation of the solvent under reduced pressure gave the oil.

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol.A stock solution of methanolic sulphuric acid was prepared by mixing (1ml)of concentrated sulphuric acid with (99ml) methanol.

The oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid.The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes .The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

2.2.2- GC-MS analysis

Sinapis alba oil was studied by GC-MS , where a Shimadzo GC-MS-QP2010 Ultra machine with a RTX-5MS column was used. Oven temperature programming and other chromatographic conditions are outlined below.

Table 2.2: Oven temperature program

Rate	Temperature(^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table2.3: Chromatographic conditions

Column oven temperature	150.0^{o}C
Injection temperature	300.0^{o}C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

2.2.3-Antimicrobial assay

Bacterial growth was maintained on Muller Hinton agar, while fungal growth was accomplished on Sabouraud dextrose agar. The media were prepared as indicated by manufacturers. Aliquots (1ml) of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.The bacterial growth was harvested and washed off with sterile normal saline, and finally

suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Plate agar diffusion assay was used to screen the antibacterial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the

compounds. Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

The agar discs were removed, alternate cup were filled with 0.1 ml samples of each compound using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

Chapter Three
Results and Discussion

3-Results and Discussion

3.1-GC-MS analysis of *Sinapis alba* oil

The constituents of *Sinapis alba* oil were characterized by GC-MS analysis. Retention times and the fragmentation pattern were used for identification of components. Twenty three constituents were identified by GC-MS. The typical total ion chromatogram (TIC) is given in Fig. (1) while the constituents of the oil are outlined in Table 1 .

Table 1: Constituents of *Sinapis alba* oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.264	0.09
2.	Pentadecanoic acid, methyl ester	15.391	0.04
3.	7,10-Hexadecadienoic acid, methyl ester	16.165	0.08
4.	3-Heptadecen-5-yne, (Z)-	16.238	0.15
5.	9-Hexadecenoic acid, methyl ester, (Z)-	16.269	0.26
6.	Hexadecanoic acid, methyl ester	16.470	5.78
7.	Heptadecanoic acid, methyl ester	17.503	0.07
8.	9,12-Octadecdienoic acid (Z,Z)-, methyl ester	18.243	16.20
9.	9-Octadecenoic acid (Z)-, methyl ester	18.301	3.81
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.329	10.17
11	Methyl stearate	18.488	3.15
12	.gamma.-Linolenic acid, methyl ester	19.996	0.58
13	cis-11-Eicosenoic acid, methyl ester	20.148	12.58
14	cis-13-Eicosenoic acid, methyl ester	20.195	3.30
15	Eicosanoic acid, methyl ester	20.336	2.31
16	Methyl 12-oxo-9-dodecenoate	20.999	0.12
17	15-Tetracosenoic acid, methyl ester, (Z)-	21.041	0.10
18	13-Docosenoic acid, methyl ester, (Z)-	21.914	32.85
19	Docosanoic acid, methyl ester	22.042	1.91
20	cis-10-Nonadecenoic acid, methyl ester	22.675	0.23
21	Tricosanoic acid, methyl ester	22.841	0.13
22	Methyl hexadec-9-enoate	23.464	4.56
23	Tetracosanoic acid, methyl ester	23.616	1.53

