

GC-MS ANALYSIS OF MUSTARD OIL (BRASSICA CAMPESTRIS) AND EXPLORATION OF THEIR ANTIOXIDANT ACTIVITIES.

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Abstract

Brassica campestris (Mustard) belongs to the genus Brassicae of the Cruciferae family. The study was aimed to analyze the potential oil, mustard oil as an antioxidant and exploration of its components by GC-MS analysis. Numerous people are calling for foods to be free of what they believe to be dangerous and unnatural additives, such as numerous preservatives and antimicrobials. As a result, there is increased interest in natural antimicrobials as possible substitutes for traditional additives to increase shelf life and fight foodborne infections. Investigating the chemical components of the fixed oil derived from Brassica campestris seeds and assessing its possible antioxidant properties were the goals of this study. The chemical constituents of Brassica campestris seeds oil was identified and quantified by GC-MS. Comprehensive evaluation of Mustard seed oil by various assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS[2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid] assay, lipid peroxidation assay and iron chelation was carried out. The study explored that mustard seed oil can be used in food preservation, cosmetics, and pharmaceutical based on its antioxidant activities. GC MS analysis proved that various components are present in mustard oil like Allyl Isothiocyanate, cyclopropane, ether, thiazole, 2,4-dimethyl, benzoic acid, methyl ester, hexanedioic acid, phthalic acid, 1, 4- Benzenedicarboxylic acid, tocopherol, Ergosta-5,22-diene-3-ol, cholesterol beta sistosterol, gamma sistosterol etc. This study provided a comprehensive framework for understanding and utilizing the essential oil of mustard seeds, particularly in relation to chemical and antioxidant.

INTRODUCTION

Reactive molecules containing oxygen atom that are naturally produced in the body during various metabolic processes especially in mitochondria is

called reactive oxygen species (ROS). They exist in the form of superoxide, hydrogen peroxide, and hydroxyl free radical. ROS at low level perform several

important functions like cell signaling and communication, immune defense, and programmed cell death known as apoptosis. When they are produced in excess they produce oxidative stress in the body. Oxidative pressure is brought about by the imbalance in the production of reactive oxygen species (ROS) and the capacity of the body to neutralize or kill them. Extracellular redox balance which is defined as a balance between reducing and oxidizing species within cell, is firmly coupled to the antioxidant (cancer prevention agent). Peptide glutathione; intracellular levels of this compound are enzymatically controlled to keep a reducing environment within the body. A portion of these reactive oxygen species (ROS) are created at low levels as by results of typical metabolic reactions within the body and are held under control by the enzymes that keep up with the intracellular redox balance. When these species are under control they do not cause any damage to the cells as various enzymes are naturally present within the body which neutralizes their effects. At the point when redox balance is upset these least reactive species will start to react with various types of transition metals and these least reactive oxygen species will turn into highly reactive oxygen species which can lead to various types of disturbances within the human body like it can damage the plasma membrane or cell membrane, can damage lipids, proteins, nucleic acids, and various other cellular organelles which are present within cell (Valko *et al.*, 2006).

Antioxidants are the substances that neutralize the effect of oxidants, free radicals or reactive oxygen species, so they help to prevent oxidative stress, cellular damage, and tissue injury. They donate electrons to stabilize free radicals, terminating chain reactions and maintaining redox homeostasis, or redox balance. Various human made or synthetic antioxidants including propyl gallate (PG) etc have been used to protect oxidative stress for nearly a century. Concerns regarding carcinogenicity have precipitated restrictions on the utilization of these substances. So the need to discover a natural antioxidant.

Oil extracted from the mustard plant is known as crude mustard oil. Mustard oil have been used for treatment of joint pain and cold. Mustard seeds paste is used to reduce back pain, to treat arthristis, and

paralysis. The use of stem, leaves and seeds of mustard plant can reduce the risk of myocardial infraction commonly known as heart attack, one sided headache (migraine) and diabetes (Neetu *et al.*, 2014). Mustard oil contain large quantity of erucic acid. This acid can be harmful when used in excess amount according to some authors. The use of mustard oil is worldwide. Seeds of mustard are used to extract oil and other plant parts are used as vegetable. So mustard is an economical plant. It is now explored that mustard oil can be used for various other commercial purposes (Jham *et al.*, 2009).

