Sudan University of Science and Technology

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Extraction and physicochemical Study of *Raphanus sativus* Seeds Oil

استخلاص زيت بذور الفجل و دراسة خصائصه الفيزيوكيميائية

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

By

Safa Elbashir Eltayb Elamin

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Supervisor by:

Dr. Kamal Mohammed Saeed

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بسم الله الرحمن الرحيم

الآيـــــة

قال تعالى:

﴿وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلاَّ قَلِيلاً﴾

صدق الله العظيم

الإسراء: 85.

Dedication

To soul of my father, mother and husband.

Acknowledgement

First of all, I would like to thank Allah almighty for making this work possible. Secondly, I would like to express my gratitude to my supervisor Dr. Kamal Mohamed Saeed for his supervision, valuable advice, kind treatment and guidance during this work period and chemistry department, colleagues and all whom support me.

Abstract

Extraction of the *Raphanus sativus* seeds oil was carried out using Soxhlet extractor. The oil percentage in the sample was found to be (33.4%) by weight. The GC-MS analysis showed nine (9) major components: palmatic acid (5.52%), Oleic acid (25.22%), linoleic acid (12.01%), 11-eicosenoic acid (10.66%), eicosanoic acid (1.90%), erucia acid (32.79%), heneicosanoic acid (2.68%), nervonic acid (2.91%) and tetracosanoic acid (1.84%). The pharmaceutical uses and health effects of these major constituents were stated.

The physicochemical tests of the oil showed that: the refractive index (1.4580), density (0.685 mg/ml), viscosity (5.01 m Pa.s), saponification value (152.899 mg/g), acid value (3.647 mg/g), Peroxide value (5 m/kg), Iodine value (63.45 g/100 g), Ester value (149.252 mg/g).

خلاصة البحث

تم استخلاص زيت بذور الفجل باستخدام جهاز السوكسليت، وشكل الزيت ما نسبته 33,4% بالوزن. تم تحديد المركبات الأساسية في الزيت بواسطة جهاز كروماتوغرافيا الغاز – مطياف الكتلة (GC-MS) وهي حمض البالمتيك (5,52%)، حمض الاوليك (25,22%)، حمض اللينوليك (12,01%)، حمض 11-ايكوسينويك (10,66%)، حمض الايكوسينويك (1,9%)، حمض اليوروسيك (32,79%)، حمض الهينيكوسانويك (2.68%)، حمض النيرفونيك (2.91) وحمض التيتراكوسانويك (1.84%). وتم تحديد الخصائص الفيزيائية والكيميائية للزيت وهي: معامل الانكسار(1.458) ، الكثافة (0.685 ملجم/مل)، اللزوجة (5.01 $N.m^{-2}.s$)، رقم الحموضة (3,647 ملجم/جرام)، رقم التصبن 152,899 ملجم/جرام، رقم اليود (63,45 جرام/100جرام)، رقم الاستر (149,252 ملجرام/جرام) ورقم البيروكسيد (5 ملجم/جرام).

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Chapter One

Introduction

1.1- Introduction:

The radish (*Raphanus sativus*) is an edible root vegetable of the Brassicaceae family that was domesticated in Europe in pre-Roman times. Radishes are grown and consumed throughout the world, being mostly eaten raw as a crunchy salad vegetable. They have numerous varieties, varying in size, flavor, color and the length of time they take to mature. Radishes of spicy varieties owe their sharp flavor to the various chemical compounds produced by the plants, including glucosinolate, myrosinase, and isothiocyanate. They are sometimes grown as companion plants and suffer from few pests and diseases. They germinate quickly and grow rapidly, smaller varieties being ready for consumption within a month while larger daikon varieties taking several months. Some radishes are grown for their seeds: daikon, for instance, may be grown for oil production. Others are used for sprouting and both roots and leaves are sometimes served cooked.

1.2-History of Radish plant:

Varieties of radish are now broadly distributed around the world, but there are almost no archeological records available to help in determining its early history and domestication.[Zuhary, 2000] However, scientists tentatively locate the origin of *Raphanus sativus* in southeast Asia, as this is the only region where truly wild forms have been discovered. India, central China, and central Asia appear to have been secondary centers where differing forms were developed. Radishes enter the historical record in 3rd century b.c.[Lewis, 1982] Greek and Roman agriculturalists of the 1st century a.d. gave details of small, large, round, long, mild, and sharp varieties. The radish seems to have been one of the first European crops introduced to the Americas. A German botanist, reported radishes of 100 pounds (45 kg) and roughly three feet in length in 1544, although

the only variety of that size today is the Japanese Sakurajima radish. The large, mild, and white East Asian form was developed in China but is mostly associated in the West with the Japanese daikon, owing to Japanese agricultural development and larger exports.

1.3-Description

Radishes (*Raphanus sativus*) are annual or biennial *brassicaceous* crops grown for their swollen tap-roots which can be globular, tapering or cylindrical. The root skin color ranges from white through pink, red, purple, yellow and green to black but the flesh is usually white. Smaller types have a few leaves about 13 cm (5 in) long with round roots up to 2.5 cm (1 in) in diameter or more slender, long roots up to 7 cm (3 in) long. Both of these are normally eaten raw in salads.[Brickell, 1992] A longer root form, including oriental radishes, daikon or mooli and winter radishes, grows up to 60 cm (24 in) long with foliage about 60 cm (24 in) high with a spread of 45 cm (18 in).[Brickell, 1992] The flesh of radishes harvested timely is crisp and sweet, but becomes bitter and tough if the vegetable is left in the ground for too long. Leaves are arranged in a rosette. They have a lyrate shape, meaning they are divided pinnately with an enlarged terminal lobe and smaller lateral lobes. The white flowers are borne on a racemose inflorescence.[Gopalakrishnan, 2007] The fruits are small pods which can be eaten when young. [Brickell, 1992].

The radish is a diploid species, and has 18 chromosomes (2n=18).[Dixon, 2007]

1.4-Cultivation

Radishes are a fast-growing, annual, cool-season crop. The seed germinates in three to four days in moist conditions with soil temperatures between 65 and 85 °F (18 and 29 °C). Best quality roots are obtained under moderate day lengths with air temperatures in the range

50 to 65 °F (10 to 18 °C). Under average conditions, the crop matures in three to four weeks, but in colder weather six to seven weeks may be required.[Seaman, 2013]

Radishes grow best in full sun in light, sandy loams with a soil pH 6.5 to 7.0, but for late season crops, a clayey-loam is ideal. Soils that bake dry and form a crust in dry weather are unsuitable and can impair germination.[Beattie, 1882] Harvesting periods can be extended by making repeat plantings, spaced a week or two apart. In warmer climates, radishes are normally planted in the autumn.[Beattie, 1882] The depth at which seeds are planted affects the size of the root, from 1 cm (0.4 in) deep recommended for small radishes to 4 cm (1.6 in) for large radishes.[Peterson, 1999] During the growing period, the crop needs to be thinned and weeds controlled, and irrigation may be required.[Beattie, 1882]

1.5-Growing radish plants

Radishes are a common garden crop in the United States, and the fast harvest cycle makes them a popular choice for children's gardens.[Faust, 1996] After harvesting, radishes can be stored without loss of quality for two or three days at room temperature, and about two months at 0 °C (32 °F) with a relative humidity of 90–95%.[Gopalakrishnan, 2007]

1.6-Companion plant

Radishes can be useful as companion plants for many other crops. This is probably because their pungent odor deters such insect pests as aphids, cucumber beetles, tomato hornworms, squash bugs and ants.[Ready, 1982] They can function as a trap crop, luring insect pests away from the main crop. Cucumbers and radishes seem to thrive when grown in close association with each other, and radishes also grow well with chervil, lettuce, peas and nasturtiums. However, they react adversely to growing in close association with hyssop.[Ready, 1982]

1.7-Pests

As a fast-growing plant, diseases are not generally a problem with radishes but some insect pests can be a nuisance. The larvae of flea beetles (Delia radicum) live in the soil but it is the adult beetles that cause damage to the crop, biting small "shot holes" in the leaves, especially of seedlings. The swede midge (Contarinia nasturtii) attacks the foliage and growing tip of the plant and causes distortion, multiple (or no) growing tips and swollen or crinkled leaves and stems. The larvae of the cabbage root fly sometimes attack the roots. The foliage droops and becomes discoloured, and small white maggots tunnel through the root making it unattractive or inedible.[Seaman, 2013]

1.8-Varieties

Broadly speaking, radishes can be categorized into four main types according to the seasons they are grown in and a variety of shapes lengths, colors, and sizes, such as red, pink, white, gray-black or yellow radishes, with round or elongated roots that can grow longer than a parsnip.

1.9-Spring or summer radishes

Sometimes referred to as European radishes or spring radishes if they are planted in cooler weather, summer radishes are generally small and have a relatively short three to four week cultivation time.[Brickell, 1992]

- The April Cross is a giant white radish hybrid that bolts very slowly.
- Bunny Tail is an heirloom variety from Italy, where it is known as 'Rosso Tondo A Piccola Punta Bianca'. It is slightly oblong, mostly red, with a white tip.
- Cherry Belle is a bright red-skinned round variety with a white interior.[Faust, 1996] It is familiar in North American supermarkets.

