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RESEARCH PAPER

Antimicrobial Efficacies of Brassica Napus L. Essential Oils Nanoparticles Composites

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ABSTRACT

Polylactide based essential oil films were formulated by incorporating polyethylene glycol, nanopowder (zinc oxide), and essential oil by solvent casting method. The films were tested against pathogens for their antibacterial activity. The effectiveness of selected oil-nanomaterial based film was tested by performing the tests. In vitro antibacterial efficacies of nanopowders/essential oil were determined by the decimal reduction concentrations and the minimum bactericidal concentrations for the pathogens. In a typical process, Brassica napus extract was obtained from supercritical fluid extraction using pressurized carbon dioxide as solvent. The composition of the essential oil was analyzed by gas chromatography (GC) and gas chromatography-mass spectrophotometry (GC-MS). 39 compounds were identified in the oil. The major compounds of the oil were 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14- (1-methylethyl) - 30,07%, Cyclohexanone, 5 -methyl-2- (1-methylethylidene) - 12.91%, 3,4-Methylenedioxypropiophenone - 9,67%, Hexadecanoic acid, ethyl ester - 8.28%, Octacosanol - 5,50%, 11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene - 4,55% and 1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl – 3,14 %.

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INTRODUCTION

Organic substances such as essential oils (EOs) are categorized as GRAS by the U.S. Food and Drug Administration as well as the European Legislation for materials intended to be in contact with food. Thyme, cinnamon, clove, basil, oregano, garlic, and basil oils have been intensively explored for the development of food packaging films due to their excellent antibacterial properties against foodborne pathogens [1-3]. A range of both biodegradable and non-biodegradable polymer films, such as chitosan, fish skin, whey protein isolate, low-density polyethylene, ethylene vinyl alcohol, poly(ethylene terephthalate), and polypropylene films, have either coated or incorporated with EO and evaluated for their antibacterial effectiveness [4]. Surprisingly, some studies are available in the literature on the use of PLA-based films as carriers of EOs for antibacterial packaging applications. In addition, there is lack

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of information with regards to the application of PLA-based EO films, especially on food systems such as cheese and meats. Recently, antibacterial activity of PLA-based films incorporated with cinnamaldehyde against gram-negative E. coli and gram-positive S. aureus was revealed [5]. Tests with these developed films were also found to be effective in reducing the mesophilic and psychrophilic bacterial counts of button mushrooms and extend its postharvest life [6]. In another similar recent study, PLA films containing an extract of Allium spp. EO were shown to have inhibitory effect against several molds, yeast, and pathogenic bacteria and those films were also observed to be effective for inhibiting the growth of aerobic, enterobacteriaceae, yeast, and mold on ready-to-eat salads for up to 7 days under refrigerated storage conditions [7-8]. Rapeseed, an annual plant belonging to the Cruciferous family (Brassicaceae), is one of the cultivated medicinal plants in Central Asia, North Africa and Western Europe [9,10]. Rapeseed oil helps lower blood cholesterol and strengthens blood vessels, preventing blood clots. Monounsaturated and polyunsaturated fatty acids, vitamins E, A, PP, B1, B2 and phytosterols were found in the chemical composition of rapeseed oil [11]. That is why it is considered as healthy, edible oil: the ratio of linoleic to linolenic acid amounts 2 and is higher balanced than in soybean oil [12]. This 7% of saturated fatty acids from canola oil is about half the level present in corn oil, olive oil or cottonseed oil. The most important in nature is the monounsaturated fatty acid (MUFA), an oleic acid. Tocopherols content in canola oil ranges from 0.5 to 0.9% [13]. Some researches show that Brassica extracts have a whitening effect and a skin-beautifying effect based on a melanin production inhibitory action and an anti-inflammatory action are specially mentioned. Like all oils with a high content of oleic acid and tocopherols, it has an accelerating healing and tissue regeneration effect, is suitable for general improvement of dry skin condition, relieves inflammation and irritation, restores elasticity, promotes better nutrition and moisturizing of the skin.