Oil extracted from mustard plant is unsaturated as it contains carbon-carbon double bond in conjugation. It also contain hydroxyl group which donates its hydrogen atom, and thus neutralize the free radical and reduce the oxidative stress in the body. In order to determine the antioxidant properties of oil in laboratory various methods have been developed. These methods includes DPPH radical scavenging activity, ABTS, metal chelating assay, lipid peroxidation etc. the oil is extracted from the plants by thiobarbituric acid reactive substances (TBARS), which is used to evaluate the lipid contents of oil which can be used to check the reducing ability of the essential oil extracted from plants. Various types of assays are available to evaluate the antioxidant potential of a compound or essential oil but it is recommended to use more than one type of assay to evaluate their antioxidant potential. There are also some limitation associated with these assays as DPPH is only dissolvable in organic solvents like methanol and ethanol. TBARS is commonly used to evaluate lipid peroxidation, but some limitations are also associated with it.

MATERIALS AND METHODS

OIL EXTRACTION

Oil used for the research purpose was extracted from a suitable source, stored in bottles made up of glass and can be used within 3 months of extraction. Oil is extracted from the seeds of *Brassica campestris* commonly known as mustard plant. Plant was taken from the local areas of Rawalakot Azad Kashmir. Contents of oil were analyzed by GC-MS analysis. Oil content can be defined as the total oil extractable by ethanol under specified conditions. 0.3g of mustard seeds were weighed and transferred to glass mortar,

and 0.2g of glass powder (Pyrex glass washed with concentrated HCl) and anhydrous sodium sulphate was added to the mixture and mixture was reduced to very fine powder by using glass pestle and mortar. The mixture powder was transferred to a small percolator whose length is 20cm and diameter is 1.5cm. The pestle and mortar were washed two times with 3ml distilled water. The mixture was allowed to remain for five minutes and then percolated by adding 7ml solvent from the top of the column. One of the disk was weighed and then added extract into the disk having 1 inch filter paper. The disk having the extract was weighed again and the difference in the weight of the disk before and after the addition of the extract. The weighted difference tells us the weight of the oil extracted. The oil content was expressed in percentage of seed weight.

SPECTROPHOTOMETRY

A quantitative technique used to measure that how much a sample substance can absorb light is known as ultraviolet spectroscopy. The sample is placed in spectrophotometer and intensity of light that passes through the sample is determined, compared with the absorbance of the standard or blank sample.

3.5 ANTIOXIDANT ACTIVITIES

3.5.1 DPPH Radical Scavenging Activity

The antioxidant potential of the sample plant determined by the DPPH radical scavenging activity. Free DPPH is 1,1-diphenyl-1-picrylhydrazyl. One of the stable organic nitrogen radical is 2,2-diphenyl-1-picrylhydrazyl can absorb the maximum light at the wavelength of 517nm in UV-VIS spectrophotometer. The color of stable DPPH radical was dark purple but with the addition of antioxidants the color of solution gradually fades as the free radical was reduced to light yellow DPPH, which could be measured spectrophotometrically. To carry out the process DPPH solution was prepared by taking 0.00247g to 25ml ethanol. Dark purple colored solution was obtained. The solution was kept in dark for 30 minutes at room temperature. Oil solution was prepared by weighing 0.001g of mustard oil and dissolving it in 25ml ethanol. Different types of solution will be prepared by taking different concentrations of oil solution. The series of solution

contain concentrations as 0, 37.5, 75, 250, and 300µl. Each dilution of the solution were mixed with 500µl DPPH solution. All 5 test tubes were incubated for 30 minutes. To check the absorbance, UV VIS spectrophotometer was used. Absorbance of solutions was checked against standard at wavelength of 517nm. The control solution was prepared by adding 500µl DPPH with ethanol without sample solution (Dedic *et al.*, 2022).

All five test tubes were incubated for 30 minutes in dark and checked the absorbance at wavelength of 517nm.

Now we used the formula to calculate percentage scavenging.

$$\% \text{ scavenging} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

3.5.2 ABTS Assay

The ABTS assay is also a radical scavenging technique. ABTS solution was prepared by taking 8mg of ABTS dissolved in 10ml of water (solution A). Then 13.6g of potassium per sulphate was dissolved in 10ml water (solution B). Subsequently; 2ml of solution A and solution B were mixed. The solution was kept in dark for 16 hours. Afterwards, 1ml of this solution was taken and serial dilutions were prepared with ethanol until the absorbance reached at 0.7nm. Finally, 0.5ml of ABTS, 1ml of ethanol and different concentrations of oil extract 37.5, 76, 150, and 300µl of extract reacted. After 10 minutes, the absorbance was measured at 734nm. Blue green colored ABTS was produced when ABTS reacted with potassium per sulphate. The color of ABTS gradually faded and ultimately become colorless with increasing concentration of antioxidants from extract. As duration of reaction between ABTS and antioxidants of oil extract was increased, it resulted in more scavenging of ABTS which resulted in increased antioxidant activity. This was probably due to the donation of hydrogen from antioxidants of mustard oil (Sabir *et al.*, 2023).

