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Chemical Differentiation and Antimicrobial Potential of Four *Brassica napus* L Seed Oils

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Abstract

The conducted study compares the phytochemical and the antimicrobial potential of four varieties of *Brassica napus* seed oils. The plant seeds were cultivated during the winter growing season. Soxhlet extractor and Gas Chromatography-Mass Spectrometer (GC-MS) were used for essential oil analysis. The micro broth dilution assay was applied to test the antimicrobial potential (MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration) of the extracted essential oils against different bacterial strains. A total of 56 phytochemicals were found, including 23 and 25 compounds in the oils of Pactol and Rapifera seed varieties, respectively, and 21 compounds in each of Bacara and Rally seed oils. Oleic acid constituted about 35.97 %, 15.62%, 7%, and 2.41 % for Rally, Bacara, Rapifera, and Pactol seed oils, respectively. Gram-positive bacteria, *Streptococcus pyogenes* and *Streptococcus agalactiae*, showed lower resistance potentials (MIC= 0.78%, 3.125% respectively) (MBC=1.36%, 6.25% respectively) to the essential oils compared with *Staphylococcus aureus*. *Escherichia coli* showed higher sensitivity (6.25% and 12.5% for MIC and MBC, respectively) than *Klebsiella pneumonia* and *Pseudomonas aeruginosa* to the *B. napus* seed oils. Gram-positive bacteria were more sensitive to the tested essential oils than Gram-negative bacteria. Overall, four different seed varieties have important chemicals and fatty acids. Oleic acid was the most common carboxylic acid (fatty acid) and 2,4-decadienal with hexanal were the most prevalent aldehydes in four seed oils. Tested *B. napus* seed essential oils showed antimicrobial activities against various Gram-positive and negative bacteria and *Candida albicans*, with Pactol seed oils exerting the highest activity.

Keywords: *Brassica napus* L, Essential oil, Antimicrobial activity, GC-MS

التمايز الكيميائي وإمكانات مضادات الميكروبات لزيوت اربعة أصناف من بذور *Brassica napus L*

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الخلاصة

تم مقارنة بعض المركبات الكيميائية النباتية وجهد مضادات الميكروبات لزيوت اربعة اصناف من بذور السلجم *Brassica* napus L والتى تم زراعتها خلال الموسم الشتوي فى حقل كرده ره شه التابعة لكلية علوم الهندسة الزراعية –اربيل . تم استخلاص الزيت باستخدام جهاز Soxhlet ثم حللت الزيوت العطرية باستخدام كروماتوجرافيا الغاز – مطياف الكتلة (GC–MS). تم تطبيق فحص السائل المخفف للمستخلصات النباتية الدقيقة في اختبار مضادات الميكروبات حيث، تم الحصول على إجمالي 56مكونًا، والتي اشتملت على 23 و 25مركبًا لزيوت بذور Pactol و ,Rapifera ، على التوالي. و21 مركبًا لكل من زيوت بذور Rally and Bacara. تشكل حامض الأوليك 35.97 %, 15.62%, 7%و 2.41% لزيت بذور كل من Rally, Bacara, Rapiferaو Pactol على التوالي. ضمن البكتيريا موجبة الجرام، أظهر كل من *Streptococcus pyogenes* و *Streptococcus agalactiae* جهد مقاومة أقل (MIC = 0.78٪، 3.125٪ على التوالي) (MBC = 1.36٪، 6.25٪ على التوالي) إلى essential oils عند مقارنتها مع *Staphylococcus aureus*. أظهرت النتائج ان *Escherichia coli* اكثر حساسية (6.25٪ و12.5٪MIC وMBC على التوالي) من *Klebsiella* و*Pseudomonas aeruginosa*. أخيرًا، كان للأصناف الاربعة من البذور مركبات كيمياوية و احماض دهنية مهمة، ان حامض الاوليك هو الاكثر شيوعا كحامض الكربوكسيل و 2,4–decadienal الديهايد كان الأكثر انتشارًا في زيوت الاصناف الاربعة . ان زيوت الاصناف الاربعة لها أنشطة مضادة للميكروبات ضد البكتيريا بما فيها إيجابية الجرام والسلبية، وكانت المبيضات البيضاء، حيث كان زيت بذور Pactol لها أعلى نشاط.

1. Introduction

Rapeseed (*Brassica napus* L.) is being used as edible oil in many countries. It is also known as canola, which is a member of the Brassicaceae family in yellow flowers. In recent years, Herbal medicine gained more popularity because of the effective phytochemicals and lesser side effects than chemical drugs. Essential oils are natural volatile compounds, synthesized naturally in various plant parts as secondary metabolic byproducts. They are known for their phytochemical contents, such as terpenoids, phenolics, and flavonoids, each playing many biological activities with more beneficial effects than a chemically synthesized pure compound. This may be related to their complex mixture content acting synergistically that can interact with multiple molecular sites [1]. Usually, their extraction is achieved by steam distillation or Soxhlet extraction and their phytochemicals are analyzed by gas chromatography-mass spectrometry. Based on the seed varieties and climate, the oil yields range between 30-45% [2].

Brassica napus is well identified as a rich oil plant with minimum saturated fatty acids (5-7%) and maximum polyunsaturated fatty acids which are estimated to contain 7-10% α-linolenic and 17-21% linoleic acids. This oil is therefore considered as very healthy edible oil [3]. Classes of fatty acids are very important when evaluating the nutritional value of vegetable oils. For example, oleic acid in *Brassica* spp. is known as a valuable unsaturated fatty acid, while the increased level of erucic acid in the seed oils of *Brassica* spp. has links to various health risks [4].

Phenolic compounds exist widely in plants. They are plant secondary metabolites. They are more abundant in rapeseed oils than most other plant seed oils. Some phenolics of *Brassica* spp. include free, insoluble bound, and esterified phenolic acids. Based on the plant oil and method of oil processing, the phenolic acid contents of different plants ranged between 6400 and 18400 μg/g. while, rapeseed flour contains nearly 30 times higher phenolic acid content than that of some plants like soybeans [5].

Plant extracts from herbs and plant seeds are mostly known for their phenolic and flavonoid contents that exhibit different antimicrobial activities [2]. For instance, some variances of *Brassica napus* L oils were shown to suppress the growth of *Klebsiella pneumonia*, *Staphylococcus epidermidis*, and *Pseudomonas originals* [6]. Canola has been introduced after nutritional up-rationing of oil by genetic alteration of *Brassica* cultivars (*Brassica campestris* and *Brassica napus*) and mustard (*Brassica juncea*), having less than 2% erucic acid in their oil [7].

Scientists have already declared that *Brassica* spp. have tremendous amounts of valuable seed oils that could be considered for frying and food purposes after refining and processing [8]. Geographical area plays a vital role in the phytochemical constituents. Thus, 4 seed varieties of *B. napus* with different origins (France, Sweden, India and Iraq) were purchased to compare their phytochemical constituents and antimicrobial activities against selected microbes isolated from different clinical specimens.