MATERIALS AND METHODS

Plant material was collected at the Kazakh Research Institute of Agriculture and Crop Production in Almaty during the ripening of seeds. Rapeseed extract was obtained by subcritical CO_2 extraction.

Study of chemical compounds

The study of the chemical composition of rapeseed extract was carried out by gas chromatography with mass spectrometric detection equipped with an Agilent 7890B / 5977A, WAXetr column (30 m × 0.25 mm, thickness 0.25 mm). Data processing included determining retention times, peak areas, and processing of spectral information obtained using a mass spectrometric detector.

Antimicrobial Study

The analysis of antimicrobial activity was carried out by the method of two serial dilutions

Fig. 1. CO_2 based extractor apparatus.

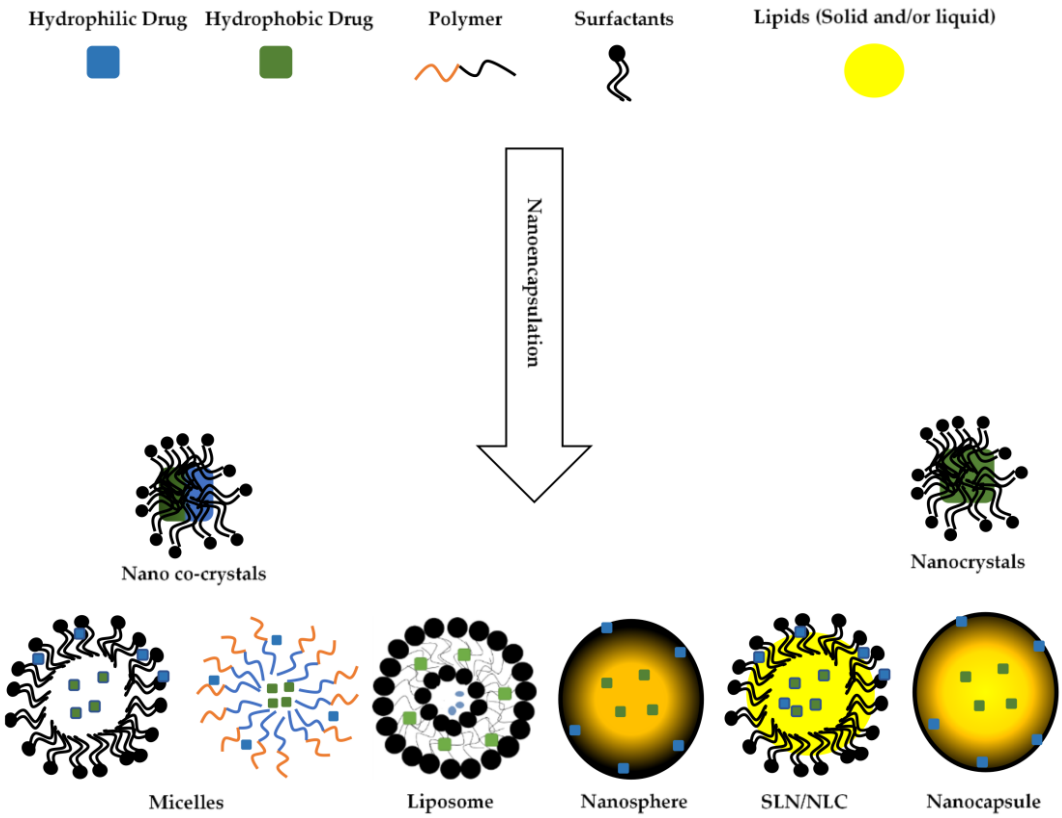


Fig. 2. Encapculation of the essential oil extract into nanoparticle core-shell.