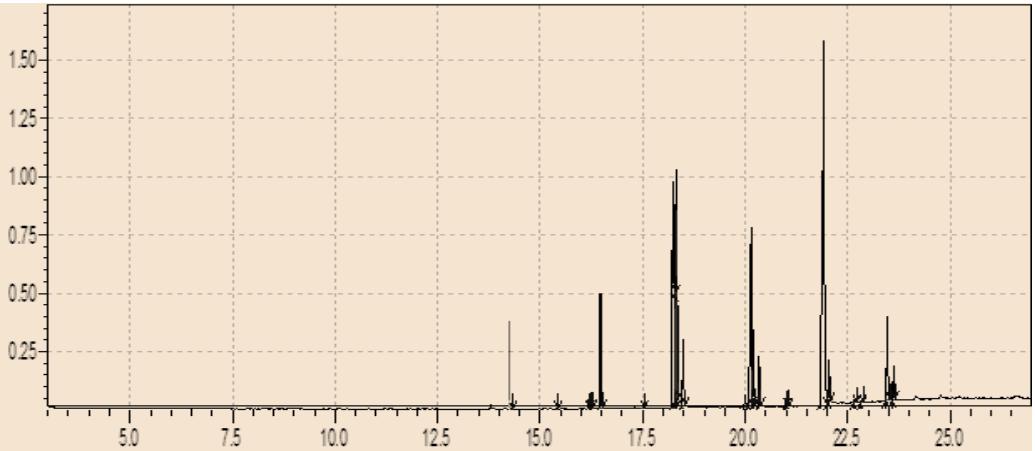


Fig.1: Total ion chromatograms

3.2-Major constituents

The following compounds were detected by GC-MS as major constituents:

a-13-Docosenoic acid methyl ester(32.85%)

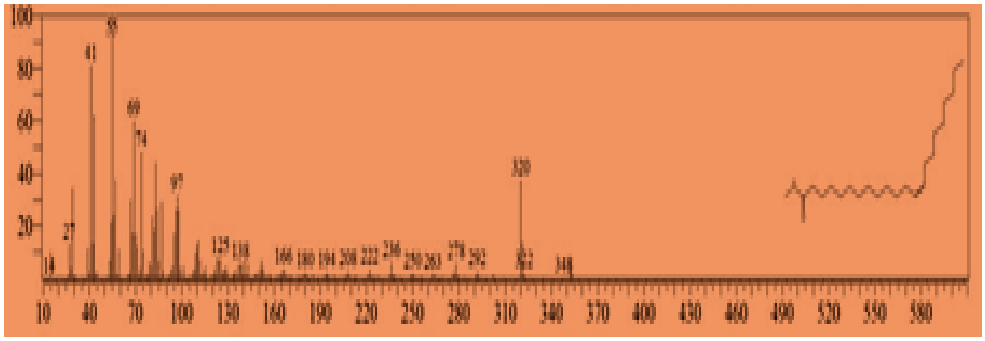


Fig. 2: Mass spectrum of 13-docosenoic methyl ester

The EI mass spectrum of 13- acid methyl docosenoic acid methyl ester is presented in Fig. 2.The molecular ion : $M^{+}[C_{23}H_{44}O_2]^{+}$ appeared at m/z 352(R.T. 21.914 in total ion chromatogram), while the signal at m/z320 is due to loss of a methoxyl.

b-9,12-Octadecadienoic acid methyl ester(16.20%)

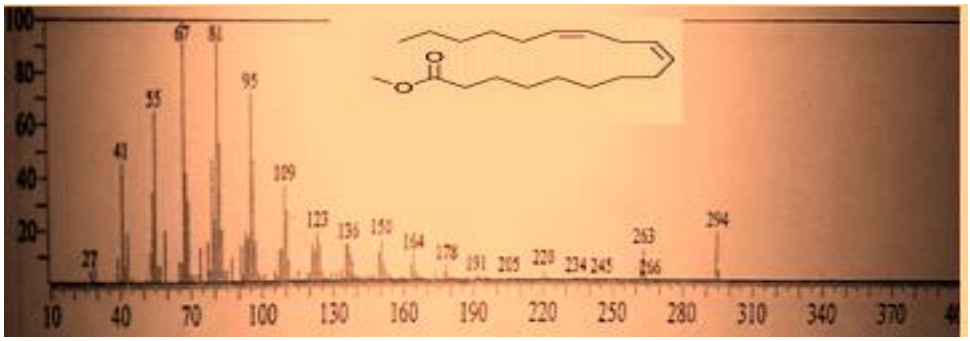


Fig. 3: Mass spectrum of 9,12-octadecenoic acid methyl ester

The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig.3.The peak at m/z 294, which appeared at R.T. 18.243 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

c-cis-11-Eicosenoic acid methyl ester(12.58%)