3.5.3 Lipid Peroxidation Assay

Lipid peroxidation assay refers to the oxidative degradation of lipids, a process that results in cell membrane damage and is a marker of oxidative stress. Evaluating the lipid peroxidation of plant extract can

provide insight into their potential as protective agent against oxidative damage (Majewski *et al.*, 2020).

3.5.4 Metal Chelation Assay

The ortho phenanthroline was used to investigate mustard oil potential to chelate iron extract. Different concentrations of extract were added to the resultant mixture, ranging from 150 μ l of freshly prepared 2Mm FeSO₄ solution (0.03g of FeSO₄ in 10ml distilled water), 168 μ l of 0.1M Tris-HCl solution (1.21g Trisma base in 100ml water), and 218 μ l of 0.9% NaCl solution (0.9g NaCl in 100ml water). After 5 minutes of incubation, 13 μ l o-phenanthroline

3.6 GC-MS ANALYSIS

GC-MS investigation is a logical testing strategy that consolidates highlights of glass chromatography and mass spectroscopy to identify different types of substances inside the sample. GC-MS is regularly used to recognize obscure example, impurities or deposits. The structure of various natural ointment (mustard oil, basil) are examined by GC-MS analysis in order to distinguish the mixtures answerable for the qualities, charming botanical or fragrance scent or taste of a few important medicinal balms. This method was utilized for portrayal of the mixtures of medicinal oil of *Brassica campestris*. The oil sample was sent to The Central Peshawar University for characterization. The oil was shifted with a strainer to isolate it from the seeds and left it in daylight for 2 days. This pre-arranged oil was named as Sweet Mustard Oil and utilized for GC-MS examination. 2.2 GC-MS Examination The investigation was led utilizing a Follow 1300 series gas chromatography (Thermo logical) with an example infusion volume of 1 ml. The GC-MS run time for the example was 48.36 minutes, with a most extreme temperature of 350 °C. The temperature was inclined multiple times to give a scope of temperatures to the mixtures. At first, the temperature was set at 50 °C for 2 minutes and afterward customized to increase to 150°C at a pace of 5 °C/min, held at this temperature for 2 minutes. Then, at that point, the temperature was raised at the pace of 8 °C/minute until it came to 200 °C, held for 2 minutes, trailed by an ascent to 240 °C at a pace of 8 °C/minute, remained at this temperature for 2 minutes. At long last, the temperature was customized to 280 °C at a pace of 10 °C/min and held at this

temperature for 8 minutes. The example was infused in split less mode, and the mass ghostly sweep range was set somewhere in the range of 40 and 500amu (nuclear mass unit) with mass spectra kept in the electron ionization (EI) mode. Information assessment of GC-MS chromatogram was performed utilizing GC/MS. Chem station programming, and the isolated mixtures' name, atomic weight, and construction were found out by contrasting the mass spectra and information from Public Organization of Norms and Innovation (NIST) libraries.

Results

Antioxidant activity shown by oil mustard oil was determined in laboratory by using various types of activities like DPPH radical scavenging activity, ABTS radical cation movement and in vitro lipid peroxidation strategy. Cell reinforcement movement of mustard oil is likewise checked by utilizing metal chelation technique.

The synthesis of various oils (mustard oil, basil) are explored by GC-MS(glass chromatography and mass spectroscopy) analysis to distinguish the mixtures answerable for the qualities, wonderful botanical or smell scent or taste of a few significant medicinal ointments.

The study showed a dose-dependent increase in DPPH activity with scavenging percentage of 79 %, 80%, 82% and 83%. This result indicates a concentration-dependent increase in DPPH radical scavenging activity, with higher concentration of oil solution exhibiting higher antioxidant activity. Now it has become obvious that flavonoids and phenolic compounds present in different plants so that present in mustard oil are the chief factor of their antioxidant potential (Ara *et al.*, 2020).