2. Materials and Methods

2.1 Sample Preparation

The Rally, Bacara, Rapifera, and Pactol seed varieties of *Brassica napus L.* were purchased from commercial markets in France, India, Sweden, and Iraq. The identification and authentication steps were achieved by the Department of Field Crop/ College of Agricultural Engineering/ University of Salahaddin /Erbil. The seeds were cultivated during the winter growing season at Grda-rasha Research Field, University of Salahaddin /Erbil. A large amount of plant seedswere freshly collected and air dry temperature of 40 °C was applied for later analysis, Figure 1.

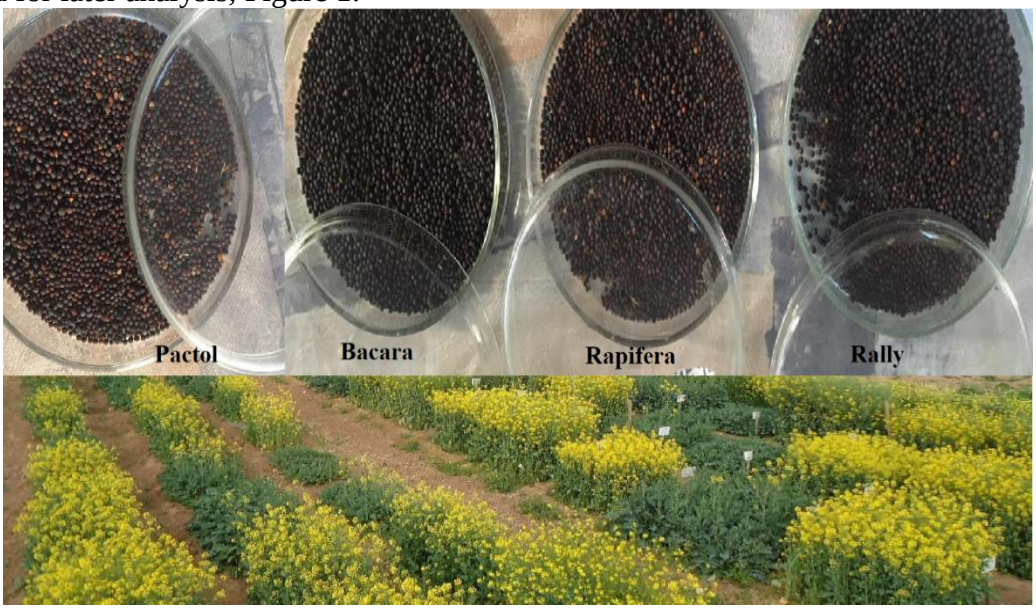


Figure -1: The harvested seed varieties of *Brassica napus* in the Grda-rasha field.

Crushing of dried seeds was performed using mortar and pestle. The crushed seed materials were transferred into the Soxhlet extractor with n-hexane being added as a solvent. The process was left to run for 3 hours. The essential oils were collected in tightened vials and stored at 10 °C [2].

2.2 Chemical analysis of essential oils

Gas chromatography-mass spectrometer (GC-MS) analysis was used for the phytochemical investigations of the essential oils contained in 4 varieties of rapeseeds, following the procedure described by Seow *et al*., Oroojalian *et al.*, and Ahmed *et al.* [2, 3, 9]. Phytochemical analysis of each oil extract was achieved by using Shimadzu QP-2010 GC gas chromatograph-mass spectrometer. The GC was equipped with HP-5 MS (5% phenylmethyl

siloxane) and capillary column (30 m × 0.25 mm i.d., film thickness 0.25μm), in the temperature program of 60°C (2') to 250 °C for 10 minutes, with a rate of 20 °C /min and helium flow rate of 1.61 ml/minute. The ion source was maintained at 250 °C with electron energy of 70 eV. The oil extract samples were added into methanol and then 1 μl of the mixture was injected into the column. Based on the Wiley library database, the unknown component was recognized depending on the comparison of its mass spectrum with that of the known components. Chemical identifications, including names, structures, and molecular weights of sample contents were eventually obtained as described previously by Fujimura *et al*. [10]. Afterward, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the oils were prepared using dimethyl sulfoxide (DMSO).

2.3 Antimicrobial Activity

2.3.1 Microbial Strains

The antimicrobial activity of four *Brassica napus* seed oils was evaluated against three Gram positive bacteria, namely *Staphylococcus aureus, Streptococcus pyogenes,* and *Streptococcus agalactiae*, three Gram negative bacteria, namely *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa,* and one *Candida albicans* isolate. All the obtained isolates were identified using VITEK 2 Compact system (bioMérieux, Nurtingen, Germany).

2.3.2 Determination of antimicrobial activity by broth microdilution Method

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of four *Brassica napus* seed oils were determined using the broth microdilution assay. The test was performed using 96 wells microtiter plate. One hundred microliters of each essential oils were diluted in 100 μL dimethyl sulfoxide (DMSO) to give a concentration of 50%. Serial twofold dilutions were made (25%, 12.5%, 6.25%, 3.125%, 1.56% and 0.78%). Amoxicillin was used as a positive control (250 μg/mL). The volume of each of the four seed oils was 100 μL per well, along with 100 μL of Brain Heart Infusion broth. A turbidity standard of 0.5 McFarland for each microorganism was prepared and 20 μL was added to the wells. Three control wells were maintained for each test; positive control (well with antibiotic), negative control (well with DMSO), and sterility control (well containing the broth without any additions). The plates were incubated at 37°C for 24 h. The MIC, MBC, and MFC values of the tested essential oils were confirmed by sub-culturing bacterial cells on the Muller Hinton agar or Sabouraud dextrose Agar. The plates were incubated at 37°C for 24h. The lowest concentration of the essential oils required to inhibit the visible growth of the tested microorganism in the well was designated as the MIC, whereas MBC and MIC were defined as the concentrations of the essential oils that completely inhibited the growth of microorganism on subculture plates. Duplicates were made and average values were recorded [11, 12].

3. Results

3.1 Chemical analysis of essential oils

The oils were obtained in 39.30%, 34.62%, 34.31%, and 34.29 yields (w/w), for Pactol, Bacara, Rapifera, and Rally seed varieties, respectively. The phytochemical study of these seed varieties showed different quantitative and qualitative results (Table1). The GC-MS analysis of seed varieties showed 56 chemicals, including 23 and 25 compounds in Pactol and Rapifera, respectively, and 21 compounds in each of Bacara and Rally seed oils, which are related to 100% of their total volatiles, as illustrated in Figures 2, 3, 4, and 5, respectively.

Collectively, different compounds were identified, which could be classified as aldehydes, esters, fatty acids, carboxylic acid, terpenoids, and alcohols. according to the obtained data, 7 phytochemicals were common to the four varieties, including pentadecanoic acid (1.23,3.30,120, and 2.56% , nonanal] (4.88,1.42,4.4, and 0.63%) , 2-undecenal

(14.27,3.80,11.10, and 1.95%) , 2-decenal (13.61,3.77,9.0, and 3.77) , 2,4-decadienal (15.64,17.28, 11.15, and 12.05%) , 9-octadecenoic acid (2.41, 15.6, 7 and 35.97%) , and hexadecane, 2,6,10,14-tetramethyl- (2.13, 10.35, 1.12, and 3.93%) for Pactol, Bacara, Rapifera, and Rally, respectively.