- Champion is round and red-skinned like the Cherry Belle, but with slightly larger roots, up to about 5 cm (2 in), and a milder flavor.[Faust, 1996]
- Red King has a mild flavor, with good resistance to club root, a problem that can arise from poor drainage.[Faust, 1996]
- Sicily Giant is a large heirloom variety from Sicily. It can reach up to two inches in diameter.
- Snow Belle is an all-white variety of radish, similar in shape to the Cherry Belle.[Faust, 1996]
- White Icicle or just Icicle is a white carrot-shaped variety, around 10–12 cm (4–5 in) long, dating back to the 16th century. It slices easily, and has better than average resistance to pithiness.[Faust, 1996][Peterson, 1999]
- French Breakfast is an elongated red-skinned radish with a white splash at the root end. It is typically slightly milder than other summer varieties, but is among the quickest to turn pithy.[Peterson, 1999]
- Plum Purple a purple-fuchsia radish that tends to stay crisp longer than average.[Peterson, 1999]
- Gala and Roodbol are two varieties popular in the Netherlands in a breakfast dish, thinly sliced on buttered bread.[Faust, 1996]
- Easter Egg is not an actual variety, but a mix of varieties with different skin colors,[Peterson, 1999] typically including white, pink, red, and purple radishes. Sold in markets or seed packets under the name, the seed mixes can extend harvesting duration from a single planting, as different varieties may mature at different times.[Peterson, 1999]

1.10-Winter varieties

Black Spanish or Black Spanish Round occur in both round and elongated forms, and are sometimes simply called the black radish or known by the French name Gros Noir d'Hiver. It dates in Europe to 1548,[Aiton, 1812] and was a common garden variety in England and France during the early 19th century.[Lindly, 1831] It has a rough black skin with hot-flavored white flesh, is round or irregularly pear shaped,[McIntoch, 1828] and grows to around 10 cm (4 in) in diameter.

Daikon refers to a wide variety of winter oilseed radishes from Asia. While the Japanese name daikon has been adopted in English, it is also sometimes called the Japanese radish, Chinese radish, Oriental radish or mooli (in India and South Asia). Daikon commonly have elongated white roots, although many varieties of daikon exist. One well known variety is April Cross, with smooth white roots.[Faust, 1996][Peterson, 1999] The New York Times describes Masato Red and Masato Green varieties as extremely long, well suited for fall planting and winter storage.[Faust, 1996] The Sakurajima radish is a hot-flavored variety which is typically grown to around 10 kg (22 lb), but which can grow to 30 kg (66 lb) when left in the ground.[Faust, 1996]

1.11-Seed pod varieties

The seeds of radishes grow in siliques (widely referred to as "pods"), following flowering that happens when left to grow past their normal harvesting period. The seeds are edible, and are sometimes used as a crunchy, spicy addition to salads.[Peterson, 1999] Some varieties are grown specifically for their seeds or seed pods, rather than their roots. The Rat-tailed radish, an old European variety thought to have come from East Asia centuries ago, has long, thin, curly pods which can exceed 20 cm (8 in) in length. In the 17th century, the pods were often pickled and served with meat.[Peterson, 1999] The München Bier variety supplies

spicy seed pods that are sometimes served raw as an accompaniment to beer in Germany.[Williams, 2004]

1.12-Nutritional value

Radishes are rich in ascorbic acid, folic acid, and potassium. They are a good source of vitamin B6, riboflavin, magnesium, copper, and calcium. One cup of sliced red radish bulbs provides approximately 19 calories, largely from carbohydrates.

Table (1) Nutritional value of radish plant:

Nutritional value per 100 g		Vitamins		Trace metals	
Energy	66 k J	Thiamine (B1)	(1%) 0.012 mg	Calcium	(3%) 25 mg
Carbohydrates	3.4 g	Riboflavin (B2)	(3%) 0.039 mg	Iron	(3%) 0.34 mg
Sugars	1.86 g	Niacin (B3)	(2%) 0.254 mg	Magnesium	(3%) 10 mg
Dietary fiber	1.6 g	Pantothenic cid (B5)	(3%) 0.165 mg	Manganese	(3%) 0.069 mg
Fat	0.1 g	Vitamin B6	(5%) 0.071 mg	Phosphorus	(3%) 20 mg
Protein	0.68 g	Folate (B9)	(6%) 25 µ g	Potassium	(5%) 233 mg
		Vitamin C	(18%) 14.8 mg	Zinc	(3%) 0.28 mg
Other constituents					
Fluoride	6 µg				

The most commonly eaten portion is the napiform taproot, although the entire plant is edible and the tops can be used as a leaf vegetable. The seed can also be sprouted and eaten raw in a similar way to a mung bean.

The bulb of the radish is usually eaten raw, although tougher specimens can be steamed. The raw flesh has a crisp texture and a pungent, peppery flavor, caused by glucosinolates and the enzyme myrosinase, which combine when chewed to form allyl isothiocyanates, also present in mustard, horseradish, and wasabi.

Radishes are mostly used in salads but also appear in many European dishes. Radish leaves are sometimes used in recipes, like potato soup or as a sautéed side dish. They are also found blended with fruit juices in some recipes.

1.13-Other uses

The seeds of Raphanus sativus can be pressed to extract radish seed oil. Wild radish seeds contain up to 48% oil content, and while not suitable for human consumption, this oil is a potential source of biofuel. The daikon grows well in cool climates and, apart from its industrial use, can be used as a cover crop, grown to increase soil fertility, to scavenge nutrients, suppress weeds, help alleviate soil compaction and prevent winter erosion of the soil.

1.14-Culture

The daikon varieties of radish are important parts of East, Southeast, and South Asian cuisine. In Japan and Korea, radish dolls are sometimes made as children's toys. Daikon is also one of the plants that make up the Japanese Festival of Seven Herbs (Nanakusa no sekku) on the seventh day after the new year.

Citizens of Oaxaca, Mexico, celebrate the Night of the Radishes (Noche de los Rábanos) on December 23 as a part of Christmas celebrations. This folk art competition uses a large type of radish up to 50 centimetres (20

in) long and weighing up to 3 kilograms (6.6 lb). Great skill and ingenuity is used to carve these into religious and popular figures, buildings and other objects, and they are displayed in the town square.

1.15-Production trends

About seven million tons of radish are produced yearly, representing roughly two percent of the global vegetable production.

1.16-Chemical Constituents

1.16.1-Alkaloids and Nitrogen Compounds

Alkaloid and nitrogen compounds present in the roots were pyrrolidine, phenethylamine, N-methylphenethylamine, 1,2´-pyrrolidin-tion-3-il-3-acid-carboxilic-1,2,3,4-tetrahydro-β-carboline, and sinapine [3,4,5]. Cytokinin (6-benzylamino-9-glucosylpurine) is a major metabolite of 6-benzylaminopurine (6-BAP) in the root radish. A minor metabolite of 6-BAP from radish has been identified as 6-benzylamino-3-β-D-glucopyranosylpurine[Coeley, et al, 1975]. Total amino acids were 0.5% of dry wt; with proline (0.5%) as the major constituent, methionine and cystine were present in traces (0.02%).

Diamines as diaminotoluene (2,4-D), 4,4´-methylenedianiline (4,4-D), and 1,6-hexanediamine (1,6-D) were isolated in the period of germination of young radish seeds. Production of thiamine is higher during germination radishes[Mal-Nam, et al, 2002].

Total protein was 6.5%[Shyamala, et al, 1987]. Two chitinases, designated RRC-A and RRC-B, were isolated from radish roots. Both compounds had a molecular weight of 25 kDa[Chen-tien, et al, 2000]. N-Bromosuccinimide and di-Etpyrocarbonate inhibited the activities of both chitinases.

Arabinogalactan proteins (AGPs) were isolated from primary and mature roots of the radish. These were composed mainly of L-arabinose and D-galactose. Structures of the carbohydrate moieties of the root were

essentially similar to those isolated from seeds and mature leaves in that they consisted of consecutive (1→3)-linked β-D-galactosyl backbone chains having side chains (1→6)-linked β-Dgalactosyl residues, to which α-L-arabinofuranosyl residues were attached in the outer regions. One prominent feature of the primary root AGPs was that they contained appreciable amounts of L-fucose[Tsumuraya, et al, 1988].

Two L-arabino-D-galactan–contained glycoproteins were isolated from the saline extract of mature radish leaves; both contained L-arabinose, D-galactose, L-fucose-4-O-methyl-D-glucuronic acid, and Dglucuronic acid residues. Degradation of the glycoconjugates showed that a large proportion of the polysaccharide chains is conjugated with the polypeptide backbone through a 3-O-D-galactosylserine linkage[Yoichi, et al, 1984].

Arabino-3,6-galactan associated with a hydroxyproline-rich protein portion and carried a unique sugar residue, α-L-fucopyranosyl-(1-2)-α-L-arabinofuranosyl [Tsumuraya, et al, 1984].

Stigma glycoproteins heritable with S-alleles (S-glycoproteins) were detected in R. sativus. Two main glycoproteins appeared on the SDS-gel electrophoretic pattern. Their molecular weights were established to be 15,000 and 100,000 Da. The carbohydrate fraction of the glycoprotein consisted of arabinose 17.3%, galactose 19.1%, xylose 8.1%, mannose 5.4%, glucose 23.7%, and rhamnose or fucose 26.4%. In the stigma surface diffusate of R. sativus, the content of protein was established to be 16% and that of carbohydrate was 11%[Yinghua, et al, 1983].

The R. sativus acanthiformis showed two ferredoxin isoproteins indicating that plants have multiple genes for ferredoxin. The relative abundance of the isoproteins varied with leaf stage[Keishiro, et al, 1985]. In the isoprotein isolated from roots of the radish, the amino acid

composition and N-terminal sequence were different from those of radish leaf ferredoxin.

Polypeptides RCA1, RCA2, and RCA3 were purified from seeds of R. sativus. Deduced amino acid sequences of RCA1, RCA2, and RCA3 have agreement with average molecular masses from electrospray mass spectrometry of 4537, 4543, and 4532 kDa, respectively. The only sites for serine phosphorylation are near or at the C terminal and hence adjacent to the sites of proteolytic precursor cleavage[Polya, et al, 1993].

