Assessing the Antimicrobial Effects of *Origanum majorana* Extracts on Canned Mackerel: A Study on Food Safety and Storage

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ABSTRACT

Origanum majorana **L. as a member of the family *Lamiaceae*, is cultivated in different areas including the Mediterranean region and Egypt. *O. majorana* extracts were used as inhibitory agents against food-poisoning microbes. This work is also aimed to assess the antimicrobial effects of *O. majorana* on canned Mackerel (*Trachurus trachurus*). It had been noticed that all extracts displayed promising antimicrobial activities toward all tested microbes including food-borne microbes. The prepared extracts exhibited brilliant antioxidant activities especially ethanolic extracts (1040.06 and 1055.44 AAE/ g). In addition, the extracts revealed promising total phenolic contents (532.94 and 900.52 mg GAE/g). GC/Mass analysis had been performed for the four extracts and the results revealed that Terpinen-4-ol (12.82%), Tricyclene (11.08%), 9-Octadecene,1,1'-[1,2-ethanediylbis(oxy)]bis-(Z,Z)(4.74%),-methoxy-6 ethylbenzoic acid (21.04%), Dodecane, 2,2,4,9,11,11-hexamethyl (13.66%) and Ethyl-9,12,15-octadecatrienoate (7.57%) were the major compounds. Results of total phenolics and antioxidant results in all extracts namely: rosmarinic acid > chlorogenic acid > kaempferol > catechin and gallic acid. The results revealed that all extracts are safe to be used as food additives (IC50 more than 200µg/ml). Therefore, we recommend incorporate these extracts into polyvinyl alcohol (PVA) films used to preserve foods from bacterial infections, as well as using these extracts as special food additives with mackerel to preserve them for a period of no less than 30 days and, the sensory characteristics has received excellent acceptance when adding fresh *O. majorana* instead of dried *O. majorana* to canned mackerel.**

Keywords: *Origanum majorana*, mackerel, antimicrobial, antioxidant, polyphenols, antibiofilm, polyvinyl alcohol.

INTRODUCTION

Natural products including herbs, herbal preparations and other botanicals are targets in livestock production (Makkar *et al*., 2007) due to their effects on the health of humans and animals in addition to the product quality and safety. The plant extracts have been found to play a crucial role in ruminant fermentation and improve their nutrient digestion (Busquet *et al*., 2005 and Patra *et al*., 2006). Bioactive compounds from plants or plants themselves, when added to feed components exhibit various effects on digestibility and blood parameters in the ruminant. *O. majorana* L., a member of the family *Lamiaceae*, is cultivated in different areas such as the Arabian Peninsula, Asia, Africa, the Mediterranean region, Europe as well as America (El-Ashmawy *et al*., 2005).

O*riganum majorana* L. is a perennial tender herb belonging to the genus *Origanum* (Vági *et al.*, 2002). It is a perennial abundant semi-hardy sub-shrub that grows yearly. They are characterized by straight stems with weak, furry round and greenish red speckles (Pimple *et al.*, 2012). This plant is characterized by smooth simple petiolate leaves exhibiting greenish to gray in colour and set divergent to each other on a quadrangular stalk. O. *majorana* is normally recognized as sugary (sweet) marjoram and it is naturally found in Cyprus, Anatolia and established partially in the Mediterranean region mainly in Egypt (Novak *et al.*, 2002). *O. majorana* L. is known by its traditional names, such as oregano as well as sweet marjoram (Al-Howiriny *et al.*, 2009 and Christman, 2010). Hippocrates mentioned that old Egyptians used *O. majorana* L. as an antiseptic agent as well as home medicine for infections of the chest, cough in addition to cardiovascular diseases, flatulence, epilepsy, insomnia, sore throat, rheumatic pain, nervous disorders, skin care as well as stomach disorders (Bremness, 1994; Yazdanparast & Shahriyary, 2008 and Zaidi *et al*., 2009).

Ancient Egyptians used oregano to cure, disinfect and preserve food. Moreover, Aristotle stated that the "turtle that ate a snake had to eat oregano not to die" which means that oregano was used as an anti-poison (Saxena *et al*., 2016). *O. majorana* L ethanolic extract. was established to treat food-borne pathogens. The inhibitory effect of *O. majorana* extract (ethanolic one) against food poisoning microbes was evaluated using *S. typhimurium* (G-ve) and *L. monocytogenes* (+ve) test microbes (Choi and Rhim, 2008). Four compounds from *O. majorana* namely: (+)-laricresol, 1H-indole-2-carboxylic acid, Procumboside B (pB) and (+)-Isolaricresol were isolated from its ethanolic extract. These compounds exhibited immunomodulatory effects and Procumboside B had significant

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immunomodulatory activity against RAW-264-7 cells. The compound pB could rise NO (nitric oxide) excretion, IL-6 (interleukin-6), TNF-a (Tumor Necrosis Factor-a), ROS (Reactive Oxygen Species) then simultaneously up-regulate CD80 and CD86 expression on the cell surface (Wang *et al.*, 2021).

Our work is undertaken to extract *O. majorana* plant aerial parts with different solvents, especially ethanol and water and evaluate their antibacterial activities, studying their antioxidant activity and measuring their phenolic and flavonoid contents through HPLC fingerprint. This work is also aimed at applying the safe and active extract (s) in keeping canned Makarel fish from bacterial attack. Additionally, the extracts could incorporate into PVA films and their antibacterial activities to be used as wrapping films to preserve foods and the sensory characteristics evaluate of the products will be conducted.

MATERIALS AND METHODS

Plant part used and preparation.

O. majorana L. aerial parts were used in green and dry states as well. These aerial green portions of the plant were first carefully cleaned using tap water and the excess water was removed by dry filter paper and extracted directly. Another part had been dried at room temperature and extracted with the proper solvents.

Extraction

Aerial parts (green and dry) were extracted with ethanol and water by soaking in the solvent overnight. The plant debris was removed by filtration and the solvents were removed by evaporation using a rotary evaporator (Model Heidolph) tell dryness and the obtained extracts were further studied.

Antimicrobial activity of different solvent extracts

S. aureus ATCC 6538-P and *L. monocytogenes* ATCC19117 (Gram-positive bacteria); *E. coli* ATCC 25933 and *S. typhimurium* ATCC14028 (Gram-negative bacteria); as well as *Candida albicans* ATCC 10231(yeast) were used to achieve the antimicrobial activities of ethanolic and aqueous of *O. majorana* extracts of its aerial parts (green and dry). The agar plate diffusion technique was applied for this purpose**.** Nutrient agar medium was used to be inoculated by each test microbe (10^6 cells/ml). The extract that exhibited potent antimicrobial effect was detected by calculating the inhibition zone diameter in millimeter (mm). The experiment was done twofold and readings was noted as means (Abdel-Aziz *et al*., 2021 and Abo-Salem *et al*., 2024). Agar diffusion disc method was interpreted to assess the antimicrobial effect of the prepared PVA incorporated with plant extract films according to Akl *et al*. (2021).

Minimum inhibitory concentrations (MIC) of different solvent extracts

S. aureus ATCC 6538-P and *L. monocytogenes* ATCC19117 (G+ve bacteria); *E. coli* ATCC 25933 and *S. Typhimurium* ATCC14028 (G-ve bacteria); in addition to *C. albicans* ATCC 10231 (yeast) were considered in this technique. The selected strains were grown on a nutrient broth and their collected cells were resuspended in 0.85% saline solution giving an absorbance of 0.5-1Au (corresponding $5x10^6$ CFU) to be used as inoculum. Membrane sterilized resazuin (67.5mg in 10 ml of distilled water) was used to assess the viability of test strain. Plant extracts (100 µl), dissolved in proper solvents, were subjected to serial dilutions (two-fold each) in the broth medium (100 µl in each well) in 96-well plates. 10µl from each test organism were added to all wells followed by 10 µl of resazurin reagent then plates were wrapped with par film and incubated at suitable temperature for 24 h. Positive results were considered as any colour change especially from dark blue to faint red or colourless. The lowermost concentration giving any change in colour was considered as the net MIC of the tested extract. MBC titer was known as the concentration of extract which didn't display any growth of bacterial colonies on the newly inoculated nutrient agar plates (Sarker *et al*., 2007).