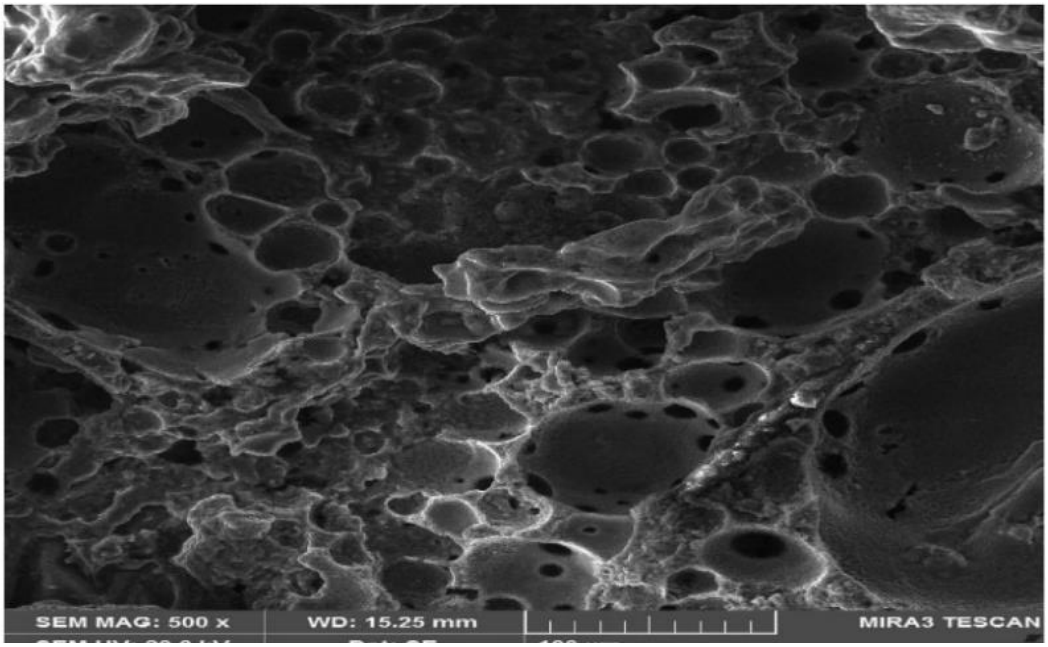


Fig. 3. SEM image of the as-prepared essential incorporated ZnO core sell nanocomposites.