Varying concentration of oil extract was used in peroxidation induced by SNP in egg yolk homogenate. The control test tube contain only yolk homogenate without oil remains yellow indicating no lipid peroxidation. Test tubes treated with SNP showed a distinct color change turning from yellow to dark yellow brown hue. Absorbance was measured spectrophotometrically at 532nm with control. These findings indicates that mustard oil is able to inhibit lipid peroxidation. These findings indicates that mustard oil can be effectively used as an antioxidant

which can prevents oxidative stress and protect our body from various types of diseases.

The results showed that crude mustard exhibit metal chelating activity, so it has potential antioxidant activity. Mustard oil diminish the accessibility of metal ion that stimulate the production of free radicals. Mustard oil is an effective chelator when used in excess, contributing to its overall antioxidant potential.

GC MS analysis:

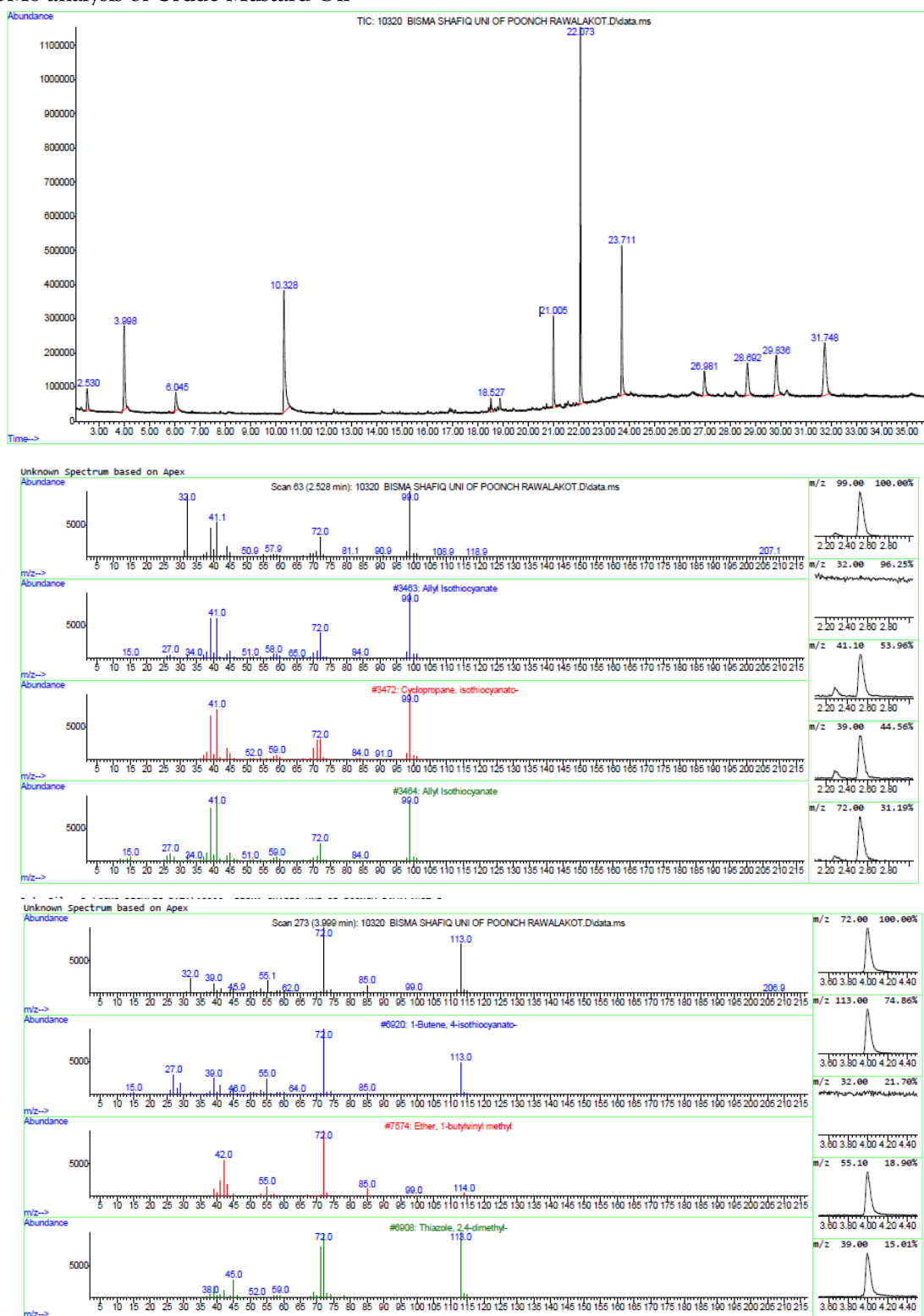
The identified components by GCMS analysis are, 4-isothiocynate(21.07%), 1-butylvinyl methyl (ether)(18.90), thiazole(15.01%),5-Ethylthiazole (28.35%), 1-Thiozol-2-yl(25.69%), Heneicosanic acid (21.83%), Benzoic acid (13.33% and 10.59%), methyl ester, 13-octadecanoic acid(26.10%), 9-octadecanoic acid (20.14%), 6-octadecanoic acid (15.80%),Hexandioic acid(29.52%), Diisooctyl adipate (24.79%), phathalic acid (15.66%), Terephthalic acid (58.15%), 1,4-Benzenedicarboxylic(49.42%), Beta tocopherol(28.04%), gamma tocopherol (60.91%),Ergosta-5,22-

