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Bioactive Compounds and Chemical Profile of Raphanus sativus

Alyaa Abubaker [1], Ahmed A. M Abdallah [2], Nidal Eisa [3], Ayman A. A. Ismail [4], Abdel Karim M. [1*]

1 (Faculty of Science, Sudan University of Science and Technology, Khartoum 11116 Sudan, E-mail: Wagnahmed30@gmail.com)

2 (Faculty of Science and technology, Omdurman Islamic University, Omdurman 14415 Sudan, E-mail: Ahmedmoneem38@gmail.com)

3 (State Key Laboratory of Metastable Materials Science and Technology, Yanshan University, Qinhuangdao 066004 People's Republic of China, E-mail: nidaleisa298@gmail.com)

4 (Department of Chemical Sciences, Joint Doctoral School, Silesian University of Technology, 44-100 Gliwice, Poland, E-mail: aymanabdall181@gmail.com)

** Corresponding author E-mail: alyaaabubaker3@gmail.com*

Abstract:

This study investigates the chemical composition and antimicrobial activity of Raphanus sativus seed oil. Using gas chromatography-mass spectrometry (GC-MS), the oil was found to contain 15 major compounds, dominated by (Z)-13-docosenoic acid, methyl ester (35.48%), 9-octadecenoic acid (Z)-, methyl ester (17.12%), and cis-11-eicosenoic acid, methyl ester (11.64%). Antimicrobial activity was tested using the disc diffusion method against several human pathogens. The oil showed partial activity against Acinetobacter baumannii, Staphylococcus aureus, Bacillus subtilis, Candida albicans, and Aspergillus flavus, but was largely ineffective against Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. At higher concentrations (100 mg/mL), the oil demonstrated limited inhibition compared to standard antibiotics and antifungal agents. While the oil exhibited some antimicrobial properties, particularly against gram-positive bacteria and fungi, it was not as potent as conventional antibiotics. These findings suggest Raphanus sativus oil may have limited but potential use in antimicrobial applications.

Keywords *— **Raphanus sativus oil, GC-MS analysis, Fatty acids, Antimicrobial activity, Bioactive compounds.***

1. INTRODUCTION

The radish (Raphanus sativus) is a flowering plant in the mustard family, Originally domesticated in Asia. The radish is sometimes considered to form a species complex with the wild radish, and instead given the trinomial name Raphanus raphanistrum subsp. sativus.[1] Being relatively easy to grow and quick to harvest, radishes are often planted by novice gardeners. Use as a cover or catch crop in winter[2] or as a forage crop.[3] Some radishes are grown for their seeds; others, such as daikon, may be grown for oil production. Others are used for sprouting.The roots obtain their color from anthocyanins. Red varieties use the anthocyanin pelargonidin as a pigment, and purple cultivars obtain their color from cyanidin.[4] 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.[5] 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).5 The flesh of radishes harvested timely is crisp and sweet, but becomes bitter and tough if the vegetable is left in the ground too long.[6]. Also the radish is a diploid species, and has 18 chromosomes (2n=18) [7].

It has been used as a medicinal plant from a long time in plant era. It has laxative effects on the intestine and acts as an appetizer,[8] used for curing liver dysfunction and poor digestion, acts as antioxidant, anti-tumorigenic, antimutagenic, anti-

diabetic and anti-proliferative[8-9] It is also very well known for its use in the treatment of bronchitis and diarrhea.[10,11] Different parts of radish fruit, including roots, seeds, and leaves have multi-nutritional medicinal properties. The radish seeds have been used to treat asthma and other chest complaints. Radish oil is known to have many different health benefits, similar to those attributed to the radishes commonly eaten in salads. It is important to note that this typeof oil is only extracted from radish seeds, not from the roots. Domings et al.,[12] reported that the seed oil was used to produce biodiesel and the condition for the ethanolysis of seed oil was optimized applying the response surface methodology and compared physicochemical properties and thermal behavior between fodder radish crude oil and biodiesel. They found that fodder radish biodiesel can meet physicochemical property specification although its acid number requires attention[12] Oilseed radish has also been observed providing additional benefits of soil compaction reduction, soil aeration, weed suppression, and nitrogen trapping.[13,14] With these benefits in mind, oilseed radish is commonly referred to as a green manure crop. The seed of the oilseed radish contains 40% oil by weight. A high value oil makes this crop a good candidate for biodiesel production [15] .