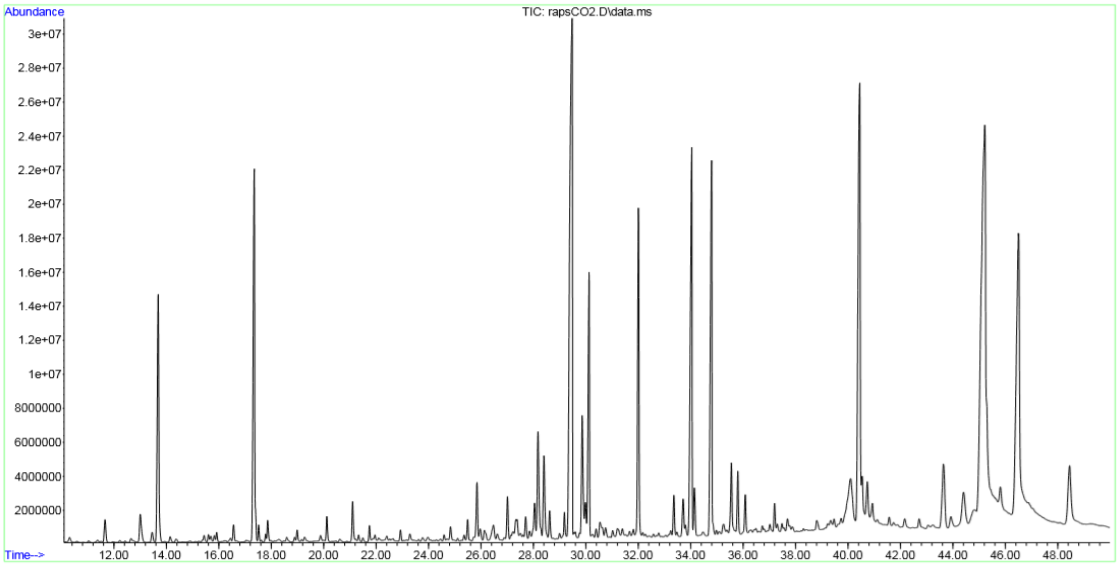


Fig. 4. Chromatogram of CO_2 analysis of rapeseed extract

in a liquid nutrient medium [14,15]. A 108-well plate was used to determine antimicrobial activity. In all wells from 1 to 8, the nutrient broth MBH (for testing bacteria) or Saburo broth (for testing fungi) was poured in an amount of 0.5 ml. The working solution (in this case, the initial extract) was made in pure form (in a volume of 0.5 ml) in the 1st test tube. Next, serial dilutions were made, which were carried out by sampling the mixture (MBB (0.1 ml) + test drug (0.5 ml)) from a 2nd test tube in an amount of 0.5 ml into a third test tube already containing 0, 5 ml of broth. Thoroughly mixed and transferred 0.5 ml of the test sample in the broth from the 3rd tube to the 4th, also containing initially 0.5 ml of broth. This procedure was repeated until the required number of dilutions was achieved. 0.5 ml of the mixture is removed from the last tube. Thus, the following dilutions were obtained: 1: 1; 1: 2; 1: 4; 1: 8; 1:16; 1:32; 1:64; 1: 128; which corresponds to wells from the 1st to 8th test tube control culture. After a series of dilutions, 0.05 ml of test strains of microorganisms at a concentration of 1.5 × 106 CFU / ml were added to all tubes. The procedure was repeated for all test samples. All samples were incubated for 18-24 hours at 37 ± 1 ° C. After the incubation time, seeding on Petri dishes was performed to determine living cells. After seeding, the plates were placed in a thermostat for 18-24 hours at 37 ± 1 ° C. The results were taken into account by the presence of visible growth of microorganisms on the surface of a dense nutrient medium. The minimum bactericidal concentration (MBC) was considered the lowest concentration in the test tube, which inhibited the growth of microorganisms. Used reagents, solutions and culture media: Muller-Hintton Agar (MHA); Mueller-Hinton Broth (ICB); Saburo Bouillon (Sab); 0.9% sodium chloride solution (saline).

Equipment used: Densitometer DEN-1 (Latvia), Comfort thermal shaker (Germany), analytical balance LB 210-A (Russia), pH meter PB11 (Germany), vertical autoclave SystecV-120 (Germany), thermostat BD-115 (Germany), BioIIA / G laminar box (Spain), IKAMS3 Digital shaker (Germany), Eppendorf dispenser (1-10 ml, 100-1000 µl, 20-200 µl, 0.5-10 µl) (Germany), HaakeP14 thermal bath (Germany), Arium611 VF water treatment system (Germany).

RESULTS AND DISCUSSIONS

Obtaining and chemical composition of CO_2-extract of seed Brassica napus

The CO_2-extract from rapeseed was developed and obtained at ZhanaPharm LLP and has valuable pharmacological properties. At present, the base of ZhanaPharm LLP is a unique production in the Republic of Kazakhstan, which receive CO_2 extracts in pre-critical conditions from plant materials.

The extraction of rapeseed is carried out with the following parameters: Extraction mass is 3 kg; Working pressure is 45-51 atm; The temperature