The highest percentage content of phytochemicals in Pactol and Bacara seed oils was that recorded for 2,4-decadienal, which constituted 15.64 % and 17.28 %, respectively of their total oil content. While hexanal (caproic aldehyde) was the most abundant chemical in Rapifera seed oils (19.72%). Rally seed oil analysis showed 9-octadecenoic acid as the prevalent phytochemical, comprising 35% of its total volatiles.

Based on the prevalence of organic class in the four seed varieties, aldehyde (mainly 2,4-decadienal and hexanal) were most common with higher percentages of 59.85% and 45.52% in Pactol, Rapifera, as compared to 31.24% and 22.88% in Bacara and Rally, respectively. The second most common compound was carboxylic acid (mainly 9-octadecenoic Acid (oleic acid)) which showed the higher percentages of 57.82% and 35.77% in Rally and Bacara, respectively, compared with 21.49% and 17.45% in Rapifera and Pactol, respectively. The four seed oils consisted of twenty-one carboxylic acids, one sesquiterpene hydrocarbons, 14 aldehydes, nine ketones, one alcohol, nine esters, and one compound of other classes.

Fatty acids (a class of carboxylic acids) were found in 6 types, including oleic acid, stearic acid, caproic acid, pentadecylic acid, caprylic acid, and pelargonic acids. the fatty acid total content values were 50.32%, 26.04%, 13.14%, and 6.11% for rally (4 types), bacara (4 types), rapifera (5 types), and pactol (3 types), respectively. oleic acid was the most common fatty acid in the 4 see
varieties (Figure 6).

Table 1- GC-MS phytochemical constituents of Pactol, Bacara, Rapifera, and Rally varieties of B. napus L

No.	Compound Name and Molecule Wight g/mol	Molecular Formula	Pactol		Bacara		Rapifera		Rally	
			tR[a]	C[%][b]	tR[a]	C[%]b	tR[a]	C[%]b	tR[a]	C[%]b
1.	Hexanal (caproic aldehyde) 100.16	$C_6H_{12}O$	3.34	8.72	2.52	3.15	3.12	19.72	NF	
2.	Hexanoic acid , Caproic acid, 116.1583	$C_6H_{12}O_2$	5.19	2.04	NF		5.11	2.89	NF	
3.	Octanal, 128.12	$C_8H_{16}O$	5.41	0.74	NF		5.41	0.70	NF	
4.	Hexanoic acid, 2-hexenyl ester, 198.30	$C_{12}H_{22}O_2$	5.82	2.40	NF		NF		NF	
5.	6-Methyl-hept-2-en-4-ol, 128.21	$C_8H_{16}O$	6.05	2.57	NF		NF		NF	
6.	Nonanal, 142.23	$C_9H_{18}O$	6.12	4.88	6.12	1.42	6.44	4.83	6.1	0.68
7.	2-Nonenal, 140.26	$C_9H_{16}O$	6.46	0.79	NF		NF		NF	
8.	Decanal, 156.2	$C_{10}H_{20}O$	6.69	1.20	NF		NF		NF	
9.	Cyclohexanone, 2-ethyl-,126.20	$C_8H_{14}O$	6.86	3.54	NF		NF		NF	
10.	Bicyclo[3.1.1]heptan-2-one, 3,6,6-trimethyl-,152.23	$C_{10}H_{16}O$	6.91	1.53	NF		NF		NF	
11.	2-Decenal, 154.25	$C_{10}H_{18}O$	6.98	13.61	6.98	3.77	7.85	9.01	6.98	3.17
12.	2,4-Decadienal, ,152.23	$C_{10}H_{16}O$	7.23	15.64	7.24	17.28	8.12	11.15	7.1	12.05
13.	Octadecanoic acid, 2-oxo-, methyl ester, 312.5	$C_{19}H_{36}O_3$	7.34	8.71	7.35	3.71	NF		NF	
14.	2-Undecenal,168.28	$C_{11}H_{20}O$	7.43	14.27	7.43	3.83	8.67	11.10	7.4	1.95
15.	6-Azabicyclo[3.2.1]octane,111.18	$C_7H_{13}N$	7.48	3.45	NF		NF		NF	
16.	1,2-Butanediol, 1-(2-furyl)-2-methyl-,170,21	$C_9H_{14}O_3$	7.89	0.76	NF		NF		NF	
17.	Trideuteriomethyl 10-Epoxy-7-Ethyl,170.25	$C_{10}H_{18}O_2$	7.92	1.23	NF		NF		NF	
18.	Hexadecane, 2,6,10,14-tetramethyl-,282.55	$C_{20}H_{42}$	7.95	2.13	8.72	10.35	9.65	1.12	8.7	3.93
19.	7-Oxabicyclo[4.1.0]heptane, 2-methyl-,112.17	$C_7H_{12}O$	7.99	1.06	NF		NF		NF	
20.	Pentadecanoic acid,242.40	CH3(CH2)13COOH	9.57	1.23	9.57	3.30	12.43	1.20	9.66	2.56
21.	9-Octadecenoic Acid (Oleic acid),282.5	$C_{18}H_{34}O_2$	10.38	2.41	10.38	15.62	13.48	7.00	10.39	35.97
22.	Octadecanoic acid (Stearic acid),284.48	$C_{18}H_{36}O_2$	NF		10.47	5.27	13.60	1.29	10.47	7.22
23.	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-,243.24	$C_{12}H_{10}FN_5$	10.47	6.36	NF		NF		NF	
24.	12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2, 240.34	$C_{14}H_{24}O_3$	11.36	0.73	NF		NF		NF	

1.	Undecanoyl chloride,	$C_{11}H_{21}ClO$	NF	6.86	1.45	7.66	4.91	NF	
2.	Nonanoic acid,158.23	$C_9H_{18}O_2$	NF	6.95	1.86	NF		6.98	0.57
3.	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl), 220.30	$C_{14}H_{20}O_2$	NF	7.92	4.58	NF		NF	
4.	Ketone, 2,2-dimethylcyclohexyl methyl,154.25	$C_{10}H_{18}O$	NF	8.83	2.12	NF		NF	
5.	Tetracosane,338.65	$C_{24}H_{50}$	NF	9.36	1.39	NF		NF	
6.	Trico sane,324.6	C23H48	NF	NF		NF		9	0.51
7.	1,2-Benzenedicarboxylic acid, diisononyl ester,418.60	$C_{26}H_{42}O_4$	NF	9.66	3.55	NF		9.66	2.5
8.	Docosane,310.60	$C_{22}H_{46}$	NF	10	3.18	12.43	0.49	NF	
9.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester,330.50	$C_{19}H_{38}O_4$	NF	13.09	5.59	15.56	1.68	15.81	8.56
10.	2,2-Diphenyl-4-pentenylamine, 235.32	$C_{17}H_{17}N$	NF	14.05	2.11	NF		NF	
11.	3,3-diphenyl-4-hexenoic acid, 266	C18 H18O2	NF	14.1	3.70	NF		NF	
12.	Octadecanoic acid, 2-hydroxy-1- C21H42O4 (hydroxymethyl)ethyl ester,358.5		NF	15.81	2.73	NF		NF	
13.	Hexanal, 3-methyl-114.19	C7 H14O	NF	NF		4.28	0.67	NF	
14.	Hexanoic acid, 2-propenyl este 156.22 C9H16O2			NF		5.99	2.24	NF	
15.	6-Methyl-hept-2-en-ol		NF	NF		6.32	3.74	NF	
16.	Octanoic acid, 144.21	$C_8H_{16}O_2$	NF	NF		6.99	0.76	NF	
17.	Cyclopentane, 1,1,3,4-tetramethyl-, cis C9H18		NF	NF		7.73	1.22	NF	
18.	cis-1,4-Dideuterio-1,4-cyclohexandiamine C6H14N2		NF	NF		7.93	1.01	NF	
19.	4-Nonanone, 7-ethyl-,170.29	C11H22O	NF	NF		8.51	6.75	NF	
20.	Decanedinitrile (Sebacontrile) 164, C10H16N2		NF	NF		8.55	2.33	NF	
21.	1,4-Epoxycyclohex-2-enole,98.14	C6H10O	NF	NF		8.76	2.95	NF	
22.	1-Eicosanol,298	CH3(CH2)19 OH	NF	NF		9.42	0.65	NF	
23.	9,12-Octadecadienoic acid, C19H34O2 methyl ester 294,		NF	NF		14.5	0.59	NF	
24.	Nonane, 3-Methyl-5- Propyl,184.36	C13 H28	NF	NF		NF		8.30	0.47
25.	Acetic acid, chloro-, hexadecyl ester,318.9	C18H35Cl O2	NF	NF		NF		2.5	11.52
26.	Dodecane, 2,6,10-trimethyl-, 212,		NF	NF		NF		7.8	0.87