Cysteine-rich peptides (Rs-AFP1 and Rs-AFP2) isolated from R. sativus showed peptides 6, 7, 8, and 9 comprising the region from cysteine 27 to cysteine 47[Terras, 1992]. Protein AFP1 isolated from radish showed peptide fragments (6-mer, 9-mer, 12-mer, and 15-mer)[Hans, et al, 1997].

Proteins RAP-1 and RAP-2 were isolated from Korean radish seeds. The molecular mass of the two purified was established to be 6.1 kDa (RAP-1) and 6.2 kDa (RAP-2) by SDS-PAGE and 5.8 kDa (RAP- 1) and 6.2kDa (RP-2) by gel filtration chromatography[Hans, et al, 1997].

1.16.2-Coumarins

Hydroxycoumarins aesculetin and scopoletin were also identified[Stoehr, et al, 1975].

1.16.3-Enzymes

A number of enzymes are present in both the cytoplasm and the cell wall, and in some cases it has been shown that the cell wall isozymes differ from those of the cytoplasmic[Matile, 1975]. When radish seedlings are grown in the dark, β-fructosidase (βF) first accumulates in the cytoplasm, then slowly increases in the cell wall. Charge heterogeneity of cytoplasmic enzymes resides in the polypeptides, while the formation of the basic cell wall occurs as a result of post-translational modifications that can be inhibited by tunicamycin[Faye, et al, 1986].

Cysteine synthase (EC 4.2.99.8) was purified to near homogeneity (275-fold) in 11.5% yield from mature roots. It was relatively stable, retaining most of its activity in standing for several days at room temperature[Tamura, et al, 1976].

A basic β-galactosidase (β-Galase) has been purified from imbibed radish. This enzyme, consisting of a single polypeptide with an apparent molecular mass of 45 kDa and pI values of 8.6 to 8.8, was maximally active at pH 4.0 on p-nitrophenyl β-D-galactoside and β-1,3-linked galactobiose. Radish seed and leaf arabino-3,6-galactan-proteins were resistant to the β-Galase[Sekimata, et al, 1989]. β-Amylase[Shigeo, et al, 1976], together with peroxidase c or paraperoxidase [Yuhei, 1973], which is an isoenzyme, were also isolated from Japanese radish roots.

A hydroxycinnamoyltransferase (EC 2.3.1.-), which catalyzes in vivo the formation of 1, 2-di-Osinapoyl-β-D-glucose, was isolated from the radish. Cotyledons exhibited activities of 1-O-acyl-glucose–dependent acyltransferases, 1-sinapoyl-glucose:L-malate sinapoyltransferase (SMT), and 1- (hydroxycinnamoyl)-glucose:1-(hydroxycinnamoyl)glucose-hydroxyl cinnamoyl-transferase (CGT), showing contrary developments depending on light conditions. Light-grown seedlings showed high Lmalate sinapoyltransferase and low 1-(hydroxycinnamoyl)glucose-hydroxyl cinnamoyl-transferase activities, while dark-grown seedlings showed low L-malate sinapoyltransferase and high 1-(hydroxycinnamoyl) glucose-hydroxyl cinnamoyl-transferase activities[Dahlbeder, 1986].

Catalase and glutathione reductase activities increased considerably in the root and leaves after 24-h exposure to cadmium, indicating a direct correlation with Cd accumulation. PAGE enzyme activity staining revealed several superoxide dismutase isoenzymes in leaves. The main response may be via activation of ascorbate-glutathione cycle for removal

of hydrogen peroxide or to ensure availability of glutathione for synthesis of Cd-binding proteins[Vitoria, et al, 2001].

A γ-glutamyl transpeptidase was found. It catalyzed the release of CySH-Gly from glutathione, the release of alanine from γ-glu-Ala, and the formation of γ-glutamyl dipeptides. A dipeptide formed from Smethylcysteine and glutathione or γ-glu-Ala was characterized as γ-glutamyl-S-methylcysteine[Thompson, et al, 1964].

Two cationic isoperoxidases (C1 and C3) and four anionic isoperoxidases (A1, A2, A3n, and A3) were isolated from Korean R. sativus L. root. All the six isoperoxidases are glycoproteins composed of a single polypeptide chain. The molecular weights of C1, C3, A1, and A2 were ca. 44,000, while anionic isoperoxidase A3n and A3 have molecular weights of 31,000 and 50,000, respectively. N-terminal amino acid sequences were determined for A1, A3n, and C3, while A2 was found to have a blocked terminal residue[Lee, et al, 1994]. Analysis of digested products of the two major N-glycans of C3 suggested that corefucosylated trimannosylchitobiose may contain a different linkage from the typical α-1,6 of native N-linked oligosaccharide[Kim, et al, 1996].

Thiamin-binding substances were found in the radish. There were two kinds of compounds; one was heat labile and Pronase sensitive, and the other was heat stable and Pronase resistant. It would be inferred that the former is protein and the latter is a nonprotein compound[Toshio, et al, 1984].

βF is an isozyme (glycoprotein) found in the cytoplasm and cell walls of the radish. The nonglycosylated cytoplasmic and cell wall βF forms have the same relative molecular mass, but glycosylated forms have different oligosaccharide side chains with respect to size and susceptibility to α-mannosidase and endoglycosidase D digestion[Loic, et al, 1986].

7-Glucoside de zeatin, isolated from radish cotyledons, occurs naturally as glycoside with β-glucose as substituent. A large number of derivatives of purine are glucosylated, but adenine derivatives with alkyl side chains at least three carbon atoms in length at position N6 are preferentially glucosylated[Faye, et al, 1986].

1.16.4-Gibberellins

The bolting (stem elongation accompanying flowering) of R. sativus L. cv. Taibyo-sobutori requires cold treatment (Vernalization) and subsequent long-day conditions. It has been suggested that gibberellins (GAs) might be involved in the control of bolting. Eleven gibberellins were identified in extracts of mature seed as 13 hydroxy-GAs [GA1, 3-epi-GA1, GA8, GA17, GA19, GA20, and a new GA, 12α-hydroxy-GA20 (GA77)] and four non-13-hydroxy-GAs [GA9, GA24, 12β-hydroxy-GA24, GA25]. The major Gas were GA8, GA20, and GA77[Nakayama, et al, 1990].

1.16.5-Glucosinolates

Glucosinolates are very stable water-soluble precursors of isothiocyanates. The relatively nonreactive glucosinolates are converted to isothiocyanates on wounding of the radish. The tissue damage releases myrosinase (EC 3.2.3.1), a glycoprotein that is physically segregated from its glucosinolate substrates. Large variations in myrosinase-specific activity have been reported in various Cruciferous plant sources. Myrosinase, purified to homogeneity from daikon, has a specific activity of 280 μMol/min/mg protein with sinigrin as a substrate[Fahey, et al, 2001]. Glucosinolate contents of seed of radish cultivar ranged from 37–87

μmol/g seed. The 5-vinyl-2-oxazolidinethione, 3-butenyl, 4-pentenyl, and phenethyl isothiocyanate were found in industrially extracted rapeseed

oils. The compounds were hydrolysis products from glucosinolates present in the seed[Daun, et al, 1977].

Desulfoglucosinolates are formed by enzyme desulfation of endogenous glucosilates. The indole glucosinolates, 4-methoxy-3-indolylmethyl glucosinolate and 1-methoxy-3-indolylmethoxy glucosinolate, were absent in seed whereas 4-hydroxy-3-indolymethyl glucosinolate was found in highest concentration in the seeds. The 3-indolymethyl glucosinolate was found in low levels in seed, but was the dominant indole glucosinolate in the leaf[Sang, et al, 1984].

1.16.6-Oil Seed Components:

The seeds of the radish contain a high percentage of oil. Chromatographic analysis of these oils showed clearly their complete similarities to cottonseed oil[El-Hinnawy]. The steam volatile constituents of fresh radish of Japanese and Kenyan origin have been studied. The overall pattern of compounds in the two materials was similar. Major components are pentyl hexyl, 4-methylpentyl isothiocyanate, dimethyl disulfide, methyl methanethiolsulfinate, and 1-methylthio-3-pentanone[Anders, et al, 1978]. Oil radish seeds contained 1.21 μmol of total alkenylglucosinolates (AG/g), consisting mostly of progoitrin and gluconapin[Jaroslav, et al, 1990].

1.16.7-Organic Acids:

Four major organic acids are present in the roots of the radish: oxalic, malic, malonic, and erythorbic acid. Lipid total was 1.23%[Shyamala, et al, 1987]. Major fatty acids in seed lipids were erucic, oleic, linoleic, and linolenic acids.

Major fatty acids in radish family lipids were linolenic acid (52–55%), followed by erucic acid (30–33%), and palmitic acid (20–22%)[39]. Also identified were stearic acid from petroleum ether extracted from

powdered R. sativus seeds. Glutamic acid is found in pickled daikon (20–100 mg%)[40].

1.16.8-Phenolic Compounds:

The content of phenolic acids in the roots of the radish were much smaller than in the leaves. Radishes and horseradish showed caffeic, p-coumaric, ferulic, hydroxycinnamic, p-hydroxybenzoic, vanillic, salicylic, and gentisic acid[Stoher, et al, 1975]. Sinapic acid esters (1-sinapolyglucose, sinapoyl-L-malate, and 6,3´-disinapoylsucrose), kaempferol glycosides, and free malic acid were isolated from cotyledons of R. sativus seedlings[Strack, et al, 1985].

Among the anthocyanins, pelargonidine and cyanidine were responsible for red and violet color in corollas and roots in all inbred progenies. The absence of pelargonidine and cyanidine resulted in a white color. The flavonoid, quercetine, was also found in both corolla and root[Narbut, et al, 1972]. Anthocyanins extracted from epidermal tissue resulted in juices with fairly low initial °Brix (1.3°), containing 400 mg anthocyanin/100 ml. This compound provided color similar to FD&C Red#40. Radish concentrate extract represents a promising natural alternative to the use of FD&C Red#40[Rodriguez, et al, 2001].