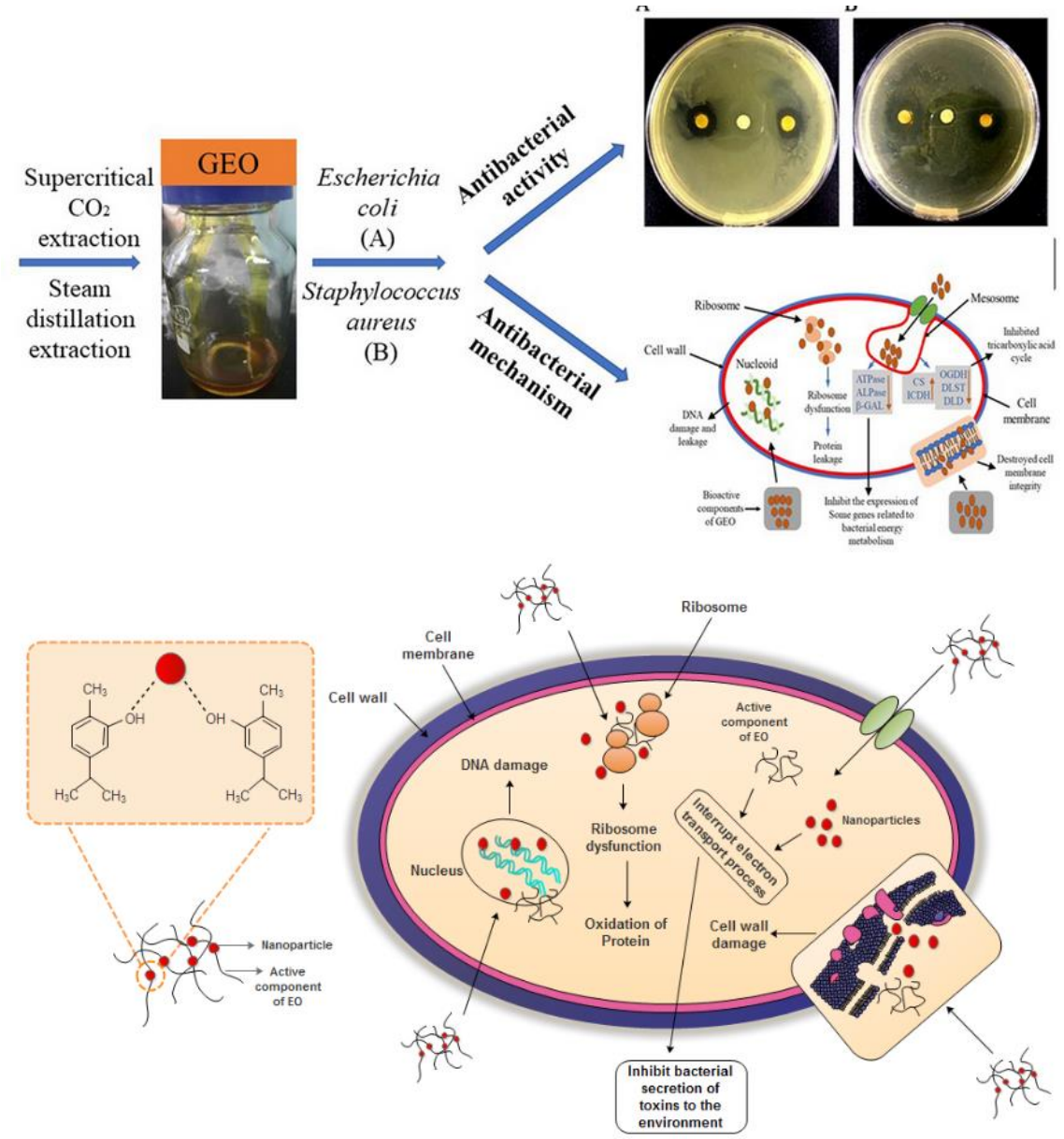


Fig. 5. Procedures for the antimicrobial activity of the synthesized nanomaterials based essential oil extract and the mechanism to degrade and oxidize the microorganism shell and protein.

of extraction is 18-21 ˚C; The extraction time is 11 h; The extract amount is 29.74 g.

Fig. 1 presents the encapsulation of the extracted essential oil into nanoparticles. Fig. 2 presents the SEM image of the as-prepared essential oil – nanoparticle hybrid sphere. The image reveals the well-formed sphere shape of the prepared nanocomposite.