This specie is used popularly to treat liver and respiratory illnesses[16]. The antibiotic activity of its extracts and its time persistence validates its effectiveness in microbial sickness as reported in traditional medicine. The root's juice showed antimicrobial activity against Bacillus subtilis, Pseudomonas aeruginosa, and Salmonella thyphosa. The ethanolic and aqueous extracts showed activity against Streptococcus mutans and Candida albicans. Aqueous extract of the whole plant presents activity against Sarcinia lutea and Staphylococcus epidermidis[17]. Aqueous extract of the leaves showed antiviral effect against influenza virus. Aqueous extract of the roots showed antimutagenic activity against Salmonella typhimurium .

Therefore the aim of the present study is extracted oil from powdered seeds of Raphanus sativus (500g) with n-hexane at room temperaturw and identify the chemical componen and biological activity.

2. MATERIALS AND METHODS

2.1 Plant material

Seeds of *Raphanus sativus* were purchased from the local Rhyad – Saudi Arabia and authenticated by direct comparison with a herbarium sample.

2.2 Extraction of oil's (Sample Preparation)

The seeds were collected, washed, dried and then ground Powdered seeds of Raphanus sativus (500g) were exhaustively extracted with n-hexane at room temperature.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4oC for further manipulation.

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

2.3 GC/MS Analysis

The extracts of the seeds analyzed with gas-chromatography mass spectrometry to obtain the number of compounds and molecular weight of the compounds present in the extracts chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used.Helium (purity; 99.99 %) was used as carrier gas[18,19]. The oven temperature was programmed to start at 150°C, where it was held for 1.00 minute, followed by an increase to 300°C at a rate of 4.00°C/min with no additional hold at the final temperature.

The chromatographic conditions for the analysis were as follows: the column oven was maintained at 150°C, and the injection port was set to 300°C. A split injection technique was used with a split ratio of 1.0. The system operated under linear flow control with a pressure of 139.3 kPa. The total flow rate through the system was 50.0 mL/min, while the

column flow was 1.54 mL/sec. The linear velocity of the helium gas was 47.2 cm/sec. Additionally, the system used a purge flow of 3.0 mL/min to maintain the cleanliness and stability of the carrier gas. These parameters ensured optimal resolution and sensitivity for the detection and quantification of the oil's components.

2.4 Antimicrobial assay

2.4.1. Bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 108-109colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

2.4.2. Fungal suspensions

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

2.4.3. Antimicrobial test

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

3. RESULTS

3.1. Chemical Composition Analysis for Raphanus sativus

The GC-MS analysis of *Raphanus sativus* seed oil revealed the presence of 15 distinct chemical components. The major constituents identified include (Z)-13-Docosenoic acid methyl ester, which dominated the composition with 35.48%, followed by 9-Octadecenoic acid (Z)-methyl ester at 17.12%. Other significant components were Cis-11-Eicosenoic acid methyl ester (11.64%), and 9,12-Octadecadienoic acid (Z,Z)-methyl ester (10.77%). Additionally, the oil contained (Z,Z,Z)-9,12,15-Octadecatrienoic acid methyl ester at 7.14% and Hexadecanoic acid methyl ester, which accounted for 5.48%. These components represent the primary fatty acids in the oil, contributing to its chemical profile.