Other purple root pigment isolated from progeny radish was an ester of cyaniding triglucoside and three kinds of cinnamic acids. The triglucoside was identified as the 2-diglucoside-5-monoglucoside of cyaniding (Rubrobrassicin)[Isikura, et al, 1965]. Other anthocyanins obtained from red radish are two diacylated pelargonidin 3-O-[2-O-β-glucopyranosyl)-6-O-(trans-p-coumaroyl)-β-glucopyranoside]-5-O-(6-O-malonyl-β-glucopyranoside).

and pelargonidin 3-O-[2-O-(β-glucopyranosyl)-6-O-(trans-p-feruloyl)-β-glucopyranoside]-5-O-(6-O-malonyl-β-glucopyranoside) and other monoacylated anthocyanins pelargonidin 3-O-[2-O-β-glucopyranosyl)-6-

O-(trans-p-coumaroyl)-β-D-glucopyranoside]-5-O-(β-glucopyranoside) and pelargonidin 3-O-[2-O-β-glucopyranosidel)-6-O-(trans-p-feruloyl)-β-glucopyranoside]-5-O-(β-glucopyranoside). Pelargonidin-3-diglucosido-5-monoglucoside is known as raphanusin[Guisti, et al, 1998].

The major anthocyanins of radishes are pelargonidin-3-sophoroside-5-glucoside acetylated with malonic acid and either ferulic or p-coumaric acid. Cinnamic acid acylation site for radish anthocyanins was determined to be at position 6 of glucose-1 of the sophorose substituents by one- and twodimensional 1HNMR-13CNMR[Wrolsta, et al, 2001]. Also 7-glucoside-pelargonidin has been identified in R. sativus[Harborne, 1963].

This compound was stable at 60°C and under light, may be used as a food colorant[Junlian, et al, 1992].

Kaempferol-7-O-rhamnoside, isorhamnetin-7-O-rhamnoside, quercetin-7-O-rhamnoside, kaempferol-3-glucoside-7 rhamnoside, kaempferol-7-glucoside-3 rhamnoside, quercetin-7-O-arabinoside-3-glucoside, and quercetin-7-glucoside-3 rhamnoside were isolated from R. raphanistrum[Kamil, et al, 1977]. Radishes have a high content of flavonoids as quercetin, kaempferol, myricetin, apigenin, and luteolin[50]. Malvidin-3,5- diglucoside was produced from the callus of radish via tissue cultivation. The callus contains 16.4% (dry wt) pigments[Kwan, et al, 1995].

1.16.9-Pigments

Salted radish roots have a characteristic yellow color, which generates during storage. 4-Methylthio-3-butenyl-glucosinolate (4-MTBG) is the substrate of the main pungent principle of radish and is one of the essential factors for the formation of the yellow pigment. The yellow compound 1-(2´-pyrrolidinethion-3´-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxilic acid is presumed to have been the condensation product from

the degradation of 4-methylthio-3-butenylisothiocyanate and L-tryptophan, which carboline compound is considered to play an important role in the formation of the yellow pigment in salted radish roots[Ozawa, et al, 1990].

1.16.10-Polysaccharides:

Pectic substances were extracted from the leaves with oxalate buffer of pH 4.25 as weakly acidic pectic polysaccharide (WAP) and pectic acid. WAP was appreciably hydrolyzed by exo- and endopolygalacturonases and the galacturonic acid content (17.3–25.8%) was much lower than the pectic acids, though the neutral sugar components of both pectic substances were almost the same. The arabinose-galactose side chains were very long or highly branched in pectine compared with those in pectic acids. These compounds are probably inherent pectic components of the cell walls of the vegetables[Matsuura, et al, 1988]. Rhamnose, glucose, and xylose were also isolated. Lipopolysaccharides (LPS) were isolated from radish roots[Genichiro, et al, 1991].

1.16.11-Proteoglycan:

An L-arabino-D-galactan–contained proteoglycan was isolated from hot phosphate-buffered saline extract of radish seeds by ethanol fractionation. The proteoglycan consisted of 86% of a polysaccharide component–contained L-arabinose and D-galactose as major sugar constituents, together with small proportions of D-xylose, D-glucose, and uronic acids, and 9% of a hydroxyproline-contained protein.Arabinogalactan from radish seed had a high content (81%) of L-arabinose and its basic structure seemed to be similar to that of the polysaccharide component of the proteoglycan[Yoichi, et al, 1987].

1.16.12-Sulfur Compounds:

Radish leaves contain only one of the sulfonium diateroisomers of S-adenosylmethionine (AdoMet), which has a remarkable variety of biochemical functions. It is an allosteric enzyme effector and a precursor of spermine biosynthesis, spermidine, and ethylene. It is also the methyl group donor for most biological transmethylation reactions, wherein transfer of its methyl group converts AdoMet to the homocysteine analog (AdoHcy). Much of the chemistry and biochemistry of AdoMet derives from the fact that it is a sulfonium compound[Creason, et al, 1985]. 1-(2´-Pyrrolidinethion-3´-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxilic acid was found in radish root. This carboline compound is considered to play an important role in the formation of the yellow pigment in salted radish roots.

1.16.13-Other Constituents:

β-Carotene was isolated from radish. Vitamin C content in fresh hotbed radishes ranged from 17.95-27.86 mg%[Bulinski et al, 1962]. Also identified was β-sitosterol from R. sativus seeds[Changdai, 1984]. The contents of raphanusol A and B in radish increased at the lighted side and decreased in the shaded side. The differential distribution of raphanusol A and B in the hypocotyls is closed correlated with growth suppression at lighted side[Koji, et al, 1989].

1.17-Biological Activities:

1.17.1-Allergic Contact:

In the radish, the allylisothiocyanate released enzymically from simigrin, a thioglycoside, was identified as a possible sensitizing substance. In some cases, it can produce allergic contact and dermatitis [Mitchell, et al, 1974]. The leaves of this plant also contained glucoparin that produced allergic contact. Antimicrobial Activity Crude juice of the radish inhibited the growth of Escherichia coli, Pseudomonas pyocyaneus, Salmonella typhi, and Bacillus subtilis in vitro. This common plant may

be an important source of antimicrobial substances[Abdou, et al, 1972]. The cysteine-rich peptides (Rs-AFP1 and Rs-AFP2) isolated from R. sativus showed substantial antifungal activity against several fungal species with minimal inhibitory concentration (MIC) of 30–60 μg/ml. Both Rs-AFPs are among the most potent antifungal proteins characterized. Moreover, their antibiotic activity shows a high degree of specificity to filamentous fungi[Terras, et al, 1992]. The active region of the antifungal protein appears to involve β-strands 2 and 3 in combination with the loop connecting those strands [De Samblanx, et al, 1996]. Rs-AFP1 and Rs-AFP2 are highly basic oligomeric proteins composed of small (5-kDa)

polypeptides that are rich in cysteine. These proteins are located in the cell wall and occur predominantly in the outer cell layers lining different seed organs. Moreover, Rs-AFPs are preferentially released during seed germination after disruption of the seed coat [Terras, et al, 1995]. Two purified antifungal proteins RAP-1 and RAP-2 isolated from Korean radish seeds (R. sativus) exhibited growth-inhibitory activities against Candida albicans and Saccharomyces cerevisiae[Jong-Heum, et al, 2001]. The protein AFP1 isolated from the radish showed antifungal activity against Fusarium culmorum[Hans, et al, 1997].

Caffeic acid showed antifungal properties in vitro against Helminthosporium maydis. It has antibacterial, antifungical activities. Ferulic acid is active against Sytaphylococcus aureus, Bacillus subtilis, Corynebacterium, diphtheria, Aspergillus niger, and Candida albicans. These acids displayed antibacterial activity against Gram-positive bacteria Bacillus subtilis and Staphylococcus aureus, and the Gram-negative Escherichia coli and Kliebsiella pneumoniae. The MIC values were 1.56–3.13 μg/ml.

These p-hydroxybenzoic acid (hydroxycinnamic, p-hydroxybenzoic) showed marked activity against Gram-positive bacteria.

The inoculation of sliced daikon roots with the bacterium Pseudomonas cichorii induced the formation of several antifungal compounds including brassinin, methoxybrassinin, spirobrassinin, and 3-indolecarbaldehydes [Takasugi, et al, 1987].

The radish released biocidal compounds, mainly isothiocyanates, produced during the enzymic degradation of glucosinolates present in the plant cell. The highest fungicidal activity depended on concentration of isothiocyanates[Smolinska, et al, 1999].

1.17.2-Antioxidative Activity:

The red radish pigment (pelargodinin-3-sophoroside-5-glucoside) had almost the same antioxidative activity as BHT at the same concentration. The inhibition ratio could reach more than 93% by the 0.01% pigment addition[Xiaoling, et al, 2001]. Also, the caffeic acid showed antioxidative activity.

1.17.3-Antitumor Activity:

A neutral fraction of kaiware radish extract aqueous in vitro showed proliferation inhibition of mouse embryo fribroblast 3T3 cells and papovavirus SV40 transformed 3T3 cells with IC50 of 17.4 and 8.7 μg/ml[Akihiro, et al, 1999]. Diaminotoluene (2,4-D) showed highest cytotoxic activity against He-La cells, 4,4´-methylenedianiline (4,4-D) intermediate, and 1,6-hexanediamine (1,6-D) lowest cytotoxicity. However, the phytotoxicity decreased in order of 4,4-D >2,4-D>1,6-D[7].

1.17.4-Antiviral Activity

Caffeic acid and pelargonidin are virucidal for several enveloped viruses[Strack, et al, 1985]. The lipopolysaccharides showed antiherpes activity.