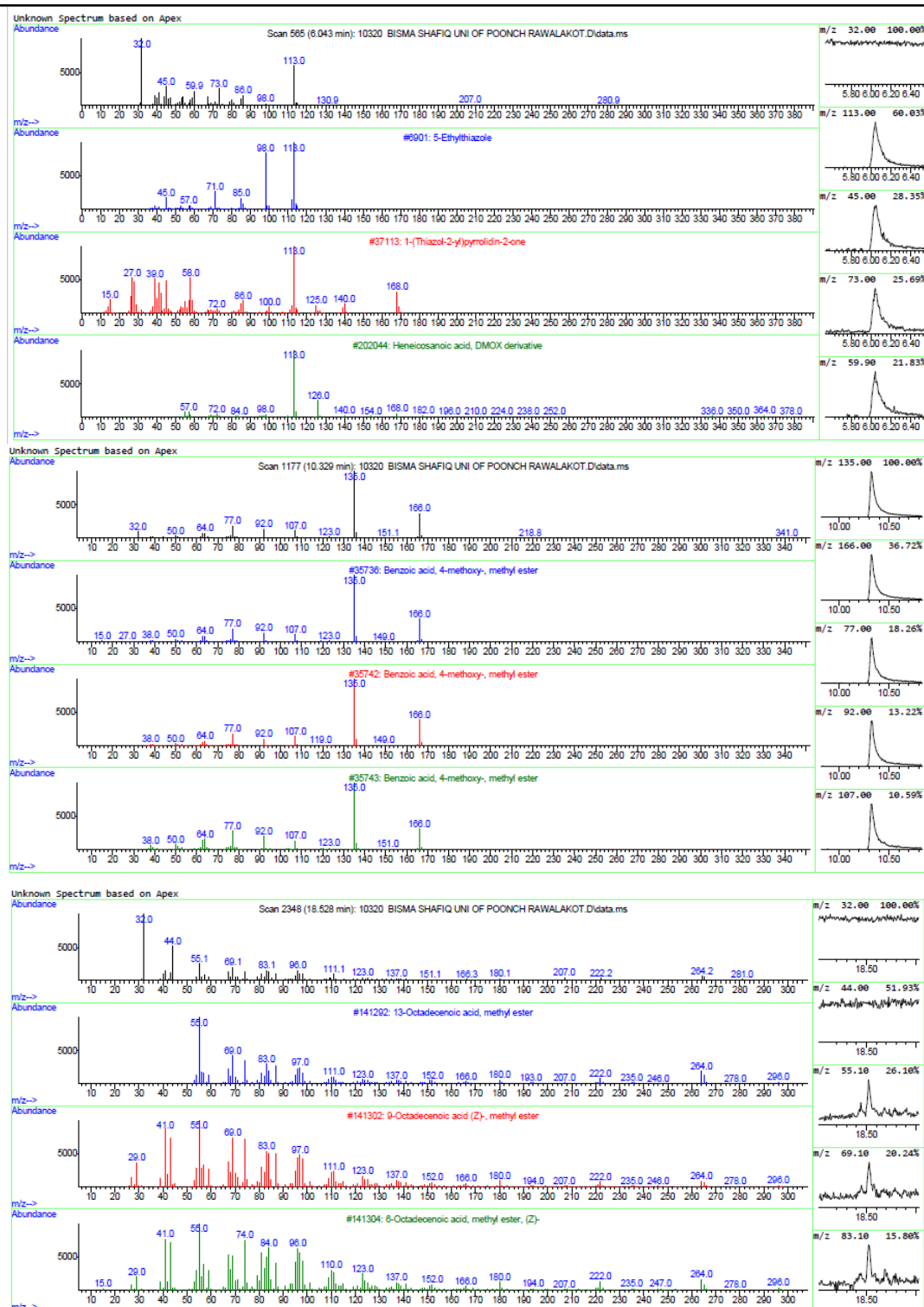
diene-3-ol (65.49%), Ergosta-5,7-diene-3-ol (64.30%), Nor-diazepam (60.38%), 5-Cholestene-3-ol,24,methyl (62.27%), cholestane (42.80%), Beta sistosterol (63.53%), and Gamma sistosterol (64.41%). All of these compounds make mustard oil a promising remedy against various diseases. So the oil should be used in daily cooking purpose. Comparing these results with the existing literature, mustard oil contain various essential components like hexanecanoic acid methyl ester, octadecanoic acid, Heneicosanic acid, methyl ester and various other types of esters. (Sarmina *et al.*, 2021). All of these results suggested that mustard oil is edible. The quality of oil is determined by free fatty acid (FFA) and Acid value (AV). The results shown by GC-MS analysis of mustard oil shows that mustard oil has low level of FFA and high level of FA. Unsaturated components (oleic acid) are also present in mustard oil due to which they act as antioxidants. Presence of these unsaturated components is useful as these components can reduce the rate of heart attack.