	C15H32						
27.	Octadecane, 1-chloro-288.9	CH3(CH2)16 CH2Cl	NF	NF	NF	7..33	1.29
28.	Isochiapin B,350.	C19H26O6	NF	NF	NF	7.9	1.10
29.	Tricosane,324.6	C23H48	NF	NF	NF	9.0	0.51
30.	Tetratriacontane,378	C34 H70	NF	NF	NF	9.45	0.46
31.	1,2-Benzenedicarboxylic acid , 166.1 C8H6O4		NF	NF	NF	9.3	1.74
32.	Dotriacontane, 45-.8	C32H66	NF	NF	NF	9.5	2.37
	Total		%100	%100	%100	%100	

Table 1-(continued)

Compound type (Total number in 4 seed varieties)	% and (number) in Pactol	% and (number) in Bacara	% and (number) in Rapifera	% and (number)in Rally
Carboxylic acids (21)	17.45 (8)	35.77(9)	21.49(10)	57.82(9)
Sesquiterpene hydrocarbons (1)	2.13(1)	10.35(1)	1.12(1)	2.47 (1)
Aldehyde(14)	59.85(9)	31.24(6)	45.40(7)	22.88(5)
Ketone(9)	6.89(2)	6.7(1)	12.91(4)	6.02(3)
Esters(9)	11.11(2)	15.94(4)	16.1(3)	10.81(3)
Alcohol(1)	2.57	NF	0.65	NF
others(1)	NF	NF	2.33	NF
Total(56)	%100	%100	%100	%100
a: Retention time (tR [min]) on a Restek Rtx-5 column. b: Peak area percentage calculated from the GC-FID chromatogram. NF: compound not found.				

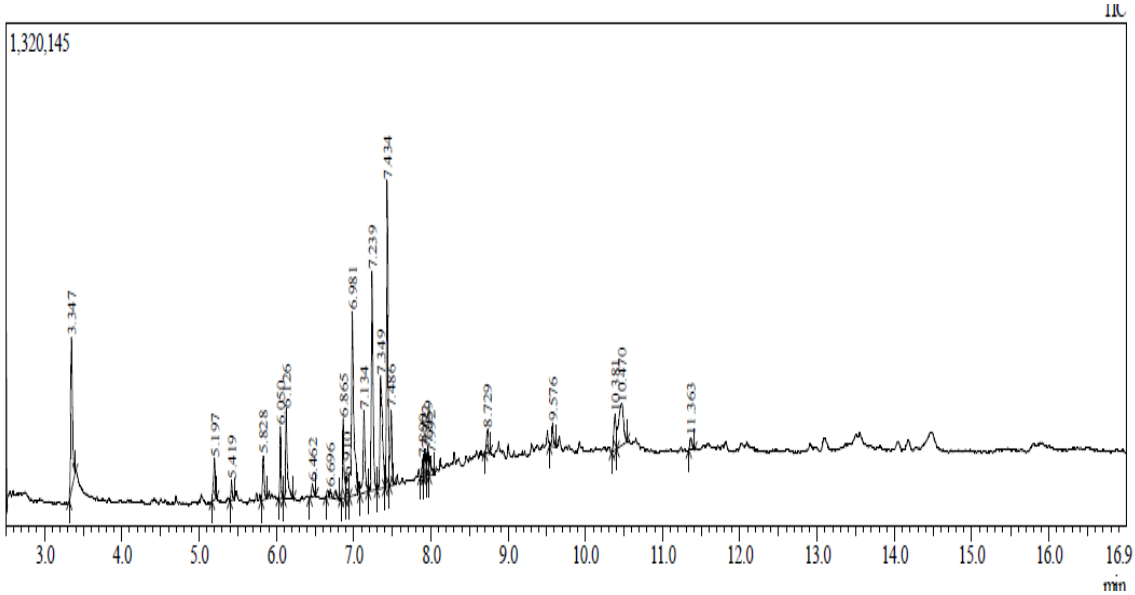


Figure 2-Typical chromatogram profile of the volatile fractions (Y-axis) at different times (X-axis) isolated from Pactol variety

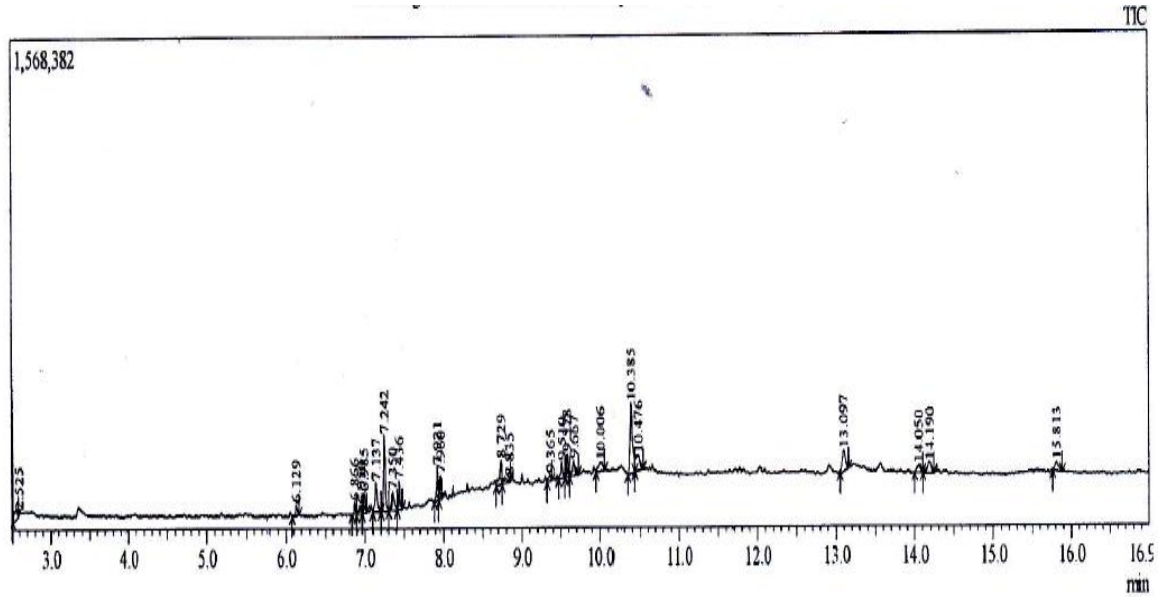


Figure 3-Typical chromatogram profile of the volatile fractions (Y-axis) at different times (X-axis) isolated from Bacara variety