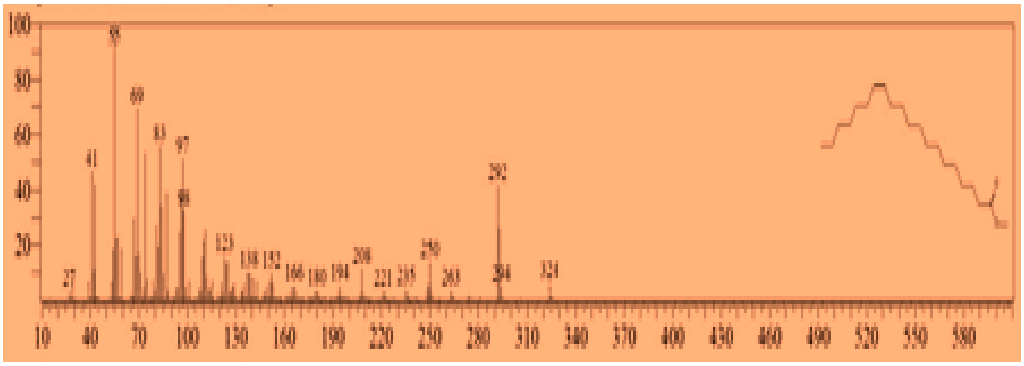


Fig. 4: Mass spectrum of 11-eicosenoic acid methyl ester

Fig.4 shows the EI mass spectrum of cis-11-eicosenoic acid methyl ester.The signal which appeared at m/z 324 at R.T. 20.148 accounts for the molecular ion :$M^+[C_{21}H_{40}O_2]^+$ .The peak at m/z292 is attributed to loss of a methoxyl group.

9,12,15-Octadecatrienoic acid, methyl ester (10.17%)

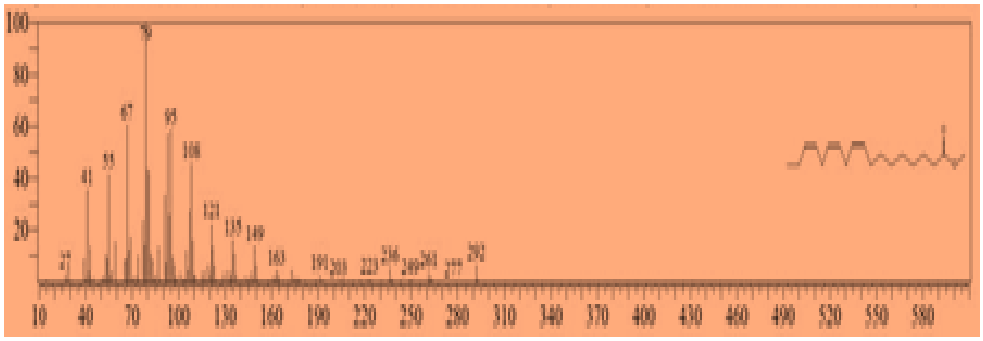


Fig.5: Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester

The EI mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester is shown in Fig.5.The peak at m/z 292(R.T. 18.329) corresponds to $M^+[C_{19}H_{32}O_2]^+$ .The signal which appeared at m/z277 is due to loss of a methyl group while the signal at m/z261 accounts for loss of a methoxyl function.

Hexadecanoic acid methyl ester(5.78%)