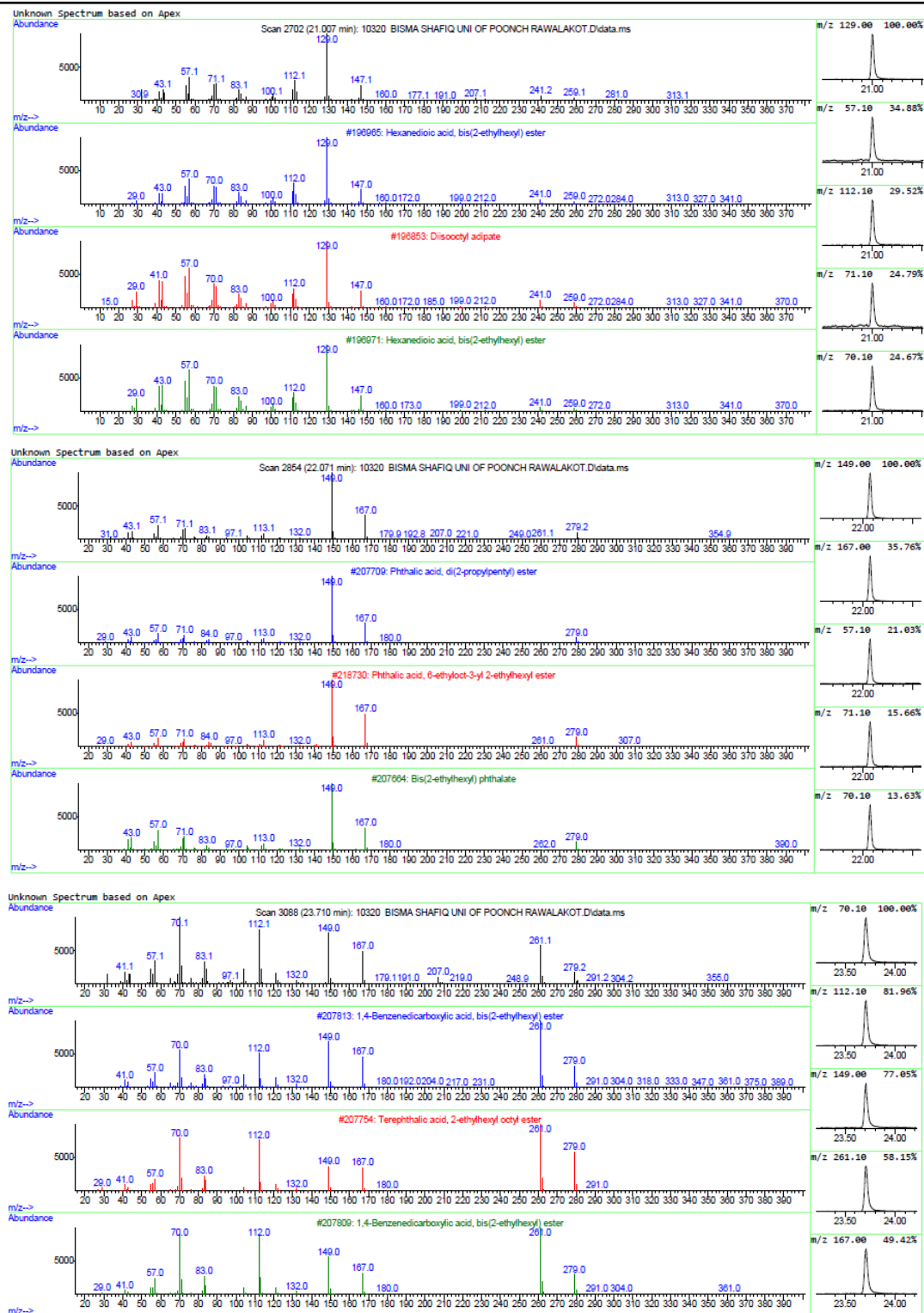
Table 4.5 GCMS analysis of Crude Mustard Oil