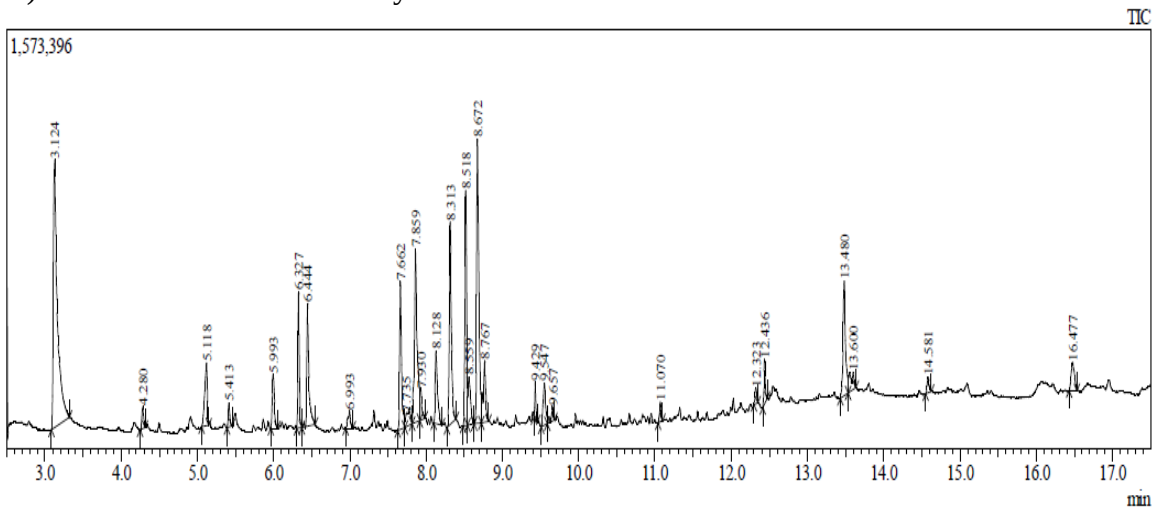


Figure 4-Typical chromatogram profile of the volatile fractions (Y-axis) at different times (X-axis) isolated from Rapifera variety

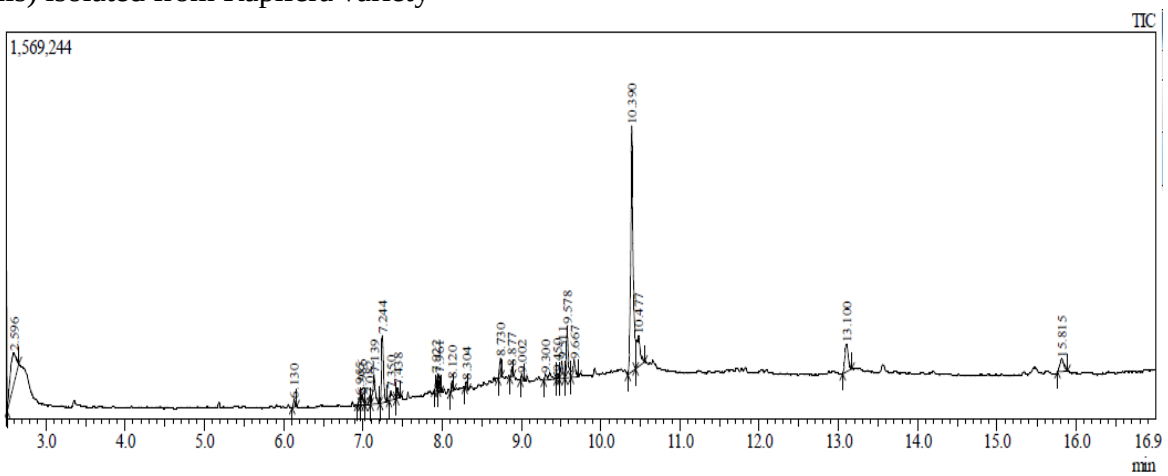


Figure 5-Typical chromatogram profile of the volatile fractions (Y-axis) at different times (X-axis) isolated from Rally variety

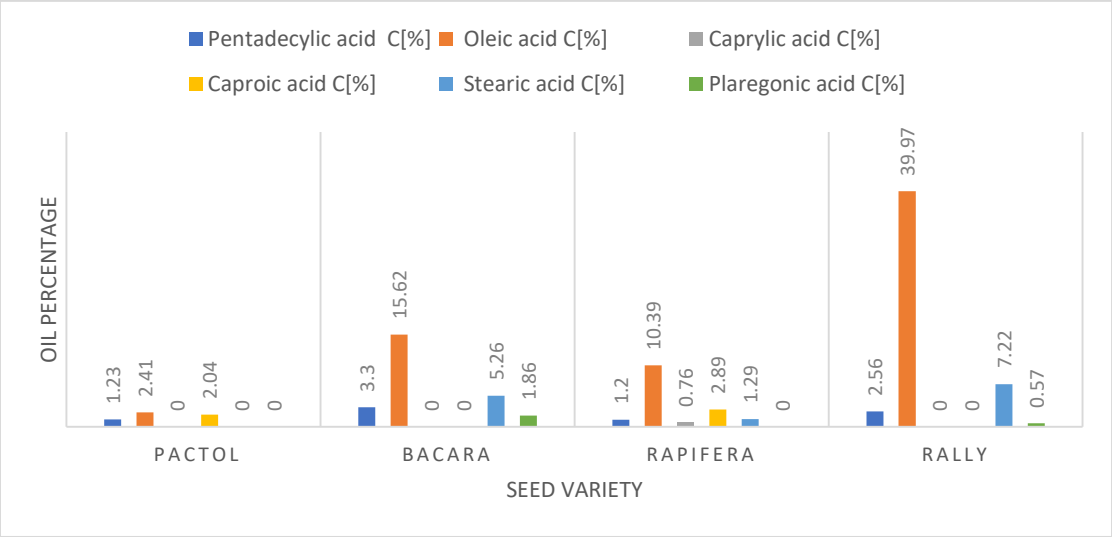


Figure 6- The percentages of the fatty contents of four seed varieties of *Brassica napus.*

3.2 Antimicrobial Activity

The results of the microbroth dilution method for testing the antimicrobial activity of the four types of *Brassica napus* seed essential oils are summarized in Tables 2 and 3. On the whole, all the essential oils showed antimicrobial activities against the tested microbial isolates, but Pactol seed essential oil showed the highest activities (both using MIC and MBC) (Figure 11).

Table 2- MIC (%) of Pactol, Bacara, Rapifera and Rally seed oils of *Brassica napus L* against tested microorganisms.