Minimum biofilm inhibitory concentration (MBIC) assay

The antibiofilm activity of ethanolic and aqueous extracts from *O. majorana* green and dry aerial parts had also been evaluated (Ceri *et al*., 2006 and Abo-Salem *et al*., 2021). 100µl of nutrient broth was dispensed in all wells of 96 microplate wells. 100 µl from each extract was added to the first raw of wells and two-fold dilution has been performed leaving the last one raw of wells as control. 10µl of stock microbial culture (0.5 McFarland standard, $5x10^5$CFU/ml) were added to all wells. Plates were incubated overnight at 35°C. The culture was gently decanted and then the plates were washed with saline phosphate buffered (PBS) buffer. The plates were left to dry for 30min and 200 µl of crystal violet 0.1% was added to each well and left for 30min and additional crystal violet was poured out and washed three times with distilled water and left to dry for 30min. Finally, 95% ethanol (200 µl) was added to each well.

Antioxidant capacity (TCA) using phosphomolybdenum method.

The antioxidant capacity of the extract of the green parts *O. majorana* was assessed according to Prieto *et al*., (1999). To 900µL of phosphomolybdate reagent (0.6M H_2SO_4, 28mM NaH_2PO_4, and 4mM $(NH_4)_6Mo_7O_{24}$). 100µL of each extract was added and

the blank was constructed at the same time using methanol. The reaction mixture was maintained by incubating at 90ºC for 90 min. The reaction mixture after cooling at ambient temperature its absorbance was noticed at 695 nm using a spectrophotometer (Shimadzu UV1024-PC). Ascorbic acid standard cuvette (0.2-1mg/ml) was originated to determine the total antioxidant equivalent.

Phenolic content (TPC) determination

Phenolic contents of *O. majorana* solvent extract aerial parts were investigated according to Kupina *et al*., (2018). 50µl extract of each was added to 950µl of water then 500µl of FCR (Folin & Ciocalteu Ragent) was added and then 2.5 ml of the 20% Na_2CO_3 solution was added. The reaction mixture was reserved for 40 minutes at 25^{0}C. The absorbance was investigated at 725 nm. A standard curve of gallic was initiated. A control solution was prepared using methanol. Total phenolic contents expressed as µg/g powder extract were calculated related to gallic acid equivalent (GAE).

Effect of *O. majorana* extracts on Cell Viability by MTT assay

The cytotoxic activity of *O. majorana* extracts on standard cell lines was done as previously done by Thabrew *et al*., (1997). All steps of this experiment were performed under aseptic conditions. Briefly, after 24h of inoculating 2x10^4 cells per well by *Human Fetal Lung Fibroblast* (Wi38) cell line (96- well plates). The medium was treated with 100µg/ml of tested extracts in triplicates for 48 h. Doxorubicin (100µM) and DMSO (0.5%) were considered as positive and negative controls, respectively. MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) technique was used to assay the cell viability (Mosmann, 1983). The cytotoxicity (%) was designed conferring to the subsequent equation: $\% \ cytotoxicity = [1- (AV_x / AV_{NC})] \times 100$. Av is known as an average, X express the absorbance of the sample whereas NC is considered as the absorbance of the negative control.

GC-MS analysis

GC/MS had been performed using Thermo Scientific with trace GC Ultra/ISQ having single quadrupole MS containing TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). Timid certification of the current compounds was attained by the assessment of the retention (RT) time and the spectra of the mass of those reserved in the NIST, and WILLY library data (Shawky *et al.*, 2019).

High-performance liquid chromatography for polyphenols profile of different solvent extracts

Phenolic and other related compounds were assessed by using reverse-phase high-performance liquid chromatography (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 1050) having an Alltima C18 column with a guard column (Alltima). Phenolic compounds were evaluated using standard calibration at 280 nm and expressed in µg/100 ml. All values were the mean of two injections (Goupy *et al*., 1999).

Preparation of Canned mackerel and preservation

Steaming of Mackerel had been done using electronic food streamer Model OSTER5711. The sample was cooked for 25min at temperature of 83.5 ± 2˚C (Figure1). Canned Mackerel fillet was reserved in half kilo-containers (cans) with water (10%) and then sealed and autoclaved for 45min at 121ºC (Figure 1). To preserve the canned fish, the prepared 1mg of extract was added with a final concentration of (10 ml/10g of canned fish). The total colony forming units (CFU) were counted before the addition of extracts and after their addition for 30 days.

Figure 1. The prepared canned Mackerel

Preparation of polyvinyl alcohol/plant extract (PVA/PE) Composites

Polyvinyl alcohol (PVA), tannic acid as well as glycerol were obtained from Sigma-Aldrich Chemicals (Cairo, Egypt). PVA/PE aqueous solutions (5% w/v) were prepared by mixing and dissolving PVA powder in distilled water at 70°C with continuous stirring for up to 6h. and mixed with different plant extracts (PE) separately by dissolving 3g of each plant extract powder in 100 mL distilled water with constant stirring for 2 h (50°C) until obtaining homogenous solution (3% w/v). By mixing the previously prepared polymer solutions followed by the steady addition of glycerol (30% of the weight of the total solids of the polymers), the PVA/PE composites were formed. Subsequently, tannic acid (5% of the weight of the total solids of the polymers) was added to the previous mixture drop by drop while being continuously stirred. The homogeneous composite suspensions (50 mL) were poured into a glass Petri dish (14cm diameter) until dryness for 72h to allow evaporation of the solvent and moulding the mixture. The formed PVA/PE composite films were removed from the mold using a spatula and stored in a desiccator before characterization (Figure 2).

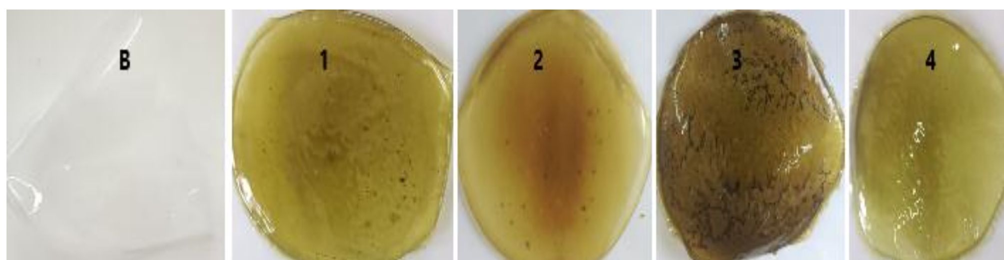

Figure 2. Preparation of PVA/plant extract. B represents the control PVA. 1, 2, 3 and 4 are PVA composite with extracts

Organoleptic evaluation of canned mackerel

According to Watts *et al.*, (1989) a nine-point hedonic scale was used for sensory evaluation. A well-trained 30-member team consisting of postgraduate and researcher staff members of the Chemistry of Natural Compounds Department, National Research Centre, Dokki, Giza, Egypt conducted the evaluation. They were asked to rate the mackerel on a 9-point Hedonic scale, with 1 denoting "dislike extremely" and 9 representing "like extremely".

Statistical analysis

IBM SPSS 25 statistics program was utilized to do statistical analysis of the data (IBM Corp, 2017). Analyses were done in triplicate for every measurement performed on every sample. The Duncan test was used to compare mean differences at a 5% significance level. Using ANOVA, the model's significance was evaluated.

RESULTS AND DISCUSSION

The green aerial parts of *O. majorana* were obtained from a farm near Giza city. The plant was washed thoroughly and cleaned using filter paper and divided into two parts. The green part was extracted directly by water and ethanol (95%). The other portion was gone to dry at 25ºC and then extracted using both water and ethanol (Figure 3). The plant solvent mixture was evaporated using a rotary evaporator (Heidolph) till dryness and the produced brownish-green extract was kept being used for further studies.