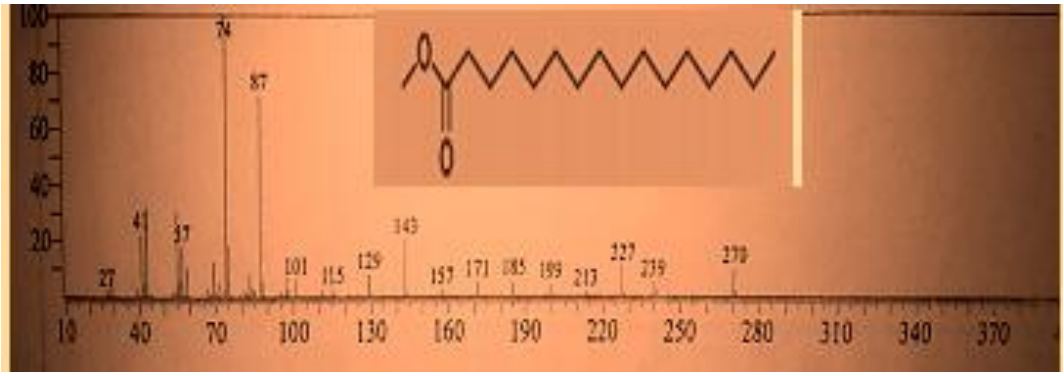


Fig. 7: Mass spectrum of hexadecanoic acid methyl ester

Fig. presents the mass spectrum of hexadecanoic acid methyl ester.The molecular ion : $M^+[C_{17}H_{34}O_2]^+$ appeared at m/z 270, with R.T.16.470 .The peak at m/z239 corresponds to loss of a methoxyl function.

3.3-Antimicrobial activity of the oil

Sinapis alba oil was assessed for antimicrobial activity against five standard microbial isolates. The diameters of the growth of inhibition zones are shown in table 2. Results were interpreted according to the following data :(< 9mm: inactive; 9-12 mm : partially active ; 13- 18 mm : active ; >18mm : very active). The oil showed moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*. Ampicilin, gentamycin and clotrimazole were used as positive controls(Tables 3 and 4) and DMSO as negative control.

Table 2: Antimicrobial activity of the oil

Sample	Ec	Pa	Sa	Bs	Ca
Sinapis alba oil (100mg/ml)	9	--	15	15	-

Table 3 : Antibacterial activity of standard drugs

Drug	Conc. (mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21

	20 10	22 17	18 14	18 15	15 12

Table 4 : Antifungal activity of standard drug

Drug	Conc. (mg/ml)	An	Ca
Clotrimazole	30 15 7.5	22 17 16	38 31 29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

Conclusion

The oil from *Sinapis alba* was analyzed by GC-MS.The analysis showed 23 components The major components are : 13-docosenoic acid methyl ester (32.85%) , 9,12-octadecadienoic acid methyl ester (16.20%), cis- 11-eicosenoic acid methyl ester (12.58%) , 9,12,15-octadecatrienoic acid methyl ester (10.17%), hexadecanoic acid methyl ester (5.78%) . Antimicrobial activity of *Sniaps alba* oil was conducted. The oil showed partial activity against *Bacillus subtilis* and *Staphylococcu aureus.*

Recommendations

The following is recommended:

1-Other constituents of *Sinapis alba* may be isolated and thoroughly studied.

2-The oil of *Sinapis alba* may be evaluated for its antimalarial,antiinflammatory properties.

References

References

1- Bruneton, J. (2001). *Farmacognosia. Fitoquímica. Plantas Medicinales*. 2nd Ed. Zaragoza: Acribia S. A.

2-Las Plantas de Extractos. Bases para un Plan de Desarrollo del Sector. Fundación Alfonso Martín Escudero. Madrid, 1999.

3-Pengelly, A. (1996). *The constituents of Medicinal Plants.* 2nd Ed. Cabi Publishing, U. K.

4-Publicaciones de la Cátedra de Farmacognosia y productos naturales. Facultad de Química, Universidad de la República Oriental del Uruguay.

5-Van Ginkel, A. (2003). Apuntes del Máster y Diplomatura de posgrado de la UAB "Plantas Medicinales y Fitoterapia. Módulo 2. Cultivo de plantas medicinales. Tecnologíay Producción."

6-IUPAC Compendium of Chemical Terminology (2nd ed.). International Union of Pure and Applied Chemistry(1997) ISBN 0-521-51150-X ; Retrieved 2007-10-31.

7-Bolsover, S. R. , "Cell Biology: A Short Course" , John Wiley and Sons, New York, P. 42(2004) ; ISBN 978-0-471-46159-3.

8-McNaught,A.D., Wilkinson,A. : Flavonoids , Isoflavonoids and Neoflavonoids ; In "IUPAC Compendium of Chemical Terminology", (2 ed.), Blackwell Scientific, Oxford(1997).

9-Ostlund, R.E., Racette, S.B., Stenson, W.F., *Am. J. Clin.Nutr*.,**77** (6), 1385(2003).