1.17.5-Calmodulin Antagonists:

The polypeptides RCA1, RCA2, and RCA3 inhibit chicken gizzard calmodulin-dependent myosin light kinase assayed with a myosin-light chain-based synthetic peptide substrate[Polya, et al, 1993].

1.17.6-Growth Inhibitors:

The hypocotyls cis- and trans-raphanusanins and 6-methoxy-2,3,4,5-tetrahydro-1,3-oxazepin-2-one (raphanusamide) were isolated from radish. Cis- and trans-raphanusanins inhibited the hypocotyls growth at concentrations higher than 1.5 μM and raphanusamide at concentrations higher than 20 μM [Hasegawa, et al, 1986]. Growth-inhibitor 2-thioxothiazolidine-4-carboxilic acid was isolated from acetone extract of lightexposed seedlings of Sakurajima radish. It inhibited the growth of hypocotyl sections of etiolated Sakurajima radish and intact hypocotyls of etiolated lettuce seedlings at concentrations greater than 3 mg/ml. Gibberellins were identified in extracts of mature seed radish and might be involved in the control of bolting (stem elongation accompanying flowering) of R. sativus [Nakayama, et al, 1990].

1.17.7-Hypotensive:

Sinapine was extracted with methanol. It is a hypotensive constituent of laifuzi (Semen raphani) and seed of R. sativus[Wilan, et al, 1987].

1.17.8-Platelet Aggregation Inhibitor:

The 6-methyl-sulfinylhexyl-isothiocyanate (MS-ITC) was isolated from wasabi horseradish (Japanese domestic) as a potential inhibitor of human platelet aggregation in vitro. It is a potential inducer of GST (glutathione S-transferase). In the mechanism of MS-ITC, the isothiocyanate moiety of MS-ITC plays an important role for antiplatelet and anticancer activities because of its high reactivity with sulfhydryl (-SH) groups in biomols (GSH, cysteine, residue in a certain protein)[Yasujiro, et al, 2000].

1.17.9-Immunological Properties:

The AGPs isolated from the radish showed immunological properties. Radish AGPs R-I, R-II, crude fraction R-C, and turnip AGP B-II reacted with eel anti-H serum, indicating that these AGPs shared common antigenic determinants[Yoichi, et al, 1984]. The root's AGPs were composed mainly of L-arabinose and Dgalactose, but were distinguishable from each other in their contents of L-fucose as well as of protein and hydroxyproline. Structures of AGPs from the root, seeds, and mature leaves were essentially similar[Kiyoshi, et al, 1988]. Proteoglycan from radish leaves and seeds appeared to share common antigenic determinant[Yoichi, et al, 1987].

1.17.10-Phytoalexins:

The inoculation of sliced daikon roots with the bacterium Pseudomonas cichorii induced the formation of several antifungal compounds including brassinin, methoxybrassinin, spirobrassinin, and 3- indolecarbaldehydes [Takasugi, et al, 1987].

1.17.11-Pungent Principle:

The pungent principle extracted from the radish root is trans-4-methylthio-3-butenyl-isothiocyanate. Also isolated was the cis-isomeride, in a trans–cis ratio of 4:1[Friis, et al, 1966]. 2-Thioxo-3-pyrrolidinecarbaldehyde (TPC)

is a major product generated from the pungent principle of radish. This compound possesses antimicrobial activity with the MIC against fungi and bacteria ranging from 50–400 μg/ml, while yeasts were more resistant. The antifungal and antibacterial actions were due to the sporicidal and bactericidal activities. A dose-dependent inhibition of the uptakes of both oxygen and the precursors for RNA and DNA was observed, suggesting that TPC caused damage to the mitochondrial functions and biosynthetic systems[Kiroki, et al, 1997].

1.17.12-Serological Activity:

AGPs were presumably responsible for expression of the serological activity. In their immunological reactions with rabbit antiradish leaf AGP antibody, the root AGPs were shown to share common antigen determinant with those of seed and leaf AGPs[Tsumuraya, et al, 1988]. Arabino-3,6-galactan associated with a hydroxyproline-rich protein portion, which might be responsible for the serological H-like activity of the AGPs[Tsumarya, et al, 1984]. Two L-arabino-D-galactan–contained glycoproteins having potent inhibitory activity against eel anti-H agglutinin were isolated from the saline extract of mature radish leaves[Yoichi, et al, 1984].

1.17.13-Intestine Motility Stimulation:

The effect of radish aqueous extract at doses of 10 μg/ml to 2 mg/ml caused a dose-dependent increase in contractions of the duodenum, jejunum, and ileum. Ileal contraction was remarkably inhibited by pretreatment of atropine (10–7 M) by 10 min. Oral administration of radish extract (300–500 mg/kg body wt) to mice improved the intestinal transit of charcoal and this was significantly attenuated by coadministration of atropine (50 mg/kg). These results suggest that radish extract stimulates gastrointestinal motility through activation of muscarinic pathways[Young, et al, 2000]. Scopoletin is an antispasmodic agent.

1.17.14-Cardiovascular Disease Prevention:

Radish powder decreased the lipid levels by increasing the fecal excretion of total lipids, triglycerides, and total cholesterol. Catalase and glutathione peroxidase (GSH-Px) activities in red blood cell (RBC) were most remarkably increased by radish. Superoxide dismutase (SOD), catalase, and GSH-Px activities in the liver were increased by radish powder. Xanthine oxidase (XOD) activities in the liver were decreased by

radish. Flavonoids and vitamin C in radish may inhibit lipid peroxidation, promote liver and RBC catalase, and inhibit XOD activities in animals tissues. Radish can be recommended for the treatment and prevention of diseases such as cardiovascular disease and cancer and for delaying aging[Jin, et al, 2001].

1.17.15-Other Activities:

Lipopolysaccharides (LPS) were isolated from radish having a macrophage activating with ED50 of 0.4-100 ng/ml. These compounds can be used as antidiabetic agents in pharmaceutical or veterinary fields.

Also the LPS showed analgesic activity[Genichiro, et al, 1991].

1.18-Applications:

1.18.1-Cooking:

Several edible vegetable and animal oils, and also fats, are used for various purposes in cooking and food preparation. In particular, many foods are fried in oil much hotter than boiling water. Oils are also used for flavoring and for modifying the texture of foods (e.g. Stir Fry).

Cooking oils are derived either from animal fat, as butter, lard and other types, or plant oils from the olive, maize, sunflower and many other species.

1.18.2-Cosmetics:

Oils are applied to hair to give it a lustrous look, to prevent tangles and roughness and to stabilize the hair to promote growth. See hair conditioner.

1.18.3-Religion:

Oil has been used throughout history as a religious medium. It is often considered a spiritually purifying agent and is used for anointing purposes. As a particular example, holy anointing oil has been an important ritual liquid for Judaism and Christianity.

1.18.4-Painting:

Color pigments are easily suspended in oil, making it suitable as a supporting medium for paints. The oldest known extant oil paintings date from 650 AD.[Rosella Lorenzi, 2008]

1.18.5-Heat transfer:

Oils are used as coolants in oil cooling, for instance in electric transformers. Heat transfer oils are used both as coolants (see oil cooling), for heating (e.g. in oil heaters) and in other applications of heat transfer.

1.18.6-Lubrication:

Given that they are non-polar, oils do not easily adhere to other substances. This makes them useful as lubricants for various engineering purposes. Mineral oils are more commonly used as machine lubricants than biological oils are. Whale oil is preferred for lubricating clocks, because it does not evaporate, leaving dust, although its use was banned in 1980.

It is a long-running myth that spermaceti from whales has still been used in NASA projects such as the Hubble Telescope and the Voyager probe because of its extremely low freezing temperature. Spermaceti is not actually an oil, but a mixture mostly of wax esters, and there is no evidence that NASA has used whale oil.

1.18.7-Fuel Synthetic motor oil:

Some oils burn in liquid or aerosol form, generating light, and heat which can be used directly or converted into other forms of energy such as electricity or mechanical work. To obtain many fuel oils, crude oil is pumped from the ground and is shipped via oil tanker or a pipeline to an oil refinery. There, it is converted from crude oil to diesel fuel (petrodiesel), ethane (and other short-chain alkanes), fuel oils (heaviest of commercial fuels, used in ships/furnaces), gasoline (petrol), jet fuel, kerosene, benzene (historically), and liquefied petroleum gas. A 42-gallon

barrel (U.S.) of crude oil produces approximately 10 gallons of diesel, 4 gallons of jet fuel, 19 gallons of gasoline, 7 gallons of other products, 3 gallons split between heavy fuel oil and liquified petroleum gases, and 2 gallons of heating oil. The total production of a barrel of crude into various products results in an increase to 45 gallons. Not all oils used as fuels are mineral oils, see biodiesel and vegetable oil fuel.

In the 18th and 19th centuries, whale oil was commonly used for lamps, which was replaced with natural gas and then electricity.

1.18.8-Chemical feedstock:

Crude oil can be refined into a wide variety of component hydrocarbons. Petrochemicals are the refined components of crude oil and the chemical products made from them. They are used as detergents, fertilizers, medicines, paints, plastics, synthetic fibers, and synthetic rubber.

Organic oils are another important chemical feedstock, especially in green chemistry.

1.19-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 has been widely heralded [who?] as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a

substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification.

1.19.1-History of GC-MS:

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.[James, 1952] These comparatively sensitive devices were originally limited to laboratory settings.

The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1964, Electronic Associates, Inc. (EAI), a leading U.S. supplier of analog computers, began development of a computer controlled quadrupole mass spectrometer under the direction of Robert E. Finnigan.[Brock, 2011][Robert, 2015] By 1966 Finnigan and collaborator Mike Uthe's EAI division had sold over 500 quadrupole residual gas-analyzer instruments.[Brock, 2011] In 1967, Finnigan left EAI to form the Finnigan Instrument Corporation along with Roger Sant, T. Z. Chou, Michael Story, and William Fies.[Brock, 2008] In early 1968, they delivered the first prototype quadrupole GC/MS instruments to Stanford and Purdue University.[Brock, 2011] When Finnigan Instrument Corporation was acquired by Thermo Instrument Systems (later Thermo Fisher Scientific) in 1990, it was considered "the world's leading manufacturer of mass spectrometers".