Figure 3. O*riganum majorana* plant part used A&B green and ground, C&D dry and ground E&F ethanol and water extracts

Antimicrobial activity of *O. majorana* methanolic and aqueous extracts

The antimicrobial sensitivity of *O. majorana* extracts were established toward different test microbes including the food-born bacteria *S. typhimurium* and *L. monocytogenes*. Results presented in Table (1) and Figure (4) revealed that all extracts showed considerable antimicrobial activities toward all test strains. Ethanolic extract of green aerial parts of *O. majorana* exhibited considerable activities against all test microbes with inhibition values of 15, 17, 14, 13 and 14mm for *S. aureus*, *E. coli*, *C. albicans* as well as *S. typhimurium* and *L. monocytogenes*, respectively. The ethanolic extract of the dry plant showed noticeable antimicrobial activities against all test microbes with inhibition zone values of 18, 19, 14, 12 and 16mm against the tested

microbes previously mentioned, respectively. Aqueous extract of green aerial parts of *O. majorana* exhibited promising antimicrobial activities against all test microbes with inhibition zone values of 16, 22, 20, 18 and 15mm for *S. aureus*, E. coli, *C. albicans*, *S. typhimuriu* and *L. monocytogenes*, respectively. Finally, the aqueous extract of dry aerial parts of *O. majorana* exhibited acceptable antimicrobial activities against all test microbes with inhibition zones of 20, 21, 19, 16 and 12 mm for the previously mentioned test microbes, respectively. Different concentrations (80, 40 and 20 mg/ml) of *O. vulgare* ethanolic extract exhibited in vitro antibacterial activity with an average size of the inhibition zones of 21.64, 15.24 and 11.45 mm for *S. aureus* , 13.31, 12.27 and 7.35 mm for *P. aeruginosa* and 12.5, 11.40 and 10.6 mm for *E. coli* (Pérez-Delgado *et al*., 2021).

Table 1.The antimicrobial activity of different solvent extracts of *O. majorana* against different test microbes

Sample name	Extracts	Clear zone (mm)				
		S. aureus	***E. coli***	***C. albicans***	*S. typhimurium*	***L.*** *monocytogene*
1	Ethanol dry	15	17	14	13	14
2	Ethanol green	18	19	14	12	16
3	Water dry	16	22	20	18	15
4	Water green	20	21	19	16	12

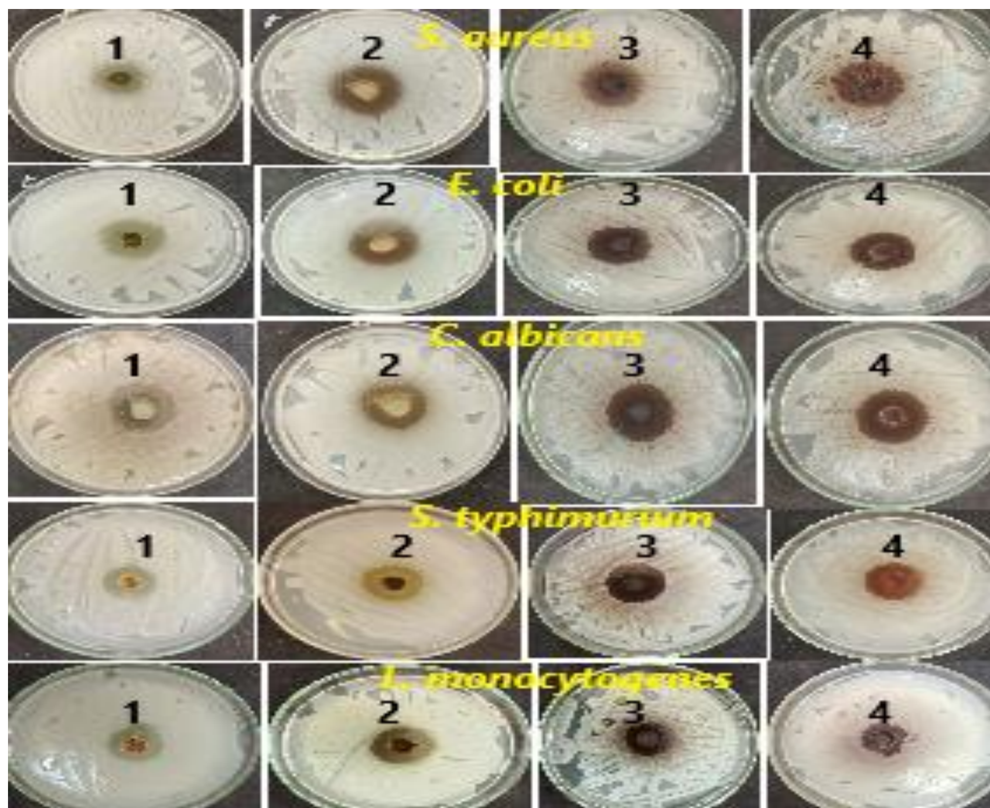

Figure 4. The antimicrobial activity of different solvent extracts of *O. majorana* against different test microbes

Minimum inhibitory concentration of different extracts from *O. majorana*

The MIC values of the different *O. majorana* extracts were assessed (Table 2 and Figure 5). Extract 1 exhibited MIC values of 156.25, 156.25, 156.25, 312.5 and 78.125µg/ml against *S. aureus*, *E. coli*, *C. albicans*, *S. typhimurium* and *S. monocytogenes*, respectively. Extract two showed acceptable MIC values with *S. aureus* (78.125 µg/ml), *E. coli* (78.125 µg/ml) and *L. monocytogenes* (39.06 µg/ml) and moderate MIC values with *C. albicans* (312.5 µg/ml) and *S. typhimurium* (156.125 µg/ml). In addition, extract 3 exhibited considerable MIC values with *S. aureus* (78.125 µg/ml), *S. typhimurium* (78.125 µg/ml) and *L. monocytogenes* (78.125 µg/ml) and moderate MIC values with *E. coli* (156.25 and 78.125 µg/ml) and *C. albicans* (312.5). Extract 4 displayed moderate MIC values with all test strains with values of 312.5, 156.25, 156.25, 312.5 and 156.25 µg/ml for *S. aureus*, *E. coli*, *C. albicans*, *S. typhimurium* and *S. monocytogenes*, respectively. *O. majorana* different extract exhibited good antimicrobial activity against *S. typhimurium* and *Listeria monocytogenes* (700 µg/ml). At this concentration, the extract of *O. majorana* inhibited the growth of *S. typhimurium* and *L. monocytogenes*. These results demonstrated the antimicrobial effects of *O. majorana* ethanolic extract against food-borne pathogens indicating that *O. majorana* is considered an effective natural antibacterial agent in food (Choi and Rhim, 2008).

Table 2. Minimum inhibition concentration (MIC) of different extracts of *O. majorana* aerial parts

Sample name	Extracts	MIC (µg/ml)				
		S. aureus	***E. coli***	***C. albicans***	***S. typhimurium***	***L. monocytogene***
1	Ethanol dry	156.25	156.25	156.25	312.5	78.125
2	Ethanol green	78.125	78.125	312.5	156.125	39.06
3	Water dry	78.125	156.25	312.5	78.125	78.125
4	Water green	312.5	156.25	156.25	312.5	156.25

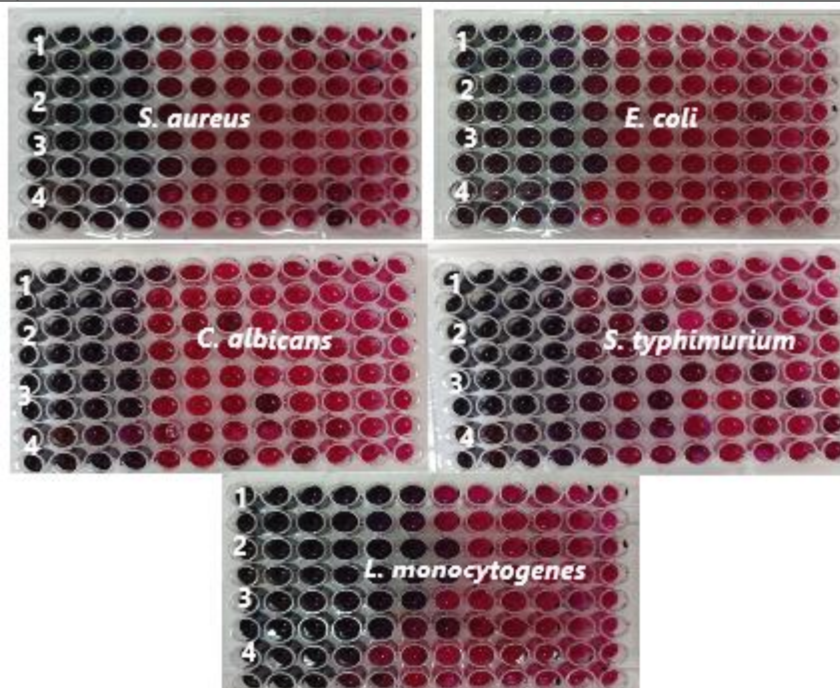

Figure 5. Minimum inhibition concentration (MIC) of different extracts of *O. majorana* aerial parts