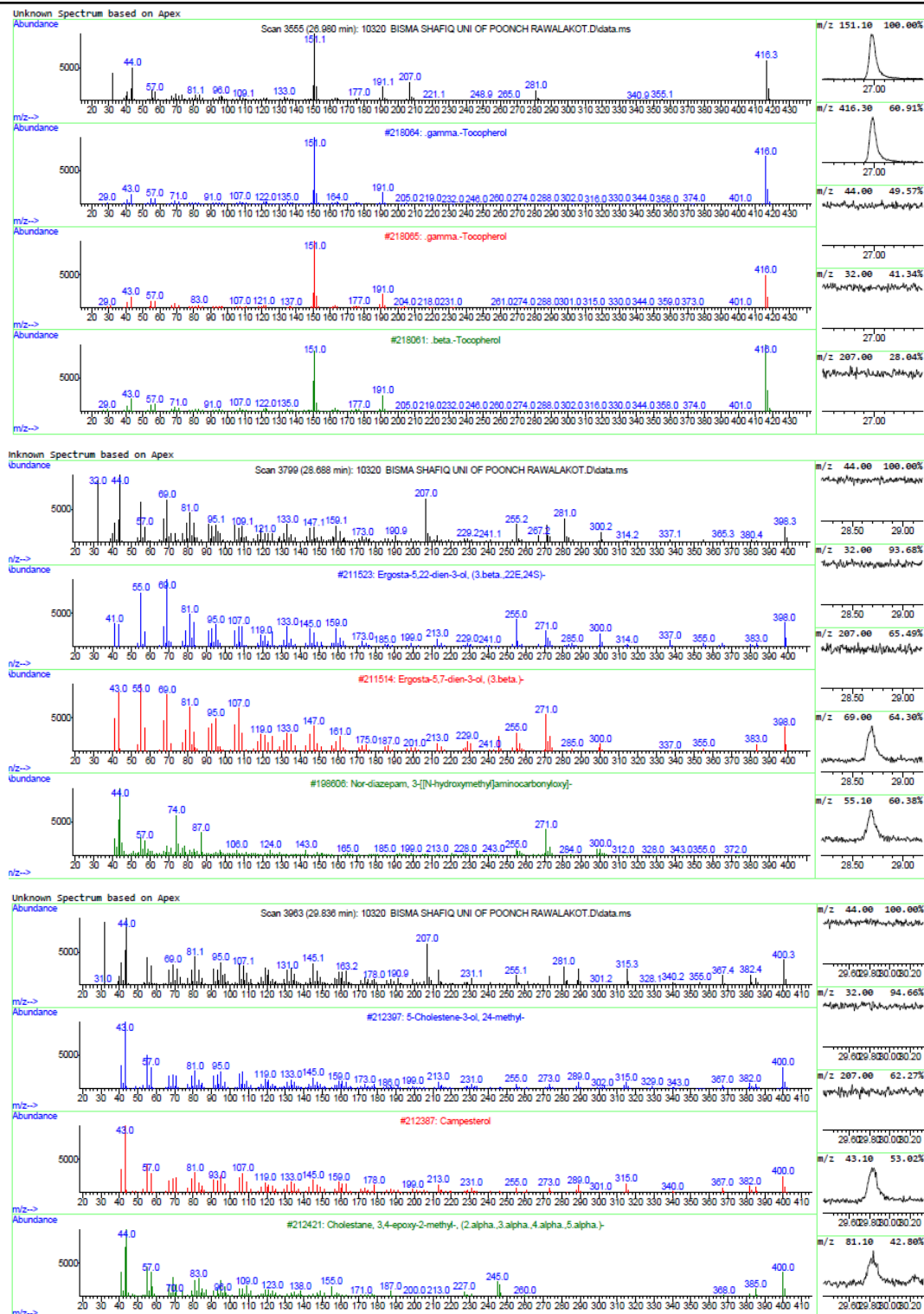
Name of compound present in oil	Percentage of compound %
Allyl isothiocynate	53.96 %
Cyclopropane 1-Butene, 4-isothiocynate	44.56 %
Ether(1-butylvinyl methyl)	18.90 %
Thiazole	15.01 %
5-Ethyl thiazole	28.35 %
Heneicosanoic acid	21.83 %
Benzoic acid	36.72 %
Methyl ester	51.93 %
Hexanedioic acid	24.67 %
Diisooctyl adipate	24.79 %
Phthalic acid	15.66 %
Terephthalic acid	58.15 %
Gamma- tocopherol	41.34 %
Beta- tocopherol	28.04 %
Ergosta-5,22-diene-3-ol	65.49 %
Ergosta-5,7-diene-3-ol	64.30 %
Nor-diazepam	60.38 %
5-Cholestene-3-ol	62.27 %
Campestrol	53.02 %
Cholestane	42.80%
Beta-Sisterol	63.53%