In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes.[Jeromejeyakumar, et al, 2013] By the 2000s computerized GC/MS instruments using quadrupole technology had become both essential to chemical research and one of the

foremost instruments used for organic analysis. Today computerized GC/MS instruments are widely used in environmental monitoring of water, air, and soil; in the regulation of agriculture and food safety; and in the discovery and production of medicine.

1.19.2-Instrumentation:

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

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.

1.19.3-Purge and trap GC-MS:

For the analysis of volatile compounds, a purge and trap (P&T) concentrator system may be used to introduce samples. The target analytes are extracted and mixed with water and introduced into an airtight chamber. An inert gas such as Nitrogen (N2) is bubbled through the water; this is known as purging. The volatile compounds move into the headspace above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber. The volatile compounds are drawn along a heated line onto a 'trap'. The trap is a column of adsorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then heated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system. P&T GC-MS is particularly suited to volatile organic compounds (VOCs) and BTEX compounds (aromatic compounds associated with petroleum).

1.19.4-Types of mass spectrometer detectors:

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, sometimes referred to by the Hewlett-Packard (now Agilent) trade name "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally one may find a magnetic sector mass spectrometer, however these particular instruments are expensive

and bulky and not typically found in high-throughput service laboratories. Other detectors may be encountered such as time of flight (TOF), tandem quadrupoles (MS-MS) (see below), or in the case of an ion trap MSn where n indicates the number mass spectrometry stages.

1.19.5-GC-tandem MS:

When a second phase of mass fragmentation is added, for example using a second quadrupole in a quadrupole instrument, it is called tandem MS (MS/MS). MS/MS can sometimes be used to quantitate low levels of target compounds in the presence of a high sample matrix background.

The first quadrupole (Q1) is connected with a collision cell (q2) and another quadrupole (Q3). Both quadrupoles can be used in scanning or static mode, depending on the type of MS/MS analysis being performed. Types of analysis include product ion scan, precursor ion scan, selected reaction monitoring (SRM) (sometimes referred to as multiple reaction monitoring (MRM)) and neutral loss scan. For example: When Q1 is in static mode (looking at one mass only as in SIM), and Q3 is in scanning mode, one obtains a so-called product ion spectrum (also called "daughter spectrum"). From this spectrum, one can select a prominent product ion which can be the product ion for the chosen precursor ion. The pair is called a "transition" and forms the basis for SRM. SRM is highly specific and virtually eliminates matrix background.

1.19.6-Ionization:

After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given time. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected.

The ionization technique chosen is independent of using full scan or SIM.

1.19.7-Electron ionization:

By far the most common and perhaps standard form of ionization is electron ionization (EI). The molecules enter into the MS (the source is a quadrupole or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. Hard ionization is considered by mass spectrometrists as the employ of molecular electron bombardment, whereas "soft ionization" is charge by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with library spectra using manufacturer-supplied software or software developed by the National Institute of Standards (NIST-USA). Spectral library searches employ matching algorithms such as Probability Based Matching[McLafferty, 1974] and dot-product[Stein, 1994] matching that are used with methods of analysis written by many method standardization agencies. Sources of libraries include NIST, Wiley, the AAFS, and instrument manufacturers.

1.19.8-Cold electron ionization:

The "hard ionization" process of electron ionization can be softened by the cooling of the molecules before their ionization, resulting in mass spectra that are richer in information.[Amirav, et al, 2008] In this method named cold electron ionization (Cold-EI) the molecules exit the GC column, mixed with added helium make up gas and expand into vacuum through a specially designed supersonic nozzle, forming a supersonic

molecular beam (SMB). Collisions with the make up gas at the expanding supersonic jet reduce the internal vibrational (and rotational) energy of the analyte molecules, hence reducing the degree of fragmentation caused by the electrons during the ionization process.[Amirav, et al, 2008] Cold-EI mass spectra are characterized by an abundant molecular ion while the usual fragmentation pattern is retained, thus making Cold-EI mass spectra compatible with library search identification techniques. The enhanced molecular ions increase the identification probabilities of both known and unknown compounds, amplify isomer mass spectral effects and enable the use of isotope abundance analysis for the elucidation of elemental formulae.[Alon, 2006]

1.19.9-Chemical ionization:

In chemical ionization a reagent gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and analyte and cause a 'soft' ionization of the molecule of interest. A softer ionization fragments the molecule to a lower degree than the hard ionization of EI. One of the main benefits of using chemical ionization is that a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced.

In positive chemical ionization (PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts.

In negative chemical ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply.

1.19.10-Analysis:

A mass spectrometer is typically utilized in one of two ways: full scan or selected ion monitoring (SIM). The typical GC-MS instrument is capable

of performing both functions either individually or concomitantly, depending on the setup of the particular instrument.

The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data.

Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC-MS. Typically, this identification done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample.

A “full spectrum” analysis considers all the “peaks” within a spectrum. Conversely, selective ion monitoring (SIM) only monitors selected ions associated with a specific substance. This is done on the assumption that

at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if the analyst has previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity of the analysis increases. So, SIM analysis allows for a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced.

1.19.10.1-Full scan MS:

When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be m/z 50 to m/z 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set to look for mass fragments too low or else one may detect air (found as m/z 28 due to nitrogen), carbon dioxide (m/z 44) or other possible interferences. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

1.19.10.2-Selected ion monitoring:

In selected ion monitoring (SIM) certain ion fragments are entered into the instrument method and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard.

1.20-Applications:

1.20.1-Environmental monitoring and cleanup:

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies. There are some compounds for which GC-MS is not sufficiently sensitive, including certain pesticides and herbicides, but for most organic analysis of environmental samples, including many major classes of pesticides, it is very sensitive and effective.

1.20.2-Criminal forensics:

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing and Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate.

1.20.3-Law enforcement:

GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs.[James, 1952] It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.

1.20.4-Sports anti-doping analysis:

GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids.[Tsivou, et al, 2006]

1.20.5-Security:

A post–September 11 development, explosive detection systems have become a part of all US airports. These systems run on a host of technologies, many of them based on GC-MS. There are only three manufacturers certified by the FAA to provide these systems,[citation needed] one of which is Thermo Detection (formerly Thermedics), which produces the EGIS, a GC-MS-based line of explosives detectors. The other two manufacturers are Barringer Technologies, now owned by Smith 's Detection Systems, and Ion Track Instruments, part of General Electric Infrastructure Security Systems.

1.20.6-Chemical warfare agent detection:

As part of the post-September 11 drive towards increased capability in homeland security and public health preparedness, traditional GC-MS units with transmission quadrupole mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field portability and near real-time detection of chemical warfare agents (CWA) such as sarin, soman, and VX.[Smith, et al, 2010] These complex and large GC-MS systems have been modified and configured with resistively heated low thermal mass (LTM) gas chromatographs that reduce analysis time to less than ten

percent of the time required in traditional laboratory systems.[Sloan, et al, 2001] Additionally, the systems are smaller, and more mobile, including units that are mounted in mobile analytical laboratories (MAL), such as those used by the United States Marine Corps Chemical and Biological Incident Response Force MAL and other similar laboratories, and systems that are hand-carried by two-person teams or individuals, much ado to the smaller mass detectors.[Patterson, et al, 2002] Depending on the system, the analytes can be introduced via liquid injection, desorbed from sorbent tubes through a thermal desorption process, or with solid-phase micro extraction (SPME).

1.20.7-Food, beverage and perfume analysis:

Foods and beverages contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful and which is often controlled by governmental agencies, for example pesticides.

1.20.8-Astrochemistry:

Several GC-MS have left earth. Two were brought to Mars by the Viking program. Venera 11 and 12 and Pioneer Venus analysed the atmosphere of Venus with GC-MS.[Krasnopolsky, et al, 1981] The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon, Titan.[Niemann, et al, 2005] The material in the comet 67P/Churyumov-Gerasimenko will be analysed by the Rosetta mission with a chiral GC-MS in 2014.[Goesmann, et al, 2005]

1.20.9-Medicine:

Dozens of congenital metabolic diseases also known as Inborn error of metabolism are now detectable by newborn screening tests, especially the

testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders. This is increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better outcome. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS.

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of 13C as the labeling and the measurement of 13C-12C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few select ions and return values as ratios.

1.21-Essential oils:

1.21.1-Pharmacology:

Although medicinal use of essential oils is seen as pseudoscience in the healthcare community, essential oils retain considerable popular use among advocates of alternative medicine. Therefore, it is difficult to obtain reliable references concerning their pharmacological merits.