GC-FID Analysis of the extract

Analysis was performed on rapeseed extract obtained by subcritical CO_2 extraction. Chromatographic analysis conditions: sample volume 1.0 µl, sample inlet temperature 240 °C, flow division 1:10. Separation was carried out using a WAXetr chromatographic capillary column with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm at a constant carrier gas (helium) speed of 1 ml / min. The chromatographic temperature is programmed from 40 °C (exposure 0 min) to 260 °C with a heating rate of 10 °C/min (exposure 20 min).

Table 1. Results of chromatographic analysis of CO_2 of rapeseed extract

No	Retention time, min	Compound	Identification probability,%	Percentage,%
1	11,7	Tetradecane	94	0,85
2	13,0	Cyclohexanone, 5-methyl-2-(1-methylethyl)	92	1,25
3	14,1	Pentadecane	90	0,22
4	15,4	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate	89	0,28
5	15,7	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	89	0,20
6	15,8	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	89	0,20
7	15,9	Cyclohexanone, 5-methyl-2-(1-methylethenyl)	90	0,28
8	16,6	Hexadecane	92	0,48
9	17,3	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	93	12,91
10	17,5	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-	92	0,43
11	17,8	1-Nonanol	90	0,20
12	17,9	Butanoic acid, 3-methyl-	84	0,64
13	21,1	2,4-Decadienal	89	1,35
14	21,7	Hexanoic acid	86	0,42
15	22,0	Dodecanoic acid, ethyl ester	71	0,26
16	23,3	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	68	0,23
17	24,6	Caryophyllene oxide	86	0,19
18	25,5	3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl)-	78	0,64
19	25,8	geranyl-α-terpinene	77	2,05
20	27,0	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl	83	1,35
21	27,7	2-Pentadecanone, 6,10,14-trimethyl-	92	0,79
22	28,4	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl	86	3,14
23	28,6	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	73	0,89
24	29,5	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)	73	30,07
25	29,9	11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	87	4,55
26	30,0	Sesquisabinene hydrate	74	1,20
27	30,1	Hexadecanoic acid, ethyl ester	88	8,28
28	30,8	Ethyl 9-hexadecenoate	78	0,40
29	32,0	3,4-Methylenedioxypropiophenone	82	9,67
30	33,8	Dodecanoic acid	69	0,35
31	36,1	Phytol	94	1,26

32	37,2	Tetradecanoic acid	80	0,88
33	37,3	cis-11-Eicosenoic acid, methyl ester	75	0,29
34	38,8	Pentadecanoic acid	71	0,45
35	40,1	Octacosanol	85	5,50
36	40,9	Hexadecenoic acid	87	0,88
37	41,6	4,8,12,16-Tetramethylheptadecan-4-olide	82	0,27
38	43,6	Squalene	95	3,78
39	44,4	Octadecanoic acid	89	2,94

Table 2. Chemical composition of Brassica napus extract.

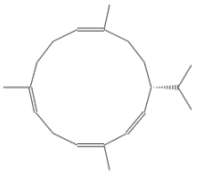
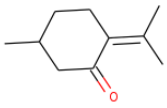
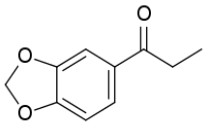
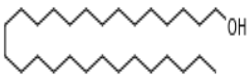
Compounds	Chemical structure	Retention time(min)	Percentage(%)
1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)		29,5	30,07
Cyclohexanone, 5 -methyl-2- (1-methylethylidene)		17,3	12,91
3,4-Methylenedioxypropiophenone		32,0	9,67
Hexadecanoic acid, ethyl ester		30,1	8,28
Octacosanol		40,1	5,50
Tetramethylhexadeca-1,3,6,10,14-pentaene		29,9	4,55
1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl		28,4	3,14

Table 3. The results of the antimicrobial activity of the extracts obtained by serial dilution