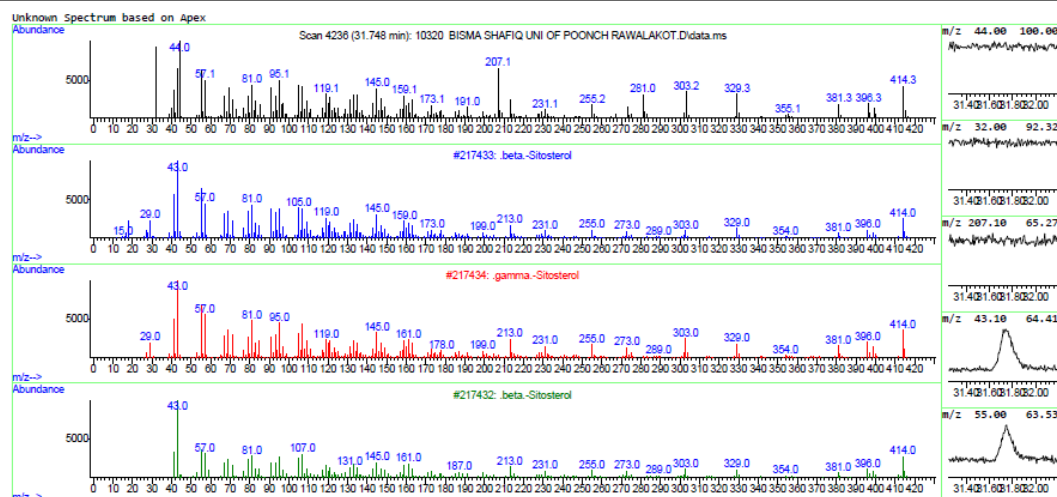
Table 1.1 GCMS analysis of Crude Mustard Oil











Figures of GC MS analysis

Discussion

These figures suggest that mustard oil contains various essential components. Each peak is shown by a specific component. The height of the peak shows the percentage of that component in the given mustard oil sample. All of these components are very essential. These results of GC-MS analysis show the percentage composition of mustard oil. All of these components indicate that mustard oil can be used in food as it acts as a strong antioxidant. Results of DPPH, ABTS, metal chelation and lipid peroxidation activities suggest that crude mustard oil can be effectively used as an antioxidant to protect our body from various types of diseases. Mustard oil protects us from the damage caused by the free radical produced in the body. Mustard oil can be used in cosmetics due to its sweet smell and can be used as hair oil. This study has various types of limitations. In this study, only an in vitro method of study was used. An in vivo study can still be performed. The GC-MS analysis provides us various types of components in crude mustard oil. About such components, further study could be performed to know how these components can be useful. Maybe there are some components which are harmful in consumption; such components could be identified and can be separated from the oil to make the oil perfect for use in daily life.

Conclusion

Mustard oil can be effectively used as an antioxidant which can protect our body from various types of fatal diseases like cancer, inflammation, aging etc.

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