Test Microorganisms	**Antimicrobial potential (** MIC %**) of tested seed oil**			
	MIC of Pactol	**MIC of Bacara**	**MIC of Rapifera**	**MIC of Rally**
Escherichia coli	6.25	12.5	25	25
Pseudomonas aeruginosa	12.5	25	12.5	25
Klebsiella pneumoniae	12.5	25	25	12.5
Staphylococcus aureus	12.5	12.5	25	12.5
Streptococcus pyogenes	3.125	25	6.26	6.25
Streptococcus agalactiae	0.78	12.5	12.5	3.125
Candida albicans	6.25	6.25	12.5	12.6

Table 3- MBC and MFC (%) of Pactol, Bacara, Rapifera and Rally seed oils of B. napus against tested microorganisms

Test Microorganisms	**Antimicrobial potential (** MBC and MFC %**) of tested seed oil**			
	MBC&MFC of Pactol	**MBC&MFC of Bacara**	**MBC&MFC of Rapifera**	**MBC&MFC of Rally**
Escherichia coli	12.5	25	50	50
Pseudomonas aeruginosa	25	50	25	50
Klebsiella pneumoniae	25	50	50	25
Staphylococcus aureus	25	25	50	25
Streptococcus pyogenes	6.26	50	12.5	12.5
Streptococcus agalactiae	1.36	25	25	6.25
Candida albicans	12.5	12.5	25	25

Regarding the effects of the tested oils against Gram-positive bacteria, both *Streptococcus pyogenes* and *Streptococcus agalactiae* showed lower resistance potentials to the essential oils when compared with *Staphylococcus aureus*. Moreover, both Pactol and Rally oils showed considerably higher activities against Gram-positive bacteria than other oil types. Nearly similar MIC and MBC effects were noticed for each oil type against the tested organisms (Figures 7, 8, and 11).

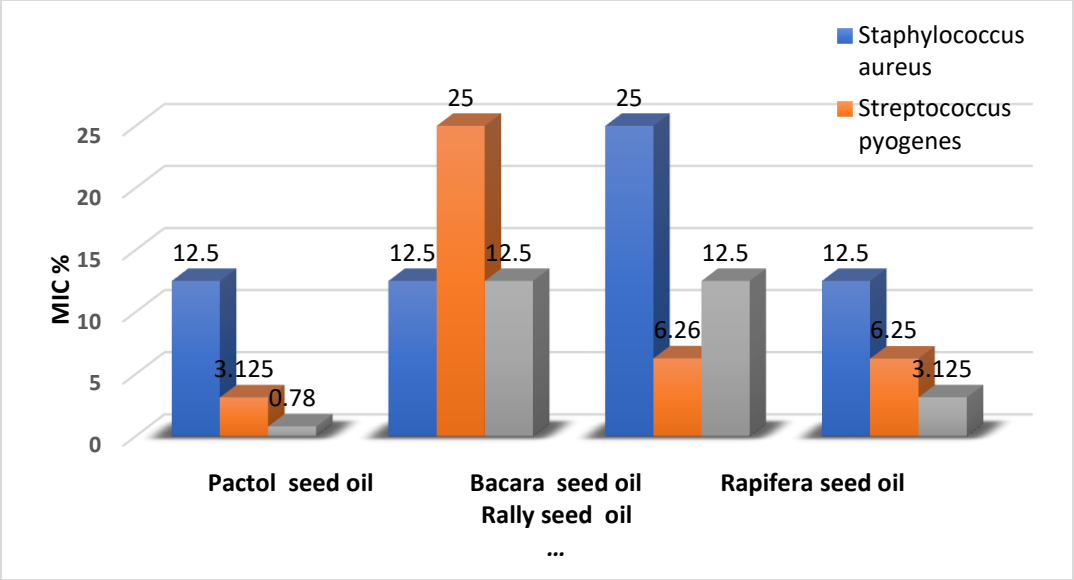


Figure 7-MIC (%) of Pactol, Bacara, Rapifera and Rally seed oils of *Brassica napus L* against Gram positive bacteria.

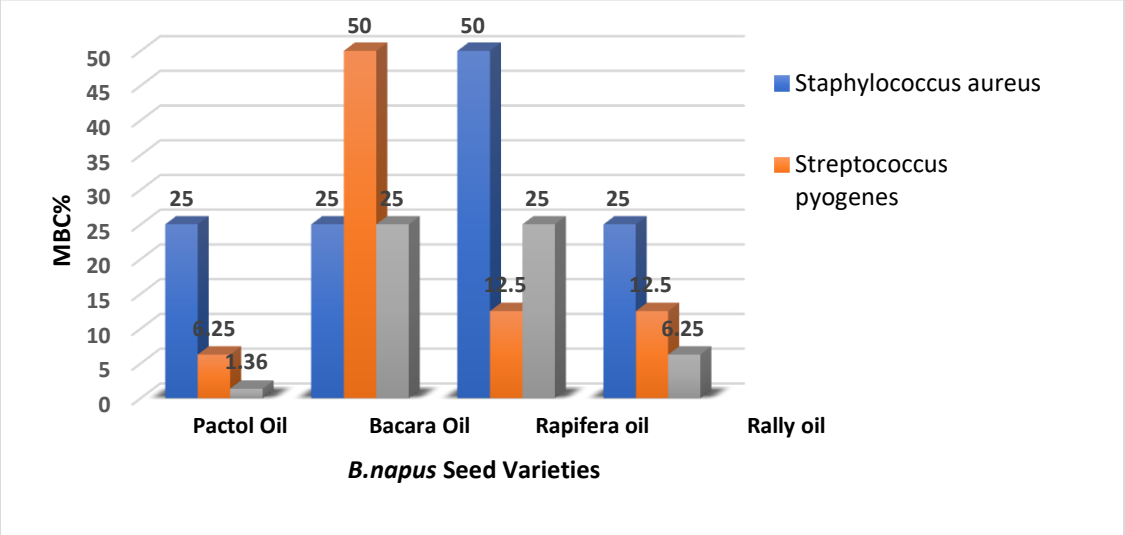


Figure 8-MBC (%) of Pactol, Bacara, Rapifera and Rally seeds oils of *Brassica napus L* against Gram positive bacteria.

On the other hand, all the oils were active against the tested Gram negative bacteria, but with different rates. For example, the strongest activities against *Escherichia coli* were exerted by oils from both Pactol (6.25% and 12.5% for MIC and MBC, respectively) and Baraca (12.5% and 25% for MIC and MBC, respectively). Rapifera essential oils showed the highest activity against *Pseudomonas aeruginosa* (12.5% and 25% for MIC and MBC, respectively), while Rally oils was found to be effective against *Klebsiella pneumonia* (12.5%

and 25% for MIC and MBC, respectively) (Figures 9 and 10). The results also show that Gram positive bacteria were more sensitive to the tested oils than Gram negative bacteria (Figure 11).