No	Retention time, min	Compound	Identification probability,%	Percentage,%
1	11,7	Tetradecane	94	0,85
2	13,0	Cyclohexanone, 5-methyl-2-(1-methylethyl)	92	1,25
3	14,1	Pentadecane	90	0,22
4	15,4	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate	89	0,28
5	15,7	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	89	0,20
6	15,8	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	89	0,20
7	15,9	Cyclohexanone, 5-methyl-2-(1-methylethenyl)	90	0,28
8	16,6	Hexadecane	92	0,48
9	17,3	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	93	12,91
10	17,5	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-	92	0,43
11	17,8	1-Nonanol	90	0,20
12	17,9	Butanoic acid, 3-methyl-	84	0,64
13	21,1	2,4-Decadienal	89	1,35
14	21,7	Hexanoic acid	86	0,42
15	22,0	Dodecanoic acid, ethyl ester	71	0,26
16	23,3	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	68	0,23
17	24,6	Caryophyllene oxide	86	0,19
18	25,5	3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl)-	78	0,64
19	25,8	geranyl-α-terpinene	77	2,05
20	27,0	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl	83	1,35
21	27,7	2-Pentadecanone, 6,10,14-trimethyl-	92	0,79
22	28,4	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl	86	3,14
23	28,6	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	73	0,89
24	29,5	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)	73	30,07
25	29,9	11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	87	4,55
26	30,0	Sesquisabinene hydrate	74	1,20
27	30,1	Hexadecanoic acid, ethyl ester	88	8,28
28	30,8	Ethyl 9-hexadecenoate	78	0,40
29	32,0	3,4-Methylenedioxypropiophenone	82	9,67
30	33,8	Dodecanoic acid	69	0,35
31	36,1	Phytol	94	1,26

Detection is carried out in the SCAN m/z 34-850 mode. To control the gas chromatography system, register and process the obtained results and data, Agilent MSD ChemStation software (version 1701EA) was used. Data processing included determination of retention times, peak areas, and processing of spectral information obtained using a mass spectrometric detector (Fig. 3). For decoding the obtained mass spectra, the Wiley 7th edition and NIST'02 libraries were used (the total number of spectra in the libraries was more than 550 thousand) (Table 1).

According to the results of the study, in the composition of the rapeseed extract in large quantities, chemical compounds were found: 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14- (1-methylethyl) - 30,07%, Cyclohexanone, 5 -methyl-2- (1-methylethylidene) - 12.91%, 3,4-Methylenedioxypropiophenone - 9,67%, Hexadecanoic acid, ethyl ester - 8.28%, Octacosanol - 5,50%, 11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene - 4,55% and 1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl – 3,14 % (Table 2).

Antimicrobial Properties

The results of a study of the antibacterial and fungicidal activity of rapeseed extract against four strains of pathogenic microorganisms S. aureus ATCC 6538-P, E. coli ATCC 8739, P. aeruginosa ATCC 9027 and C. albicans ATCC 10231 are presented in Table 3.

From the data presented in table 2 it is seen that the rapeseed extract exhibits the expected biological activity against strains of C. albicans ATCC 10231 at a dilution of 1:32 and E. coli ATCC 8739 at a dilution of 1: 8, respectively. In relation to P. aeruginosa ATCC 9027 and S. aureus ATCC 6538-P, no antivicity was detected.

Fig. 5 presents the general antimicrobial activity test of the as-prepared nanomaterials based essential oil extract.

CONCLUSIONS

We conducted a study of the chemical composition of CO_2 of the rape seed extract (Brassica napus) by gas chromatography with mass spectrometric detection. The analysis was carried out using a WAXetr capillary column, in the detection mode SCAN m / z 34-850. The retention time and peak areas were determined using a detector. As a result, 39 types of chemical compounds were revealed in the composition of rape extract, in which terpenoids, diterpenes, sesquiterpenes, and other organic substances predominate. The tested Rapeseed extract exhibits the expected biological activity against yeast fungi of the genus Candida. The obtained results of the antimicrobial activity of the tested samples indicate the prospect of their further study for use as anti-infective (anti-inflammatory) drugs in medicine. The study indicated that nanomaterial-based EOs were effective against tested microorganisms. The PLA-based films formulated with the oil extract showed excellent antibacterial efficacy against both gram-positive and gram-negative pathogens.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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