Phenolic and Antioxidant capacity of *O. majorana* extracts

Total free-radical scavenger antioxidant activity was measured using the phosphate molybdenum method. Results in Table (3) and Figure (6) revealed that both ethanolic and water extracts from green and dry leaves of *O. majorana* exhibited relevant antioxidant activity with special attention to ethanolic extracts of green and dry plant extracts which gave potent activities (1040.06 and 1055.44 AAE/ g dry extract, respectively). The water extracts of green and dry plant extracts showed applicable antioxidant activities (613.40 and 668.31 AAE/g dry powder extract, respectively). The total phenolic contents of different extracts had been assessed using the Folin-Ciocalteu method (Table 3 and Figure 6). It was found that all extracts exhibited high total phenolic contents, especially the aqueous extracts of green and dry plants (936.88 and 1061.73 mg GAE/g dry powder extract, respectively). Additionally, the ethanolic sample extract of green aerial parts showed moderate phenolic content (523.94 mg GAE/g dry powder extract) but the ethanolic extract of dry plant exhibited noticeable phenolic content (900.52 mg GAE/g dry extract). These results are consistent with Benshikha *et al*. (2013) showed that leaf extract of *O. majorana* exhibited good polyphenol content (266.86mg GAE/g dry extract) and high antioxidant activity. Sweet marjoram (*O. majorana*) extracts from Egypt and Serbia were noticeable as more hopeful owing to their maximum contents of total phenolics as well as flavonoids with the greatest antioxidant, antibacterial in addition to tyrosinase inhibitory activity (Duletić-Laušević *et al*., 2018).

Table 3.Antioxidant activity and total phenolics different solvent extracts from *O. majorana* aerial parts extract

Extract no.	Extracts	Total antioxidant capacity (AAE/ g dry extract)	Total phenolics content (GAE/g dry extract)
1	Ethanol dry	1040.06 ±4.725	532.94 ± 9.106
2	Ethanol green	1055.44 ± 3.349	900.52 ± 15.885
3	Water dry	613.40 ± 13.657	936.88 ± 17.209
4	Water green	668.31 ± 6.532	1061.73 ± 18.917

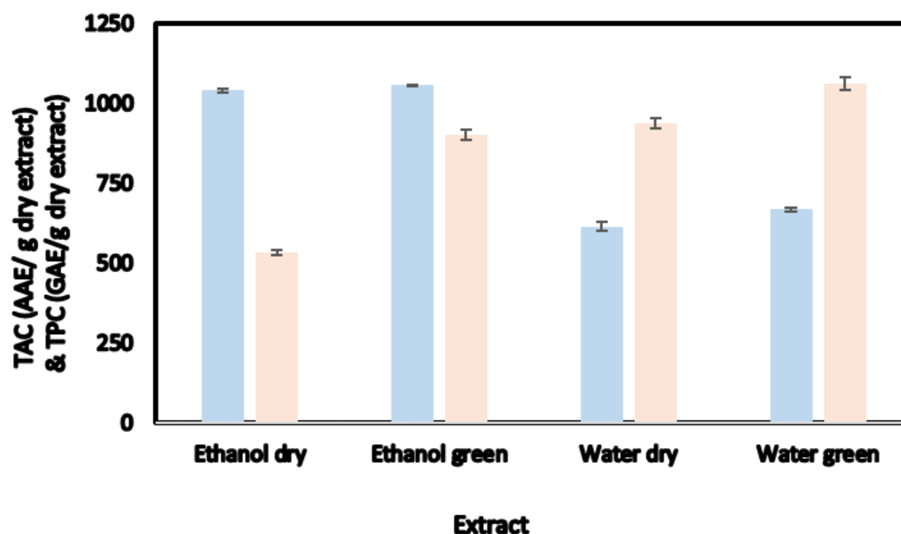

Figure 6.Antioxidant activity and total phenolics of different solvent extracts from *O. majorana* aerial parts extract

Effect of different extracts of *O. majorana* on cell viability by MTT assay

The Cytotoxic activity against human fetal lung fibroblast normal cell line (Wi38) is illustrated in Figure 7(1,2,3 and 4). All extracts were highly safe, i.e. low cytotoxic activity on normal cell lines. The dose-response study was done according to the method of Elshahid *et al*. (2021). The final tested concentrations were 1000, 500, 250, 125, and 62.5 µg/ml to reach 31.25 µg/ml in triplicates. The IC50 values presented in Table (4) were calculated through the concentration-response curve fit to the non-linear regression model using Graph Pad Prism® v6.0 (GraphPad Software Inc., San Diego, CA, USA). Accordingly, Figure (8) shows the effect of different concentrations of extract on the normal cells. Table (4) and Figure (8) showed that a little change of the normal cells had been observed at very high concentrations of extracts. Following the United States National Cancer Institute search program, it was found that any crude extract is generally known to have talented in vitro cytotoxic activity if the IC50 value is located between 30–40µg/ml. In agreement with our results, all extracts showed an IC_{50} value ≥100 µg/ml, so they were all considered to be safe (Mahmoud *et al*., 2022). The cytotoxicity of *O. majorana* hydro-ethanolic extract was evaluated using the MTT colorimetric test. However, the obtained results of the examined extract were lacking cytotoxic activity. *O. majorana* exhibited good potential to prevent diseases caused by the overproduction of free radicals, though it can be used as a natural antioxidant agent and cell proliferation dependent on concentration (Ennaji *et al*., 2020).

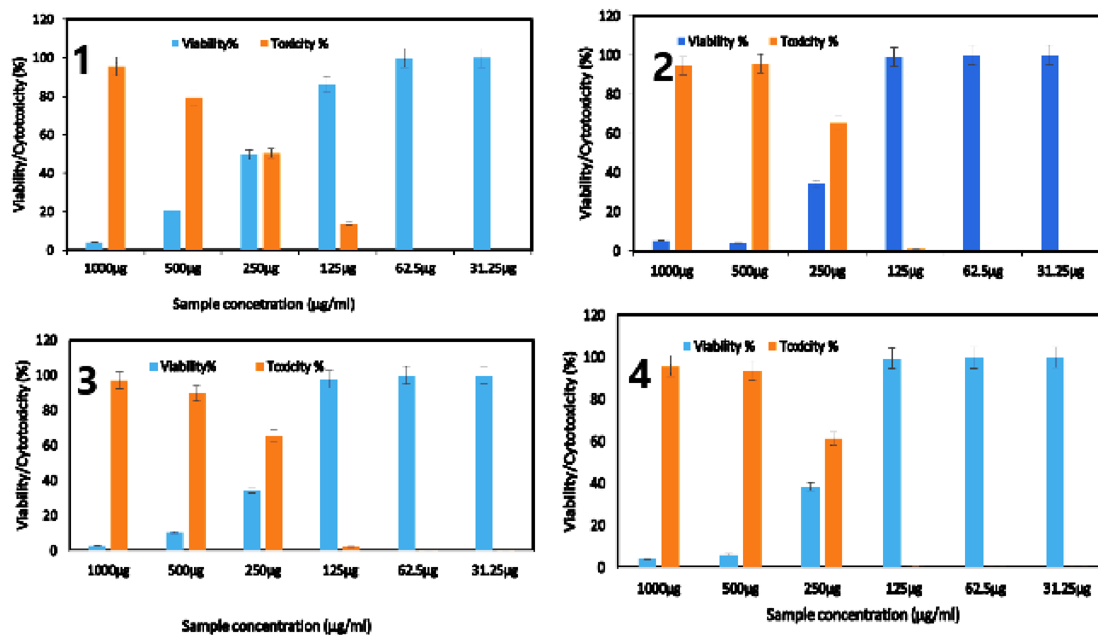

Figure 7. The effect of different extracts of *O. majorana* on Cell Viability by MTT assay

Table 4. The IC50 cytotoxicity of different extracts of *O. majorana* on Cell Viability by MTT assay

No	Extract no.	IC50 (µg/ml)
1	Ethanol dry	303.96 ± 4.18
2	Ethanol green	212.98 ± 2.97
3	Water dry	212.87 ±1.57
4	Water green	220.71 ± 2.49