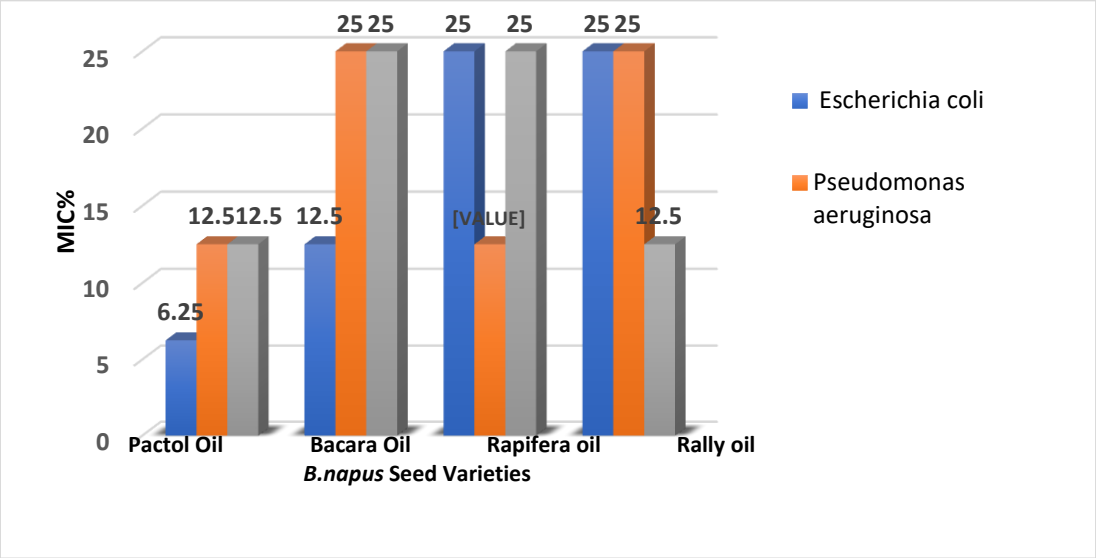


Figure 9-MIC (%) of Pactol, Bacara, Rapifera and Rally seeds oils of *Brassica napus L* essential oils against Gram negative bacteria.

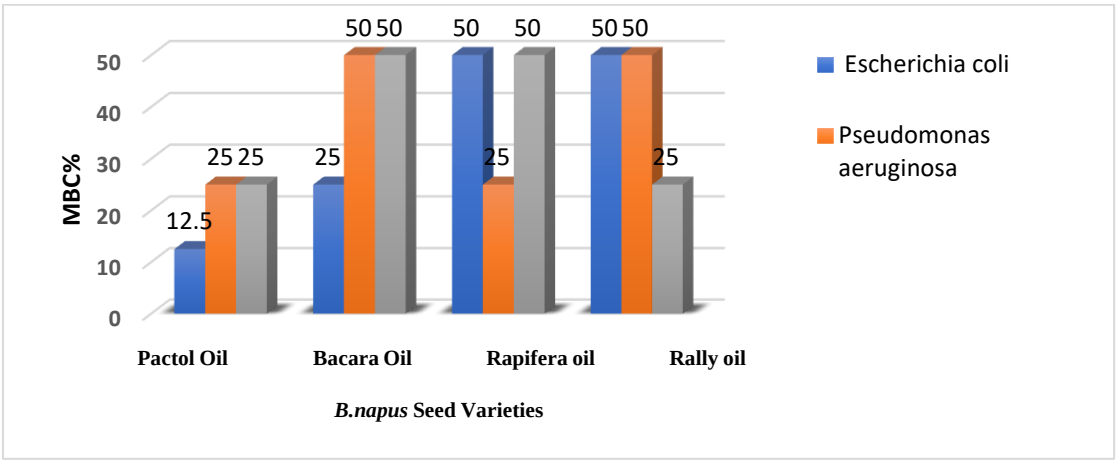
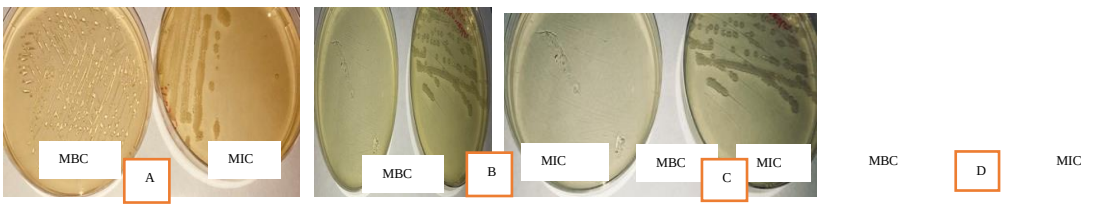
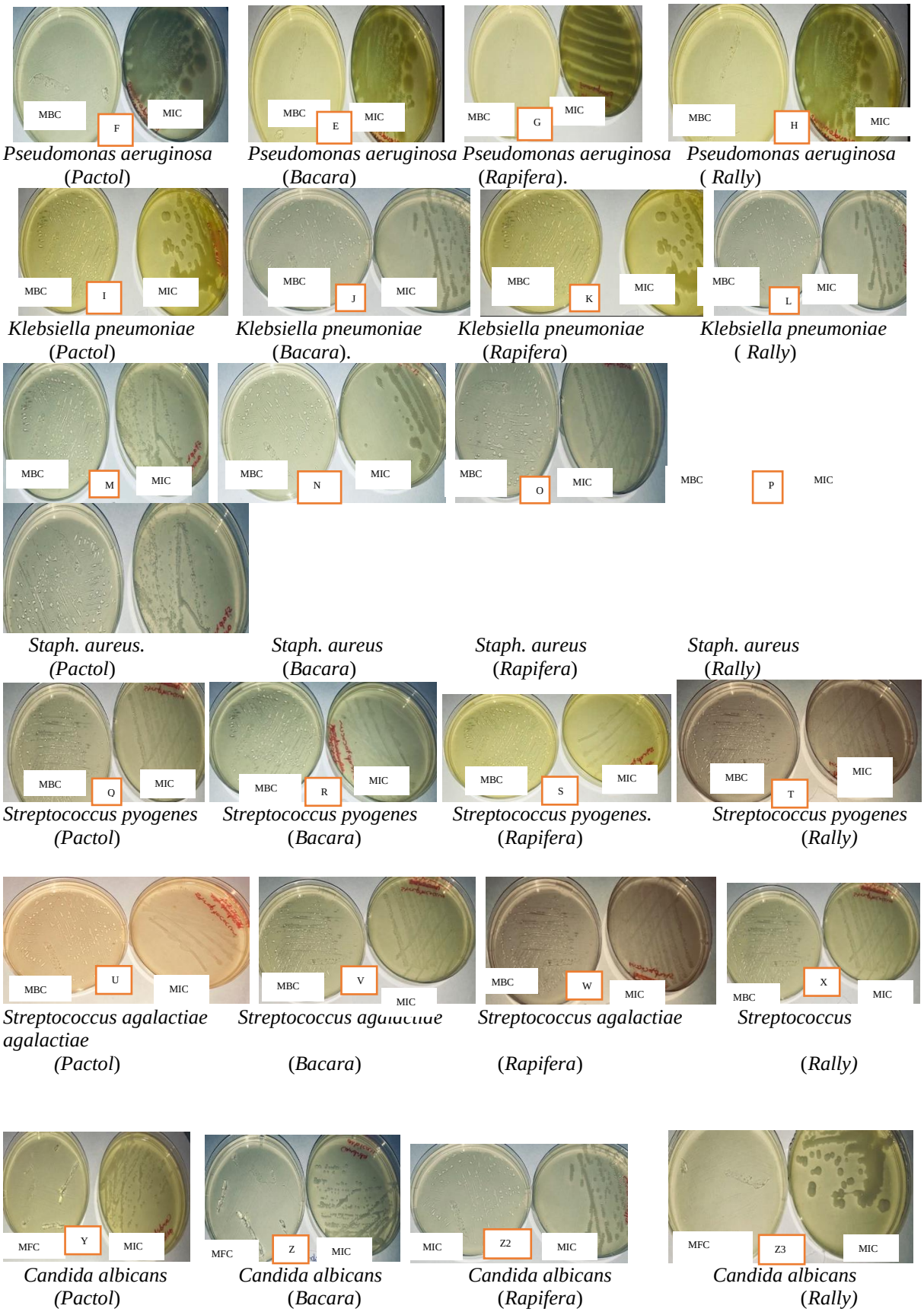


Figure -10 MBC (%) of Pactol, Bacara, Rapifera and Rally seeds essential oils *of Brassica napus L* essential oils against Gram negative bacteria.