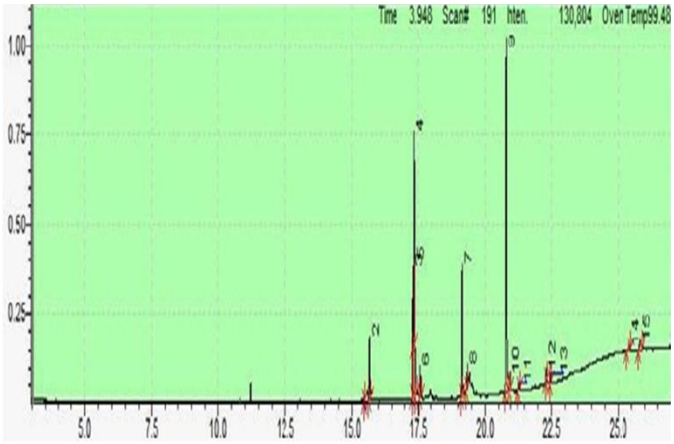

Figure1 *Total ion chromatogram of Raphanus sativus oil*

Table 1: Constituent of Raphanus sativus oil

Peak#	R.Time	Area	Area%	Name
1	15.472	61565	0.11	9-Hexadecenoic acid, methyl ester, (Z)-
2	15.664	3157853	5.48	Hexadecanoic acid, methyl ester
3	17.316	6204550	10.77	9,12-Octadecadienoic acid (Z,Z)-, methyl e
4	17.361	9856155	17.12	9-Octadecenoic acid (Z)-, methyl ester
5	17.385	4110173	7.14	9,12,15-Octadecatrienoic acid, methyl este
6	17.579	1415747	2.46	Methyl stearate
7	19.140	6705320	11.64	cis-11-Eicosenoic acid, methyl ester
8	19.338	1336160	2.32	Eicosanoic acid, methyl ester
9	20.786	20431171	35.48	13-Docosenoic acid, methyl ester, (Z)-
10	20.961	798998	1.39	Docosanoic acid, methyl ester
11	21.288	468825	0.81	Ethyl 9-hexadecenoate
12	22.312	695356	1.21	15-Tetracosenoic acid, methyl ester, (Z)-
13	22.468	544165	0.94	Tetracosanoic acid, methyl ester
14	25.432	767230	1.33	Olean-12-en-28-oic acid, 2.beta.,3.beta.,23-
15	25.844	1032770	1.79	Urs-12-en-28-al
		57586038	100.00	

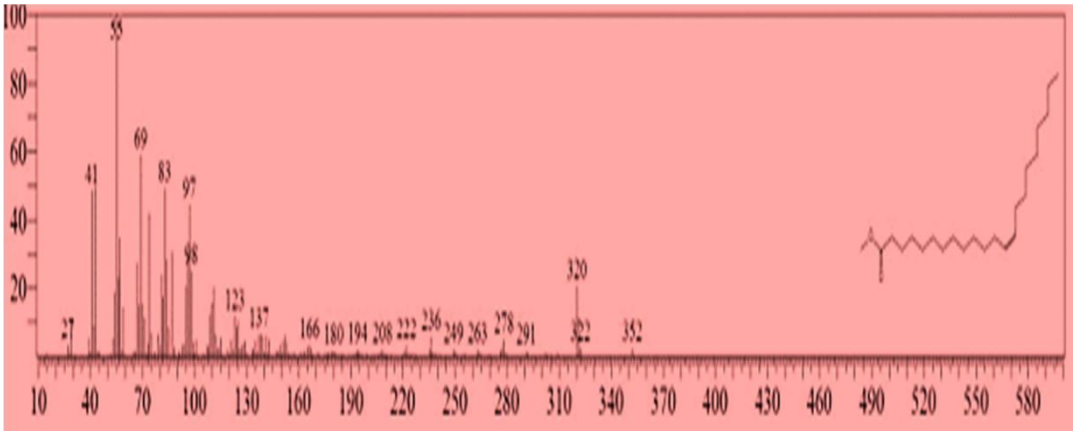

Fig. 2: The mass spectrum of 9-octadecenoic acid(z)methyl ester

In the above figure, the peak at m/z 296,RT(17.316) corresponds to the molecular ion : M+[C19H36O2]+ , while the signal at m/z 265 accounts for loss of a methoxyl function.