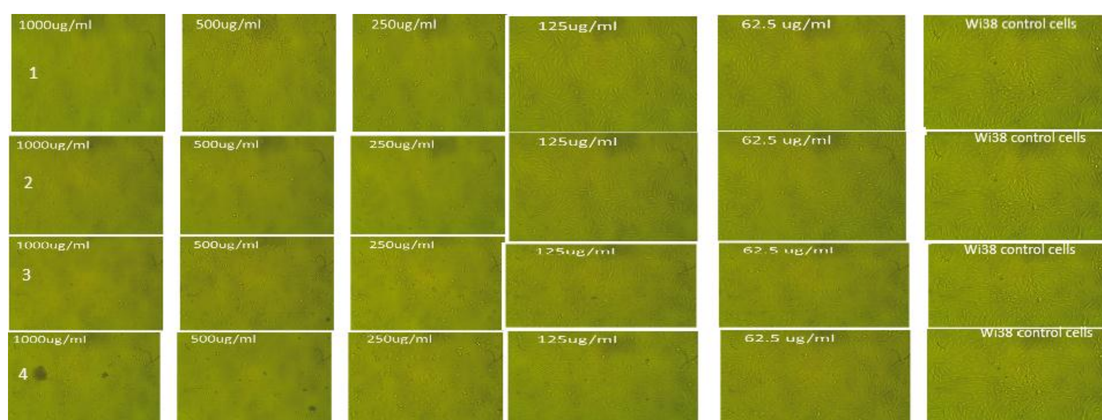

Figure 8. The effect of different concentrations of extracts on the normal cells

Antibiofilm formation of different ethanolic and aqueous extracts of *O. maorana*

The minimum biofilm inhibitory concentrations (MBIC) of different *O. maorana* extracts from aerial parts (green and dry) were evaluated against *S. aureus*, *E. coli*, *S. typhimurium* and *L. monocytogenes* test microbes. The MBIC values varied from extract to other and from extract to other (Table 5 and Figure 9). Extract 1 (ethanolic from dry plant) exhibited acceptable MBIC values (156.25, 39.06, 39.06 and 312.5µg/ml) against *S. aureus*, *E. coli, S. typhimurium and L. monocytogenes*, respectively. Extract 2 (ethanolic extract from a green plant) exhibited considerable MBIC values (39.06, 156.25, 156.25 and 156.25 µg/ml) against the same test microbes. The MBIC values of extract 3 (aqueous extract of a green plant) showed the highest MBIC values (312.5, 625, 625 and 1250 µg/ml) against the mentioned microbes, respectively. Extract revealed MBIC values of 78.125, 78.125, 78.125 and 19.53 µg/ml against *S. aureus*, *E. coli, S. typhimurium and L. monocytogenes*, respectively. Broad-spectrum antimicrobial and anti-biofilm activities were observed with ethanolic extracts from *O. vulgare* considering them as a talented source of bioactive compounds that could control oral biofilms. This effect was considered at non-cytotoxic concentrations (0.195-0.781 mg/ml) (Idir *et al*., 2022).

HPLC fingerprint of polyphenols present in *O. majorana* extracts.

Ethanolic and aqueous extracts of green and dry aerial parts of *O. majorana* were evaluated using HPLC fingerprint studies. The chromatogram presented by HPLC studies of *O. majorana* extracts was compared with standard polyphenolics (Table 6). HPLC examination showed that rosmarinic acid is the major polyphenol present in most extracts having the following values of 16066.28, 10418.98, 4236.28 and 1648.77 µg/g for extracts 1, 2, 3 and 4, respectively. Chlorogenic acid was also found in high contents in all extracts (5331.02, 1365.85, 14612.02 and 6940.53 µg/g for extracts 1, 2, 3 and 4, respectively. In addition, Catechin was found in a high ratio in extracts 1, 2 and 3 (4127.18, 1682.83 and 1094.22 µg/g, respectively. Kaempferol was present in higher amounts in the ethanolic extracts (1 and 2) having the values of 4904.99 and 2069.46 µg/g, respectively. Methyl gallate was only present in a considerable amount in extract 3 (aqueous of green parts) with a value of 5290.48 µg/g. Extract 2 exhibited a better amount of caffiec acid (1181.18 µg/g).

Table 5. The minimum biofilm inhibitory concentration (MBIC) of different solvent extracts of *O. majorana* green and dry aerial parts

No	Extracts	MIC of Biofilm inhibition (µg/ml)			
		S. aureus	***E. coli***	***S. typhimurium coli***	***L. cytomonogenes***
1	Ethanol dry	156.25	39.06	39.06	312.50
2	Ethanol green	39.06	156.25	156.25	156.25
3	Water dry	312.50	625.00	625.00	1250.0
4	Water green	78.125	78.125	78.125	19.53

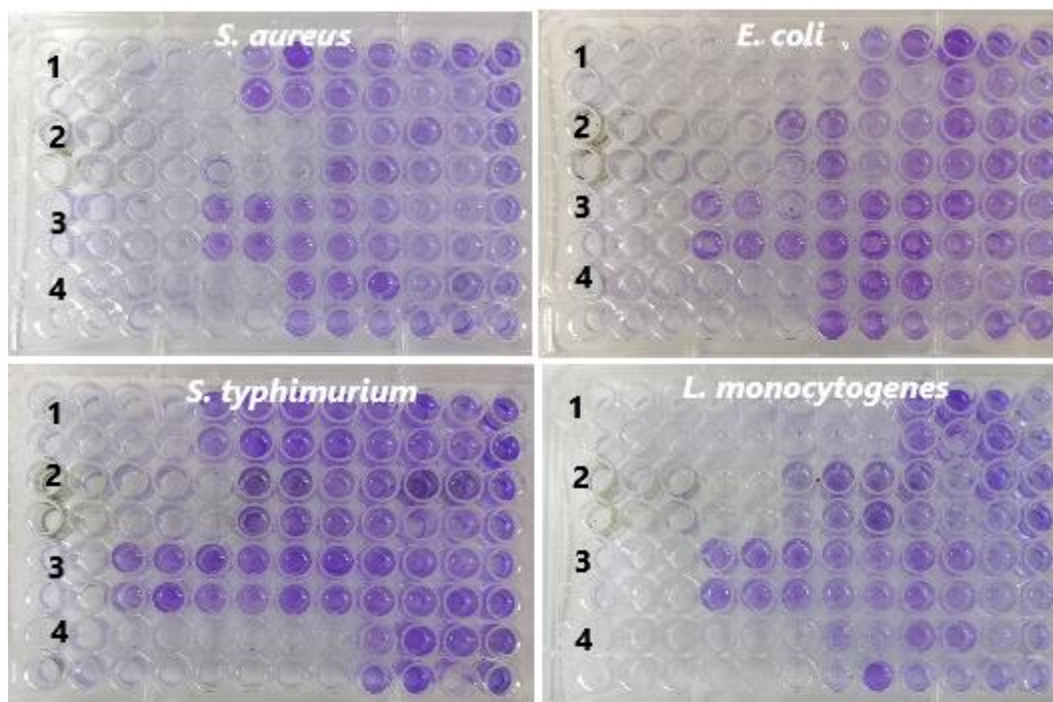

Figure 9. The minimum biofilm inhibitory concentration (MBIC) of different solvent extracts of *O. majorana* green and dry aerial parts

Table 6. Polyhenols of *O. majorana* extracts of green and dry aerial parts

Polyphenols		Sample concentration Conc. (µg/g)			
Standards	**Conc. (µg/ml)**	**Ethanol dry**	**Ethanol green**	**Water dry**	**Water green**
Gallic acid	20	1592.72	1419.30	782.11	1371.23
Chlorogenic acid	50	5331.02	1365.85	14612.02	6940.53
Catechin	75	4127.18	1682.83	1094.22	298.50
Methyl gallate	15	233.90	317.88	5290.48	857.78
Coffeic acid	18	793.88	1181.18	728.03	435.87
Syringic acid	17.2	401.89	322.73	895.87	191.40
Pyro catechol	40	31.87	0.00	235.98	75.96
Rutin	50	90.08	515.55	451.27	197.47
Ellagic acid	60	30.48	18.09	0.00	0.00
Coumaric acid	20	7.86	538.97	53.76	184.53
Vanillin	12.9	76.60	22.86	26.52	15.50
Ferulic acid	20	5.28	13.43	593.36	126.61
Naringenin	30	13.74	35.74	358.62	69.35
Rosmarinic acid	50	16066.28	10418.98	4236.28	1648.77
Daidzein	20	32.42	60.40	272.38	18.12
Quercetin	40	804.36	257.63	103.59	88.87
Cinnamic acid	10	796.87	340.94	37.44	14.47
Kaempferol	20	4904.99	2069.46	262.23	58.02
Hesperetin	20	873.21	204.96	12.65	103.47

The other polyphenols were represented in moderate to low values in all extracts. Ethanolic extracts from *O. majorana* revealed the presence of flavonoids including apigenin, catechin, arbutin, hesperidin, rutin in addition to amentoflavone as well as phenolic acids like caffeic acid, rosmarininc acid as well as coumaric acid in addition to tannins especially gallic acid (Janicsák *et al*.,

1999; Sellami *et al*., 2009 and Khan & Abourashed, 2011).