Studies have shown that certain essential oils may have the ability to prevent the transmission of some drug-resistant strains of pathogen, specifically Staphylococcus, Streptococcus and Candida.[Kurt, 1999]

Taken by mouth, many essential oils can be dangerous in high concentrations. Typical effects begin with a burning feeling, followed by salivation. In the stomach, the effect is carminative, relaxing the gastric sphincter and encouraging eructation (belching). Further down the gut, the effect typically is antispasmodic.[Wanda, 2001]

Typical ingredients for such applications include eucalyptus oils, menthol, capsaicin, anise and camphor. Other essential oils work well in

these applications, but it is notable that others offer no significant benefit. This illustrates the fact that different essential oils may have drastically different pharmacology. Those that do work well for upper respiratory tract and bronchial problems act variously as mild expectorants and decongestants. Some act as locally anaesthetic counterirritants and, thereby, exert an antitussive effect.[Wanda, 2001]

Some essential oils, such as those of juniper and agathosma, are valued for their diuretic effects.[unreliable medical source?] With relatively recent concerns about the overuse of antibacterial agents, many essential oils have seen a resurgence in off-label use for such properties and are being examined for this use clinically.[Robert, 1995]

Many essential oils affect the skin and mucous membranes in ways that are valuable or harmful. Many essential oils, particularly tea tree oil, may cause a contact dermatitis. They are used in antiseptics and liniments in particular. Typically, they produce rubefacient irritation at first and then counterirritant numbness. Turpentine oil and camphor are two typical examples of oils that cause such effects. Menthol and some others produce a feeling of cold followed by a sense of burning. This is caused by its effect on heat-sensing nerve endings. Some essential oils, such as clove oil or eugenol, were popular for many hundred years in dentistry as antiseptics and local anaesthetics. Thymol is well known for its antiseptic effects.

1.21.2-Dangers:

The potential danger of an essential oil is generally relative to its level or grade of purity. Many essential oils are designed exclusively for their aroma-therapeutic quality; these essential oils generally should not be applied directly to the skin in their undiluted or "neat" form. Some can cause severe irritation, provoke an allergic reaction and, over time, prove

hepatotoxic. Non-therapeutic grade essential oils are never recommended for topical or internal use.

Essential oils should not be used with animals, as they possess extreme hepatotoxicity and dermal toxicity for animals. Some essential oils, including many of the citrus peel oils, are photosensitizers, increasing the skin's vulnerability to sunlight.

Industrial users of essential oils should consult the material safety data sheets (MSDS) to determine the hazards and handling requirements of particular oils. Even certain therapeutic grade oils can pose potential threats to individuals with epilepsy or pregnant women.

1.21.3-Handling:

Exposure to essential oils may cause a contact dermatitis[K.H.C. Baser, et al, 2010] Essential oils can be aggressive toward rubbers and plastics, so care must be taken in choosing the correct handling equipment. Glass syringes are often used, but have coarse volumetric graduations. Chemistry syringes are ideal, as they resist essential oils, are long enough to enter deep vessels, and have fine graduations, facilitating quality control. Unlike traditional pipettes, which have difficulty handling viscous fluids, the chemistry syringe has a seal and piston arrangement which slides inside the pipette, wiping the essential oil off the pipette wall.

1.21.4-Pregnancy:

The use of essential oils in pregnancy is not recommended due to inadequate published evidence to demonstrate evidence of safety.[citation needed] Pregnant women often report an abnormal sensitivity to smells and taste, essential oils can cause irritation and nausea.

1.21.5-Gynecomastia:

Estrogenic and antiandrogenic activity have been reported by in vitro study of tea tree oil and lavender essential oils. Case reports suggest the

oils may be implicated in some cases of gynecomastia, an abnormal breast tissue growth, in prepubescent boys. However, these claims have been challenged [unreliable medical source?] and the European Commission's Scientific Committee on Consumer Safety has dismissed the claims saying "Since the hormonal active ingredients of Tea Tree Oil were shown not to penetrate the skin, the hypothesized correlation of the finding of 3 cases of gynecomastia to the topical use of Tea Tree Oil is considered implausible."

1.21.6-Pesticide residues:

There is some concern about pesticide residues in essential oils, particularly those used therapeutically. For this reason, many practitioners of aromatherapy buy organically produced oils.[citation needed] Not only are pesticides present in trace quantities, but also the oils themselves are used in tiny quantities and usually in high dilutions. Where there is a concern about pesticide residues in food essential oils, such as mint or orange oils, the proper criterion is not whether the material is alleged to be organically produced, but whether it meets the government standards based on actual analysis of its pesticide content.

1.21.7-Ingestion:

Essential oils are used extensively as GRAS flavoring agents in foods, beverages and confectioneries according to strict Good Manufacturing Practice (GMP) and flavorist standards. Therapeutic grade essential oils are generally safe for human consumption in small amounts.[citation needed] Pharmacopoeia standards for medicinal oils should be heeded. Some oils can be toxic to some domestic animals, cats in particular. The internal use of essential oils can pose hazards to pregnant women, as some can be abortifacients in dose 0.5–10 ml, and thus should not be used during pregnancy.

Research Objectives:

- To extract Raphanus sativus seeds oil.
- To detect the major constituent of the oil.
- To study the physicochemical properties of the oil and to establish a frame of a specification for the oil according to the international specifications.
- To identify the health effects and pharmaceutical uses.

Chapter Two

Material and Method

2- Material and methods

2.1- Material

Radish seeds were purchased from Vico Company, the seeds were grounded and kept labeled self-sealing polyethylene bag and stored for further use.

2.2-Methods

2.2.1-Extraction of the oil

100g of the seed powder were extracted with diethyl ether for 4 hours in a soxhelt apparatus. the oil was collected after removal of slovent.

2.2.2-Physicochemical properties of the oil

2.2.2.1- Refractive index

The refractometer was used to determine the refractive index of the oil. The refractometer was adjusted first using distilled water (1.330) few drops of oil were placed on the prism and the prism was closed firmly by lightning screw heat the instrument was then left to start for few minutes before reading in order to equilibrate the oil temperature with that of the instrument.

2.2.2.2-Viscosity

10 ml of hexane was taken by pipette to Ostwald viscometer and allowed to stand in water path at 27 ºC, then the flow time between the two marks was measured (t_1), repeat the measurement for the oil t_2 then determination the viscosity of oil $\eta_1 / \eta_2 = t_1\rho_1 / t_2\rho_2$

Where:

η_1 = is the viscosity of hexane

η_2 = is the viscosity of oil

ρ_1 and ρ_2 are the density of water and oil respectively

2.2.2.3-Density

A pyconometer (50ml) was weighed empty (m) then filled with distilled water at room temperature and weighed (m_1).

The pyconometer then was dried and filled with the oil at the same temperature and weight (m_2).

Oil density = $m_2 – m/m_1$-m

2.2.2.4-Acid value (A.V)

(19 g) of the oil was weighed and dissolved in 50 ml of mixture of equal volumes of ethanol and diethyl ether. After complete dissolution, the oil was titrated against standard (0.1m) KOH using 0.5 ml of phenolphthalein solution indicator.

The acid value was then calculated using the formula:

(A.V) = 5.61 v/w

When

V = volume (in ml) of KOH solution

W = weight (in gm) of oil sample.

2.2.2.5- Peroxide value (P.V)

19 g of oil weight in a 250 ml conical flask, then added mixture of 4.8 ml volumes of chloroform and 7.2 ml volumes glacial acetic acid was added followed by addition 0.2 ml saturated potassium iodide solution. After shaking for one min, the mixture was titrated with a standard (0.1N) sodium thiosulphate using starch as indicator the yellow color was discharged.

The peroxide value was calculated using the following equation:

Peroxide value = V×N×10001W m eq/kg.

V = is the volume (in ml) of $Na_2S_2O_3$ used in test sample

W = is the weight (in gm) of sample.

2.2.2.6- Iodine value (I.V)

19 g of oil weighed and dissolved in 50 ml of chloroform and added 25 ml of iodine mono chloride solution (0.2M) close the flask and keep it in dark for 30 min, unless otherwise prescribed shaking frequently added 10 ml of 100 g/1 solution of potassium iodide and (10 ml chloroform) titrated with almost discharged added 10 ml of potassium iodide and continue the titration added (0.5M) sodium thiosulphate drop wise until the color is discharged carried out a blank test under same condition.

$V_i = 1.269 (n_2-n_1)$ lm

2.2.2.7-The saponification value (S.V)

6 ml of 1:1 mixture of ether and ethanol 95% solution was added to 19 g of the oil and the mixture was then refluxed on steam bath for half hour with frequent shaking.

The excess of alkali was titrated with a standard hydrochloric solution (0.5M) while it was still hot using phenolphthalein as indicator back titration was carried out at the same condition.

The saponification value was calculated from the expression:

28.05 V/W

Where

V = is the different in ml, between the titrations

W = is the weighting of oil sample

2.2.2.8- Ester value (E.V)

Ester value = Saponification value - acid value.

2.3 Sample preparation

2ml of oil was placed in a test tube and 7 ml of alcoholic sodium hydroxide was added, followed by 7 ml of alcoholic sulfuric acid. The tube was shaken gently and left overnight. Then sodium chloride was added to saturation. (2ml) of n-hexane were added and the contents were

shaken. The layers were separated. One drop from hexane layer was diluted with other and 1 microliter was injected in GC-MS.

Chapter Three

Results and Discussion

3-Results and Discussion

Table (3.1) shows the physiochemical properties of the raphanus seeds oil.

Table 3.1: physiochemical properties of the oil extracted from seeds of raphanus.

Refractive index	1.4580
Density	0.685 g/ml
Peroxide value (PV)	5 m/kg
Viscosity at 27 ^{o}C	5.01 N.m^{-2}S
Acid value (AV)	3.647 mg/g
Saponification value (SA)	152.899 mg/g
Iodine value	63.45 g/100g
Ester value	149.252 mg/g

Percentage of the oil in the sample was found to be: 33.4%

Percent of compound = (peak of area/total area) × 100

1. Palmatic acid = (9238187/167409081) ×100 = 5.52%
2. Oleic acid = (42212344/167409081) ×100 =25.22%
3. Linoleic acid = (20111924/167409081) ×100 =12.01%
4. Erucic acid = (548968886/167409081) ×100 = 32.79%
5. Nervonic acid = (4866199/167409081) ×100 = 2.91%
6. Tetracosanoic acid = (3074101/167409081) ×100 = 1.84%
7. 11-Eicosenoic acid = (17838358/167409081) ×100 = 10.66%
8. Eicosenoic acid = (3178513/167409081) ×100 = 1.90%
9. Heneicosanoic acid = (4488216/167409081) ×100 = 2.68%

Table (3.2) shows the results of GC-MS Chromatography.