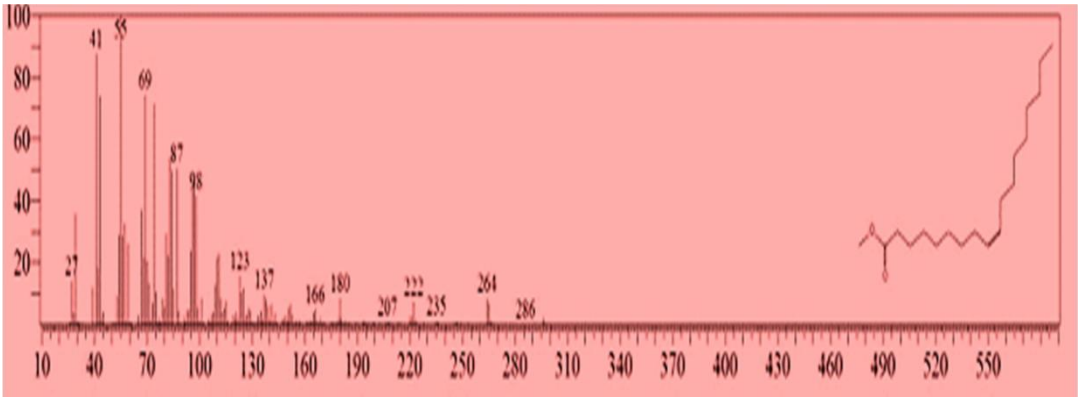

Fig. 3 : The mass spectrum of 9-octadecenoic acid(z)methyl ester

In the above figure, the peak at m/z 296,RT(17.316) corresponds to the molecular ion : $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z 265 accounts for loss of a methoxyl function.

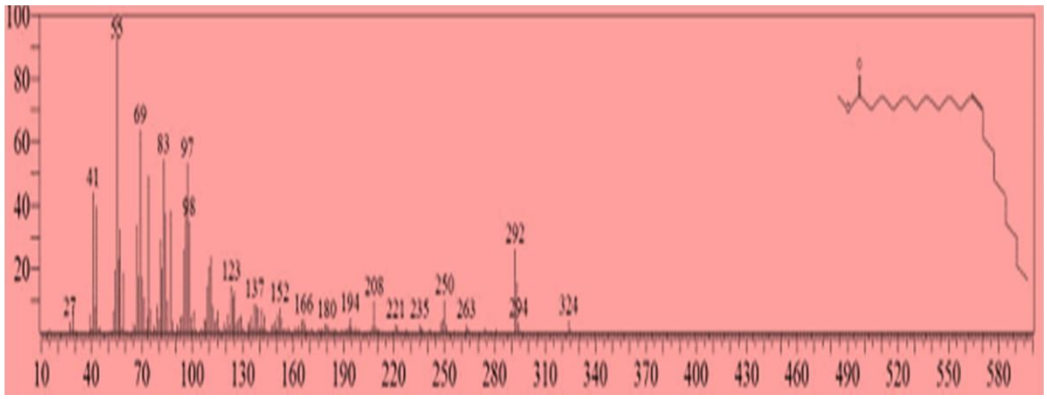

Fig. 4: The mass spectrum of cis-11-eicosenoic acid,methyl ester

In Fig.4 ,the peak at m/z 324,RT(19.140) accounts for the molecular ion:$M^+[C_{21}H_{40}O_2]^+$.The signal at m/z 293 corresponds to loss of a methoxyl.

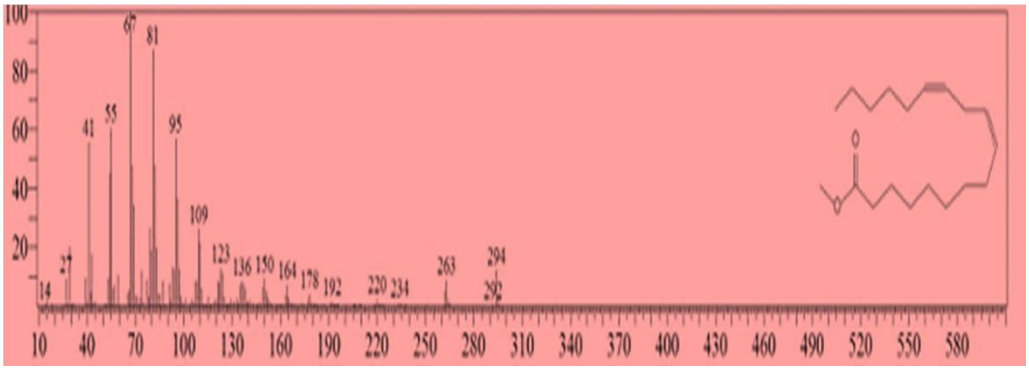

Fig. 5 : The mass spectrum of 9,12-octadecadienoic acid (z,z) methyl ester

The peak at m/z 294,RT(17.316) corresponds to $M^+[C_{19}H_{34}O_2]^+$, while the signal at m/z 263 corresponds to loss of a methoxyl function.