GC-MS investigation of different extracts of *O. majorana*

Thirty-three major compounds were identified for the ethanolic extract *O. majorana* aerial dry plant parts (Figure 10). The total areas of the identified peaks comprised 71.93% of the whole constituents. Table 7 (a, b, c and d) represents the identified compounds, their molecular weight as well as their molecular structure. The major compounds are Terpinen-4-ol (12.82%), Tricyclene (11.08%), 9-Octadecene,1,1'-[1,2-ethanediylbis(oxy)]bis-,(Z,Z) (4.74%), 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl (2.95%), and α-Pinene, (-) (2.53%). The GC/mass analysis of the ethanolic extract of green aerial parts of *O. majorana* exhibited 36 key compounds (Figure 10). These peaks comprised total peak areas of 83.86 and the chemical structure and the molecular weight of the identified compounds were represented in Table 7 (a-d). The main distinguished compounds are 2-methoxy-6-methylbenzoic acid (21.04%), Dodecane, 2,2,4,9,11,11-hexamethyl (13.66%), Ethyl-9,12,15-octadecatrienoate (7.57%), 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl (2.87%), 3-Oxo-20-methyl-11à-hydroxyconanine-1,4-diene (2.66%), and Octadecanoic acid, ethyl ester (2.55%). GC/MS of extract 3 comprises 32 compounds (Figure 10) constituting 97.54%. The mjaor detected compounds are Hydroquinone di-TMS (62.56%), 6-(T-Butyl)-2,4-dimethyl-2h-thiopyran (7.59%), 1,3-Bis (trimethylsiloxy) benzene (3.14%), Acetamide, n-[2-(3-ethyl-1-methyl-9h-carbazol-2-yl)ethyl]-n-methyl- (3.10%), Syn-9-methyl-1,6-methanofluorene (2.96%), 3,6-Dioxa-2,7-disilaoctane, 2,2,4,5,7,7-hexamethyl- (2.79%), and 1-Methyl-1-n-hexyloxy-1-silacyclohexane (2.66%). Finally, extract 4 showed 32 comprising 90.73% of the total peaks. The main distinguished compounds are 1,3-Bis(trimethylsiloxy)benzene (20.60%), 2-Furanacetaldehyde, tetrahydro-à,3,4,5-tetrakis[(trimethylsilyl)oxy]- (9.11%), Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo- (7.82%), Benzenepropanoic acid, 3,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester (6.90%), Benzothieno[2,3-c]quinoline-6(5h)-thione (5.73%), and 5-Hydroxy-1,3,4-trimethoxy-7-methyl-6-propargy naphthalene (4.62%). Twenty-one compounds were identified as the major components in methanolic extract of O. *majorana* leaves. The major compounds include phytols, 1,6-Octadien-3-Ol, 3,7-Dimethyl (Linalool), Bicyclo[3.1.1]Hept-2-Ene, 2,6-Dimethyl-6-(4-Met), Tau.-Cadinol, Phytol, Silane, [(3,7,11,15-Tetramethyl-2-Hexadecenyl)Oxy] Trimethyl and Squalene (Bhardwaj and Dubey, 2019). The ethanol leaf extract of *O. majorana* showed the following major constituents (%): squalene (2.95%), dl-à-tocopherol (3.46%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol(3.75%), tetracosane, 11-decyl- (3.85%), 1-phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a -dimethyl- 7 - (1-methylethyl)-, [1S-(1à,4aà,10aá)]- (4.69%), phthalic acid, di (2-propylpentyl) ester (5.06%), ursolic acid (5.48%), neophytadiene (6.47%), octadeca-9,12,15-trienoic acid (8.65%), hexadecanoic acid (6.64%) and ç-sitosterol (12.93%) (El-Shintinawy *et al*., 2021).

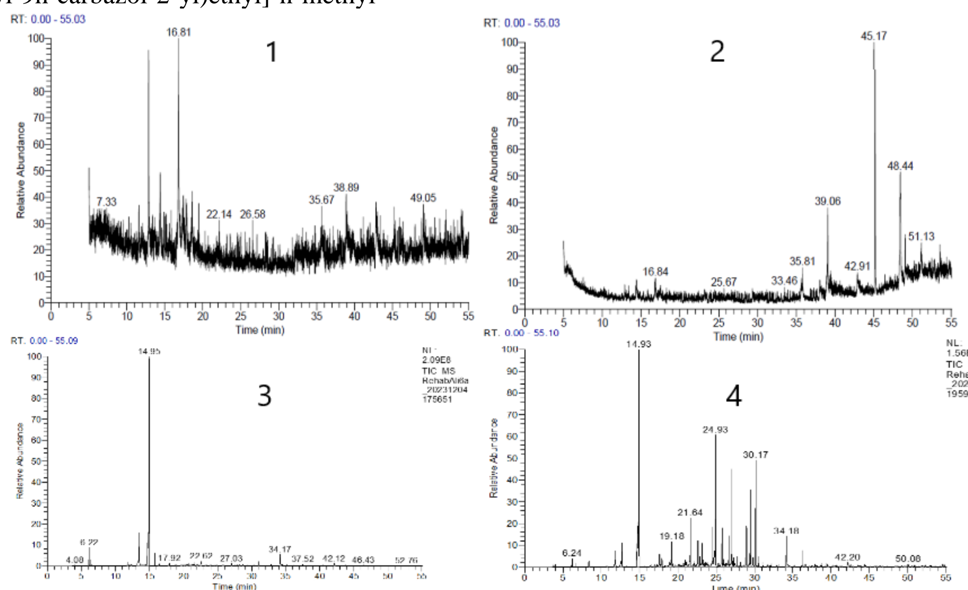

Figure 10. GC/MS Chromatogram of ethanolic and water extracts of green and dry extracts of *O. majorana*