E. coli (*Pactol*) *E. coli* (*Bacara*) *E. coli* (*Rapifera*) *E. coli* (*Rally*)



Pseudomonas aeruginosa (*Pactol*) *Pseudomonas aeruginosa* (*Bacara*) *Pseudomonas aeruginosa* (*Rapifera*). *Pseudomonas aeruginosa* (*Rally*)

Klebsiella pneumoniae (*Pactol*) *Klebsiella pneumoniae* (*Bacara*). *Klebsiella pneumoniae* (*Rapifera*) *Klebsiella pneumoniae* (*Rally*)

Staph. aureus. (*Pactol*) *Staph. aureus* (*Bacara*) *Staph. aureus* (*Rapifera*) *Staph. aureus* (*Rally*)

Streptococcus pyogenes (*Pactol*) *Streptococcus pyogenes* (*Bacara*) *Streptococcus pyogenes.* (*Rapifera*) *Streptococcus pyogenes* (*Rally*)

Streptococcus agalactiae agalactiae (*Pactol*) *Streptococcus agalactiae* (*Bacara*) *Streptococcus agalactiae* (*Rapifera*) *Streptococcus* (*Rally*)

Candida albicans (*Pactol*) *Candida albicans* (*Bacara*) *Candida albicans* (*Rapifera*) *Candida albicans* (*Rally*)

Figure -11 MIC, MBC, and MFC% of Pactol, Bacara, Rapifera, and Rally seed varieties on Gram negative and positive bacteria and *Candida albicans*.

4. Discussion

The total oil yields of 4 seed varieties of *Brassica napus L* were in agreement with those found by previous findings [13, 14]. GC-MS results showed different chemicals, each of which has been known for its biological activities. 9-octadecenoic acid, also known as oleic acid, is a mono-unsaturated fatty acid that was found to be prevalent in all four seed varieties. A previous study has demonstrated the advantage of oleic acid in immunomodulation, treatment, and prevention of different types of disorders, such as cardiovascular and autoimmune diseases, metabolic disturbances, skin injury, and cancer, beside exerting a prominent role in drug absorption [15]. Additionally, oleic acid was reported to have a good antibacterial activity [16]. Aldehyde and ketone classes are other prevalent organic classes. the most prevalent compounds belonging to these groups include nonanal, 2-undecenal, 2-decenal, hexanal, and 2,4-decadienal. The antimicrobial activities of these individual chemicals were previously assessed by exposing bacterial cultures to vapours of selected chemicals. The research concluded that individual aldehydes, such as pentanal, hexanal, and heptanal exhibited antimicrobial activities against *Staphylococcus epidermidis* and *Klebsiella pneumonia* but only at very high concentrations [17]. The carboxylic acid is the second most prevalent organic class that has been known as an antibacterial agent. A previous study reported the same finding regarding seed phytochemicals of *Brassica napus* L [18]. Carboxylic acids are commonly applied as food preservatives and disinfectant, but also in the control of fermentation and the extraction of solvent of biological compounds and substrates for biopolymer manufacturing [19]. The most available terpenoid in all four seed varieties was phytan (Hexadecan 2,6,10,14-tetramethyl), a long-chain alkane with a fuel-like odour that is considered as the natural compound source in biofuel production [20].

Fatty acids, such as stearic acid, pentadecanoic acid, and caproic acid, were also found in the tested seed varieties. The antibacterial role of these fatty acids was reported in previous studies [21-23]. A pelargonic acid was also detected by a previous GC-MC analysis of Bacara seed oil and is known for herbicidal effects [13]. Its industrial applications include lubricant cosmetics and metalworking fluids. Thus, it is included among raw materials used in synthetic lubricants [24]. Earlier experimental results indicated that pelargonic acid forms a good surface-active agent, but the high cost of the process makes it favourable to use oleic acid as it is known to be cheaper [25]. Many other compounds mentioned in Table 1 are not discussed here. In general, various cultivars had different phytochemical constituents and consequently showed different rates of biological activities, including antimicrobial activities.

The antimicrobial sensitivity test generally revealed that all the four tested essential oils had antimicrobial activities. Our result is in accordance with a previous study on the antimicrobial activities of *Brassica napus* [26], [27]. These activities are undoubtedly due to the antimicrobial potential of multiple phytochemical compounds mentioned above. These are the reasons behind the growth inhibition of different microorganisms (bacteriostatic) or the destruction of bacterial cells (bactericidal). The antimicrobial activity of Pactol essential oils was considerably higher than those of the other tested oils due to its unique phytochemicals with higher percentages of aldehydes (mainly 2,4-decadienal and hexanal). A similar notice was found regarding the efficiency of Pactol seed oils [28, 29]. In addition, previous studies reported that rapeseed oil possessed higher antimicrobial activities than refined rapeseed oil [26, 30]. Previous works showed that Gram positive bacteria isolates were more sensitive to the action of the investigated essential oils than Gram-negative bacteria. Our results are in agreement with those of previous studies [31-33]. The difference in the sensitivity between Gram-positive and Gram-negative bacteria may result from the impermeable of the outer membrane of Gram-negative bacteria, since it is negatively charged and contains channels and porin proteins that allow the diffusion of small solutes and ions into the periplasmic space. In contrast, large, hydrophobic, and negatively charged molecules like fatty acids are difficult to

diffuse through these channels [34, 35]. Another reason for the low sensitivity of Gram-negative pathogens is the barrier of the outer membrane for most polyphenols, such as phenolic acids, that are present in the ionized form at the neutral pH (7.0) and are too polar to penetrate the semi-permeable bacterial membrane and react with the cytoplasm or cellular proteins [36, 37].

5. Conclusions

The GC-MS analysis was shown to be an effective method to determine the phytochemical compounds of *brassica napus* l seed varieties. The oleic acid was the most common carboxylic acid (fatty acid) and 2,4-decadienal with hexanal were the most prevalent aldehydes in the four seed oils. In general, the tested *B. napus* seed essential oils showed antimicrobial activities against various Gram positive and negative bacteria and Candida albicans, with Pactol seed oils exerting the highest activity. Future research is required to investigate cytotoxicity and edibility effects before using *Brassica* seed oil as a nutritional and medicinal source.

6. Conflict of interest

The authors declare that they have no conflicts of interest.

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