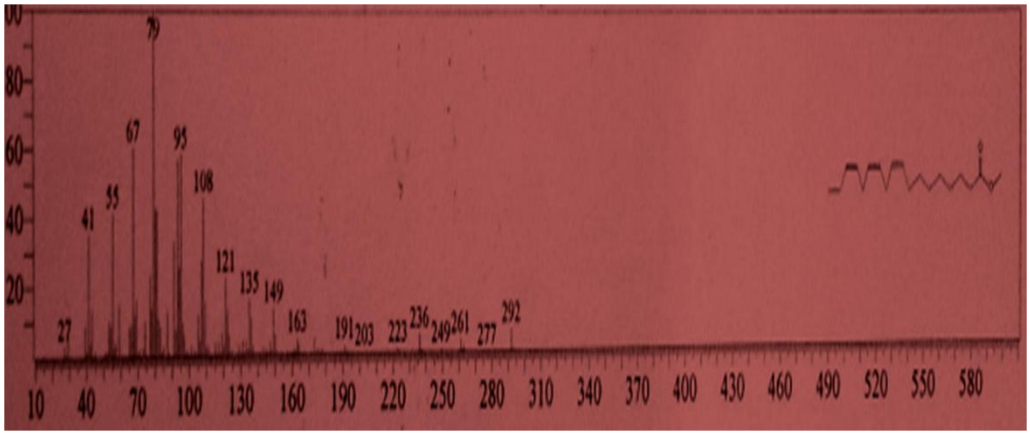

Figure6: the mass spectrum of 9,12,15-octadecatrienoic acid,methyl ester

In Figure 6 ,the peak at m/z 292,RT(17.358) accounts for : $M^+[C_{19}H_{32}O_2]^+$.The signal at m/z 261 is due to loss of a methoxyl.

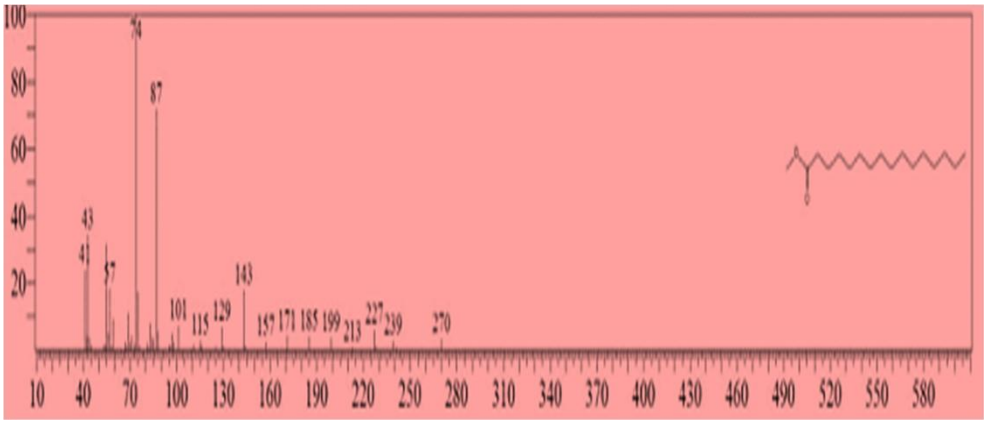

***Fig.** 7: The mass spectrum of hexadecanoic acid,methyl ester*

The mass spectrum of hexadecanoic acid,methyl ester is displayed in figure 7. The peak at m/z 270,RT(15.664) is attributed to the molecular ion : $M^+[C_{17}H_{34}O_2]^+$.The signal at m/z 239 corresponds to loss of a methoxyl group.