Table 7a. Chemical compounds of ethanol dry extract

No.	R_t	Area%[a]	M.W.	M.F.	Identified compounds
1	10.49	1.32	264	$C_{17}H_{28}O_2$	Methyl hexadecatrienoate
2	11.57	**2.53**	136	$C_{10}H_{16}$	α-Pinene, (-)
3	12.86	**11.08**	136	$C_{10}H_{16}$	Tricyclene
4	13.38	1.77	294	$C_{16}H_{22}O_5$	6-Methyl-5-oxo-11-propenyl-12,13-dioxatricyclo[7.3.1.0(1,6)]tridecane-8-carboxylic acid
5	13.68	1.59	1022	$C_{69}H_{98}O_6$	Docosahexaenoic acid, 1,2,3-propanetriylester
6	14.39	**4.74**	562	$C_{38}H_{74}O_2$	9-Octadecene,1,1'-[1,2-ethanediylbis(oxy)]bis-,(Z,Z)
7	14.90	**2.08**	378	$C_{26}H_{50}O$	4-Heneicosanone, 1-cyclopentyl
8	15.06	1.07	578	$C_{30}H_{42}O_{11}$	9-Desoxo-9-xi-hydroxy-3,7,8,9,12-pentaacetateingol
9	15.11	1.0	502	$C_{32}H_{54}O_4$	7,8-Epoxylanostan-11-ol, 3-acetoxy
10	15.26	1.15	550	$C_{40}H_{54}O$	Anhydrolutein II
11	15.56	1.13	574	$C_{30}H_{38}O_{11}$	Anodendrosidee 2
12	16.81	**12.82**	154	$C_{10}H_{18}O$	Terpinen-4-ol
13	17.29	1.12	312	$C_{20}H_{40}O_2$	Olealdehyde, dimethyl acetal
14	17.40	1.34	408	$C_{24}H_{40}O_5$	Cholic acid
15	18.58	**2.95**	410	$C_{30}H_{50}$	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl
16	23.91	1.11	620	$C_{39}H_{72}O_5$	DI-(9-Octadecenoyl)-glycerol
17	24.91	1.07	547	$C_{38}H_{33}N_3O$	6-benzyl-3,5-diphenyl-4-(phenylimino)-1-N-thiomethyl-1,2,3,4-tetrahydro-1,3-diazin-2-one
18	26.14	1.44	184	$C_{11}H_{20}O_2$	2-Methyl-2-butenoic acid, 3-methylpentylester, Z isomer
19	28.40	1.11	502	$C_{32}H_{54}O_4$	7,8-Epoxylanostan-11-ol, 3-acetoxy
20	32.37	1.61	686	$C_{41}H_{66}O_8$	4-O-Methylphorbol-12,13-didecanoate
21	32.57	1.22	440	$C_{28}H_{40}O_4$	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester
22	33.22	0.99	324	$C_{23}H_{48}$	Heptadecane, 9-hexyl
23	34.36	1.30	564	$C_{40}H_{52}O_2$	Canthaxanthin
24	35.67	1.01	214	$C_{14}H_{30}O$	3-Tetradecanol
25	35.74	1.21	578	$C_{38}H_{74}O_3$	9-Octadecenoic acid, 2-(octadecyloxy)ethyl ester
26	35.83	1.34	596	$C_{40}H_{52}O_4$	Astaxanthin
27	38.86	2.43	420	$C_{24}H_{36}O_6$	8,14-Seco-3,19-epoxy androstane-8,14-dione,17-acetoxy-3á-methoxy-4,4-di methyl
28	39.37	1.04	354	$C_{22}H_{42}O_3$	n-Butyl ricinoleate
29	39.82	1.10	309	$C_{20}H_{39}NO$	cis-11-Eicosenamide
30	42.38	1.0	414	$C_{27}H_{42}O_3$	Pseduosarsasapogenin-5,20-dien
31	42.80	2.38	550	$C_{36}H_{46}N_4O$	Octaethylporphyrin N-oxide
32	45.63	1.17	542	$C_{40}H_{62}$	Phytofluene
33	53.56	1.71	462	$C_{25}H_{42}N_4O_4$	2-Nonadecanone-2,4-dinitrophenylhydrazine
		71.93%			

Rt: Retention time; **M.W.:** Molecular weight; **M.F.:** Molecular formula.

Table 7b. Chemical compounds of ethanol green extract

No.	R_t	Area%[a]	M.W.	M.F.	Identified compounds
1	12.90	1.14	71	C_4H_9N	3-Buten-2-amine
2	13.40	1.06	154	$C_{10}H_{18}O$	cis-Sabinenehydrate
3	14.37	2.16	154	$C_{10}H_{18}O$	3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)
4	16.80	2.11	594	$C_{36}H_{42}N_4O_4$	(2RS)1,3,8-trimethyl-4-propyl-5-ethyl-2-(1-hydroxyethyl)-7-methoxycarbonyl ethyl-6,.gmma.methylene carbonyl porphine
5	18.13	0.62	686	$C_{41}H_{66}O_8$	4-O-Methylphorbol 12,13-didecanoate
6	22.70	0.97	689	$C_{45}H_{31}N_5O_3$	2-Methoxy-3-nitro-5,10,15,20-tetraphenyl-2,3-dihydroporphyrin
7	23.52	0.77	644	$C_{44}H_{32}N_6$	5,10-bis(3-aminophenyl)-15,20-diphenylporphyrin
8	23.80	0.76	696	$C_{40}H_{56}O_{10}$	Nephthoside 1,2',3',4'-Tetraacetate
9	24.32	0.64	542	$C_{40}H_{62}$	Phytofluene
10	29.40	0.90	310	$C_{22}H_{46}$	Docosane
11	31.57	0.69	1022	$C_{69}H_{98}O_6$	Docosahexaenoic acid, 1,2,3-propanetriylester
12	32.55	0.72	312	$C_{20}H_{40}O_2$	Ethanol, 2-(9-octadecenyloxy),(Z)
13	35.64	1.88	502	$C_{32}H_{54}O_4$	7,8-Epoxylanostan-11-ol, 3-acetoxy
14	35.71	0.77	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)
15	35.81	**2.55**	312	$C_{20}H_{40}O_2$	Octadecanoic acid, ethyl ester
16	37.28	0.85	678	$C_{45}H_{58}O_5$	7,13,19,25-Tetratertbutyl-27,28,29,30-tetrahydroxy-2,3-bishomo-3-oxacalix[4] Arene
17	37.70	0.77	528	$C_{32}H_{48}O_6$	2-(16-Acetoxy-11-hydroxy-4,8,10,14-tetramethyl-3-oxohexadecahydrocyclo penta[a]phenanthren-17-ylidene)-6-methylhept-5-enoicacid, methyl ester
18	38.03	**2.66**	341	$C_{22}H_{31}NO_2$	3-Oxo-20-methyl-11à-hydroxyconanine-1,4-diene
19	39.06	**7.57**	306	$C_{20}H_{34}O_2$	Ethyl-9,12,15-octadecatrienoate
20	39.46	1.38	254	$C_{18}H_{38}$	Dodecane, 2,2,4,9,11,11-hexamethyl
21	39.88	1.18	338	$C_{22}H_{42}O_2$	2-Heptadec-5''-en-1''-yloxytetrahydrofuran
22	40.77	0.77	324	$C_{23}H_{32}O$	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde
23	42.90	2.20	470	$C_{26}H_{38}N_4O_4$	Ceanothine C
24	42.99	1.27	324	$C_{15}H_{24}N_4O4$	O,N-Permethylate D ACALAHIS
25	44.62	1.26	576	$C_{38}H_{72}O_3$	9-Octadecenoicacid(Z), 2-(9-octadecenyloxy)ethyl ester, (Z)
26	45.17	**21.04**	166	$C_9H_{10}O_3$	2-methoxy-6-methyl benzoic acid
27	45.72	0.67	436	$C_{26}H_{44}O_5$	Ethyl isoallocholate
28	46.42	1.12	440	$C_{28}H_{40}O_4$	Azafrin methyl ester
29	47.07	0.69	346	$C_{19}H_{22}O_6$	Isochiapin B
30	48.42	**13.66**	254	$C_{18}H_{38}$	Dodecane, 2,2,4,9,11,11-hexamethyl
31	49.03	**2.87**	410	$C_{30}H_{50}$	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl
32	50.19	1.53	490	$C_{35}H_{70}$	17-Pentatriacontene
33	52.77	1.88	402	$C_{23}H_{46}O_5$	Docosanoic acid, 8,9,13-trihydroxy, methyl ester
34	52.84	1.01	450	$C_{27}H_{46}O_5$	Cholestan-26-oicacid, 3,7,12-trihydroxy,(3à,5á,7à,12à)
35	54.30	0.68	430	$C_{30}H_{54}O$	Tetrahydro dammaradienol
36	54.69	1.06	610	$C_{27}H_{30}O_{16}$	Lucenin 2
		83.86%			