Table (3.2) percentage area in GC-MS:

Area%	Name
018	Butylated hydroxytoluene
0.06	Methyl tetradecanoat
0.04	7,10-Hexadecadienoic acid, methyl ester
0.04	Methyl 8,11,14-heptadecatrienoate
0.15	9-Hexadecenoic acid, methyl ester, (z)-
5.52	Hexadecanoic acid, methyl ester
0.02	6-Octadecenoic acid, methyl ester, (z)-
0.03	Hexadecanoi acid, 15-methyl-, methyl ester
12.01	9,12-Octadecenoic acid, (Z,Z)-, methyl ester
25.22	9-Octadecenoic acid (Z)-, methyl ester
2.51	Methyl stearate
0.32	9,12,15-Octadecatrienoic acid, methyl ester
10.66	11-Eicosenoic acid, methyl ester
0.99	Cis-11-Eicosenoic acid, methyl ester
1.90	Eicosanoic acid, methyl ester
0.15	Phenol, 2,2'-methylenebis [6-(1,1-dimethyle ester)]
32.79	13-Docosenoic acid, methyl ester
2.68	Methyl 20-methyl-heneicosanoate
2.91	15-Tetracosenoic acid, methyl ester, (Z)-
1.84	Tetracosanoic acid, methyl ester.
100.00	

Figure (3.1) shows the peaks of radish oil contents.

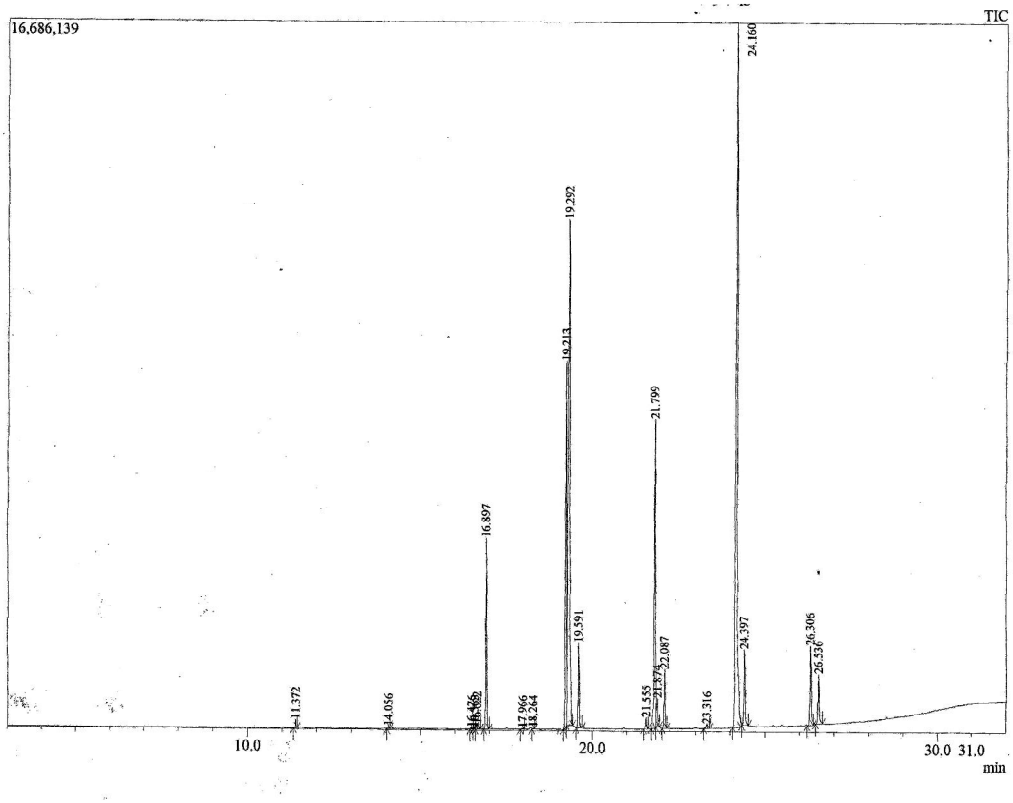

Figure (3.1) Chromatogram for Radish oil.

Table (3.3) shows the major compounds in radish oil.

Table (3.3): Major compounds in radish oil

No.	Name	Area %
1.	Palmatic acid	5.52
2.	Oleic acid	25.22
3.	Linoleic acid	12.01
4.	11-Eicosenoic acid	10.66
5.	Eicosanoic acid	1.90
6.	Erucic acid	32.79
7.	Heneicosanoic acid	2.68
8.	Nervonic acid	2.91
9.	Tetracosanoic acid	1.84

This study was about extraction of essential oils from *Raphanus sativus* seeds using (solvent extraction technique).

The study covered the percentage yield, physical and chemical properties, of the oil. The study followed GC-MS chromatography analysis to identify the oil components.

The physical properties of the oil were found to be: refractive index (1.4580), density (0.685) g/ml, viscosity (5.01) m Pa S, saponification value (152.899) mg/g, acid value (3.647) mg/g, Peroxide value (5) m/kg, Iodine value (63.45) g/100g, Ester value (149.252) mg/g.

While the GC-MS analysis showed nine (9) major components with its pharmaceutical uses and health effects, as follows:

1- Heptanoic acid: Heptanoic acid is used to esterify steroids in the preparation of drugs such as testosterone enanthate, trenbolone enanthate, drostanolone enanthate and methenolone enanthate (Primobolan). It is also one of many additives in cigarettes.

2- Decanoic acid: is used in the manufacture of esters for artificial fruit flavors and perfumes. It is also used as an intermediate in chemical syntheses. It is used in organic synthesis and industrially in the manufacture of perfumes, lubricants, greases, rubber, dyes, plastics, food additives and pharmaceuticals.

Pharmaceuticals: decanoate ester prodrugs of various pharmaceuticals are available. Since decanoic acid is a fatty acid, forming a salt or ester with a drug will increase its lipophilicity and its affinity for fatty tissue. Since distribution of a drug from fatty tissue is usually slow, one may develop a long-acting injectable form of a drug (called a Depot injection) by using its decanoate form. Some examples of drugs available as a decanoate ester include nandrolone, fluphenazine, bromperidol, and haloperidol.

Health effects: While studies done on laboratory animals in the early 1970s, show that erucic acid appears to have toxic effects on the heart at

high enough doses, an association between the consumption of rapeseed oil and increased myocardial lipidosis or heart disease has not been established for humans. While there are reports of toxicity from long-term use of Lorenzo's oil (which contains erucic acid and other ingredients), there are no reports of harm to people from dietary consumption of erucic acid.

3- 13-Decosonic acid: Publication of animal studies with erucic acid through the 1970s led to governments worldwide moving away from oils with high levels of erucic acid, and tolerance levels for human exposure to erucic acid have been established based on the animal studies. In 2003, Food Standards Australia set a provisional tolerable daily intake (PTDI) of about 500 mg/day of erucic acid, based on "the level that is associated with increased myocardial lipidosis in nursling pigs. There is a 120-fold safety margin between this level and the level that is associated with increased myocardial lipidosis in nursling pigs. The dietary exposure assessment has concluded that the majority of exposure to erucic acid by the general population would come from the consumption of canola oil. The dietary intake of erucic acid by an individual consuming at the average level is well below the PTDI, therefore, there is no cause for concern in terms of public health and safety. However, the individual consuming at a high level has the potential to approach the PTDI. This would be particularly so if the level of erucic acid in canola oil was [sic] to exceed 2% of the total fatty acids.

4- 15-tetracosenoic acid: Nervonic acid is a monounsaturated omega-9 fatty acid. Nervonic acid has been identified as important in the biosynthesis of nerve cell myelin. It is found in the sphingolipids of white matter in human brain.

Nervonic acid is used in the treatment of disorders involving demyelination, such as adrenoleukodystrophy and multiple sclerosis where there is a decreased level of nervonic acid in sphingolipids.

Industrial uses: Linoleic acid is used in making quick-drying oils, which are useful in oil paints and varnishes. These applications exploit the easy reaction of the linoleic acid with oxygen in air, which leads to crosslinking and formation of a stable film.

Reduction of linoleic acid yields linoleyl alcohol. Linoleic acid is a surfactant with a critical micel concentration of 1.5 x 10−4 M @ pH 7.5.

Linoleic acid has become increasingly popular in the beauty products industry because of its beneficial properties on the skin. Research points to linoleic acid's anti-inflammatory, acne reductive, and moisture retentive properties when applied topically on the skin.

5- Palmitic acid: -Health effects: According to the World Health Organization, evidence is "convincing" that consumption of palmitic acid increases risk of developing cardiovascular diseases. Retinyl palmitate is an antioxidant and a source of vitamin A added to low fat milk to replace the vitamin content lost through the removal of milk fat. Palmitate is attached to the alcohol form of vitamin A, retinol, to make vitamin A stable in milk.

Rats fed a diet of 20% palmitic acid and 80% carbohydrate for extended periods showed alterations in central nervous system control of insulin secretion, and suppression of the body's natural appetite-suppressing signals from leptin and insulin (the key hormones involved in weight regulation).

6- Oleic acid (Octadec-9-enoic acid): - Health effects: Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein

(LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol. However, its ability to raise HDL is still debated.

Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil. Adverse effects also have been documented, however, since both oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer, although the consumption of oleate in olive oil has been associated with a decreased risk of breast cancer.

3.4 Suggestions for future studies:

- To study the volatile oils out of *Raphanus Sativus* seeds oil.
- To test the specific rotation of the oil.
- To measure the hydroxyl and acetyl value of the oil.
- To detect the main functional group of the oil by infrared spectroscopy (IR).

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