The composition of *Raphanus sativus* oil, as revealed by GC-MS, shows a rich mixture of unsaturated fatty acids, predominantly methyl esters of long-chain fatty acids. The most abundant compound, (Z)-13-Docosenoic acid methyl ester (also known as erucic acid), is commonly found in plant oils and is associated with various biological activities, including antioxidant and anti-inflammatory properties. The presence of 9-Octadecenoic acid (Z)-methyl ester (oleic acid) and 9,12-Octadecadienoic acid (Z,Z)-methyl ester (linoleic acid) suggests potential health benefits, as

these compounds are well-known for their roles in maintaining healthy lipid profiles and providing anti-inflammatory effects. The presence of (Z,Z,Z)-9,12,15-Octadecatrienoic acid (linolenic acid) further enhances the oil's nutritional value, as it is an essential omega-3 fatty acid involved in various physiological functions.

Hexadecanoic acid methyl ester (palmitic acid), though present in lower amounts, is a common saturated fatty acid that contributes to the structural integrity of cell membranes. The variety of unsaturated and saturated fatty acids in *Raphanus sativus* oil suggests that it may have diverse applications in both food and pharmaceutical industries, particularly due to its potential anti-inflammatory and antioxidant properties.

3.2 Antimicrobial Activity

The antimicrobial activity of *Raphanus sativus* oil was evaluated against several pathogens using a disc diffusion assay. The oil exhibited partial activity against *Acinetobacter baumannii*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus flavus* at 100% concentration, with inhibition zones ranging from 7 mm to 10 mm. However, the oil showed minimal to no activity against *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, with inhibition zones of 6-7 mm at both 50% and 100% concentrations. Compared to standard antibiotics such as Ampicillin and Kanamycin, the oil's antimicrobial activity was significantly lower. For fungal strains, *Candida albicans* and *Aspergillus flavus* exhibited slight sensitivity to the oil, with inhibition zones of 7 mm and 9 mm, respectively. In contrast, Nystatin demonstrated stronger antifungal effects, particularly against *Aspergillus flavus*. These results suggest that *Raphanus sativus* oil has limited antimicrobial potential, particularly against gram-negative bacteria, while showing some promise against gram-positive bacteria and fungal strains at higher concentrations.

Table 2: Antimicrobial Activity of Raphanus sativus Oil

	Concn. mg/ml					
	oil			Amp	Kan	Nys
Strain	0%	50%	100%	10	10	10
Escherichia coli	-	6±0.1	6±0.7	25±0.4	21±0.3	N
Klebsiella pneumoniae	-	6±0.2	7±0.4	22±0.4	24±0.3	N
Acinetobacter baumannii	-	7±0.4	10±0.2	22±0.3	16±0.3	N
Pseudomonas aureginosa	-	6±0.1	7±0.4	29±0.3	11±0.3	N
Staphylococcus aureus	-	6±0.4	9±0.4	32±0.1	19±0.1	N
Bacillus subtilis	-	6±0.1	8±0.4	19±0.4	21±0.4	N
Candida albicans	-	6±0.1	7±0.4	N	N	12±0.1
Aspergillus flavus	-	6±0.7	9±0.4	N	N	15±0.1

-ve: gram negative, +ve: gram positive, C: colony forming, F: filameous, -: no activity, N: Not Valid

4. CONCLUSIONS

In conclusion, the chemical analysis and biological evaluation of Raphanus sativus seed oil revealed its promising but limited antimicrobial properties. The GC-MS analysis identified 15 significant compounds, with (Z)-13-docosenoic acid, methyl ester being the most abundant. While the oil demonstrated partial antimicrobial activity against certain gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis) and fungal strains (Candida albicans, Aspergillus flavus), it was generally less effective against gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. The oil's efficacy was notably weaker in comparison to standard antibiotics like Ampicillin and Kanamycin, which showed greater zones of inhibition across all tested strains. However, the oil did exhibit some inhibitory effects at 100 mg/mL concentration, particularly against Acinetobacter baumannii, which suggests that certain constituents of Raphanus sativus oil may possess selective antimicrobial capabilities. Despite its modest antimicrobial activity, the oil's bioactive composition highlights its potential for further investigation in the development of natural antimicrobial agents. Nonetheless, more comprehensive studies are required to optimize its efficacy and determine its suitability for medicinal applications.

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