Table 7c. Chemical compounds of water dry extract

No.	R_t	Area%	M.W.	M.F.	Identified compounds
1	4.08	0.28	160	$C_7H_{16}O_2Si$	Butanoic acid, trimethylsilyl ester
2	6.06	1.17	234	$C_{10}H_{26}O_2Si_2$	3,6-Dioxa-2,7-disilaoctane, 2,2,4,5,7,7-hexamethyl-
3	6.22	2.79	234	$C_{10}H_{26}O_2Si_2$	3,6-Dioxa-2,7-disilaoctane, 2,2,4,5,7,7-hexamethyl-
4	6.46	0.28	353	$C_{26}H_{27}N$	3-Cyclohexen-1-amine, n,n-dimethyl-2,5,6-triphenyl-, (1à,2á,5á,6á)-
5	11.86	0.92	308	$C_{12}H_{32}O_3Si_3$	3,7-Dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl-5-[(trimethylsilyl)oxy]-
6	12.35	0.17	308	$C_{12}H_{32}O_3Si_3$	Trimethylsilyl ether of glycerol
7	12.42	0.17	322	$C_{13}H_{34}O_3Si_3$	2,2,4,8,8-Pentamethyl-5-[(trimethylsilyl)oxy]-3,7-dioxa-2,8-disilanonane
8	13.36	2.96	194	$C_{15}H_{14}$	Syn-9-methyl-1,6-methanofluorene
9	13.47	7.59	182	$C_{11}H_{18}S$	6-(T-Butyl)-2,4-dimethyl-2h-thiopyran
10	14.42	0.29	292	$C_{15}H_{17}BrO$	4a,10a-Methanophenanthren-9á-ol, 11-syn-bromo-1,2,3,4,4a,9,10,10a-octahydro-
11	14.69	3.14	254	$C_{12}H_{22}O_2Si_2$	1,3-Bis(trimethylsiloxy)benzene
12	14.95	62.56	254	$C_{12}H_{22}O_2Si_2$	Hydroquinone di-TMS
13	15.76	2.66	214	$C_{12}H_{26}OSi$	1-Methyl-1-n-hexyloxy-1-silacyclohexane
14	16.42	0.85	282	$C_{17}H_{14}O_4$	9,10-Anthracenedione, 1,8-dimethoxy-3-methyl-
15	17.91	0.59	336	$C_{13}H_{32}O_4Si_3$	Butanal, 2,3,4-tris[(trimethylsilyl)oxy]-, (r*,r*)-
16	18.98	0.27	282	$C_{14}H_{26}O_2Si_2$	2-(O-Hydroxyphenyl)ethanol 2TMS
17	20.57	0.60	286	$C_{22}H_{22}$	Benzo[b]triphenylene, 1,2,3,4,10,11,12,13-octahydro-
18	20.68	0.39	288	$C_{14}H_{32}O_2Si_2$	1,2-Cyclooctanediol, bis-O-(trimethylsilyl)-
19	21.63	0.74	286	$C_{16}H_{18}N_2OS$	(3-Ethylthio-5-isopropyl-4-methoxy-2,4,6-cycloheptatrienyl idene)malononitrile
20	22.10	0.33	224	$C_{15}H_{16}Si$	9,9-Dimethyl-9-sila-9,10-dihydrophenanthrene
21	22.63	1.08	376	$C_{15}H_{32}O_5Si_3$	Hexanedioic acid, 3-oxo-, tris(trimethylsilyl) deriv.
22	25.80	0.57	688	$C_{30}H_{56}N_2O_{10}Si_3$	5-Allyl-1,3-dimethyl-5-(3-hydroxy-1-methylbutyl) barbituric acid o-tris(trimethylsilyl)glucuronide methyl ester
23	27.03	0.49	398	$C_{24}H_{30}O_5$	2H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one, 5-hydroxy-8,8-dimethyl-6-(2-methyl-1-oxobutyl)-4-pentyl-, (.+-.)-
24	27.85	0.23	325	$C_{19}H_{23}NO_2Si$	1H-Indole, 6-methoxy-5-(phenylmethoxy)-1-(trimethylsilyl)-
25	28.70	0.26	328	$C_{19}H_{40}O_2Si$	Hexadecanoic acid, trimethylsilyl ester
26	30.31	0.29	740	$C_{20}H_{60}O_{10}Si_{10}$	Cyclodecasiloxane, eicosamethyl-
27	31.06	0.88	227	$C_{14}H_{29}NO$	Tetradecanamide
28	32.85	0.24	578	$C_{16}H_{50}O_7Si_8$	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
29	34.16	3.10	308	$C_{20}H_{24}N_2O$	Acetamide, n-[2-(3-ethyl-1-methyl-9h-carbazol-2-yl)ethyl]-n-methyl-
30	34.55	0.38	241	$C_{16}H_{35}N$	1-Hexadecanamine
31	37.52	0.43	532	$C_{16}H_{48}O_6Si_7$	Heptasiloxane, hexadecamethyl-
32	42.12	0.84	337	$C_{22}H_{43}NO$	*trans*-13-Docosenamide
		97.54			

Table 7d.Chemical compounds of water green extract

No.	R_t	Area%	M.W.	M.F.	Identified compounds
1	6.24	0.49	234	$C_{10}H_{26}O_2Si_2$	3,6-Dioxa-2,7-disilaoctane,2,2,4,5,7,7-hexamethyl-,(R*,R*)-(.+-.)-
2	8.39	0.73	234	$C_9H_{22}O_3Si_2$	Propanoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester
3	11.84	1.61	308	$C_{12}H_{32}O_3Si_3$	Trimethylsilyl ether of glycerol
4	12.68	1.95	262	$C_{10}H_{22}O_4Si_2$	Butanedioic acid, bis(trimethylsilyl) ester
5	14.61	0.60	254	$C_{19}H_{26}$	2-Ethyl-1,3,4,5,6,7,8-heptamethylnaphthalene
6	14.70	1.30	254	$C_{20}H_{14}$	11,12-Dihydrobenzo[b]fluoranthene
7	14.78	3.77	254	$C_{12}H_{22}O_2Si_2$	Silane, [1,4-phenylenebis(oxy)]bis[trimethyl-
8	14.92	20.60	254	$C_{12}H_{22}O_2Si_2$	1,3-Bis(trimethylsiloxy)benzene
9	17.60	1.03	466	$C_{18}H_{42}O_6Si_4$	Mannoonic acid, 2,3,5,6-tetrakis-o-(trimethylsilyl)-, lactone
10	17.91	0.77	410	$C_{16}H_{42}O_4Si_4$	Threitol, 1,2,3,4-tetrakis-o-(trimethylsilyl)-, D-
11	18.96	0.48	336	$C_{13}H_{32}O_4Si_3$	Propanoic acid, 2-methyl-2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester
12	19.18	2.04	336	$C_{13}H_{32}O_4Si_3$	(R*,S*)-2,3-Dihydroxybutanoic acid, tris(trimethylsilyl) deriv.
13	20.97	0.45	438	$C_{16}H_{38}O_6Si_4$	Succinic acid, 2,3-bis(trimethylsiloxy)-, bis(trimethylsilyl) ester
14	21.64	4.62	286	$C_{17}H_{18}O_4$	5-Hydroxy-1,3,4-trimethoxy-7-methyl-6-propargy naphthalene
15	22.63	2.02	314	$C_{22}H_{18}O_2$	1(3AH)-Pentalenone, 4,6a-dihydro-4-(4-methylbenzoyl)-3-phenyl-, (3aà,4à,6aà)-
16	23.17	1.89	310	$C_{15}H_{26}O_3Si_2$	Hydrocinnamic acid, p-(trimethylsiloxy)-, trimethylsilyl ester
17	24.55	3.05	462	$C_{19}H_{42}O_5Si_4$	1-Cyclohexene-1-carboxylic acid, 3,4,5-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester, [3R-(3à,4à,5á)]-
18	24.77	0.47	384	$C_{17}H_{32}O_4Si_3$	Benzeneacetic acid, 3,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester
19	24.92	9.11	466	$C_{18}H_{42}O_6Si_4$	2-Furanacetaldehyde, tetrahydro-à,3,4,5-tetrakis[(trimethylsilyl)oxy]-
20	25.78	3.52	346	$C_{25}H_{18}N_2$	9,9'(10H,10'H)-Spirobiacridine
21	25.97	0.61	365	$C_{17}H_{31}NO_2Si_3$	1H-Indole, 1-(trimethysilyl)-2,5-bis[(trimethylsilyl)oxy]-
22	27.06	6.90	398	$C_{18}H_{34}O_4Si_3$	Benzenepropanoic acid, 3,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester
23	27.24	1.26	275	$C_{15}H_{21}NO_2Si$	1H-Indole-3-acetic acid, 1-(trimethylsilyl)-, ethyl ester
24	27.75	0.78	247	$C_{13}H_{17}NO_2Si$	1H-Indole-2-carboxylic acid, 1-methyl-, trimethylsilyl ester
25	28.71	0.79	328	$C_{19}H_{40}O_2Si$	Hexadecanoic acid, trimethylsilyl ester
26	29.32	0.95	235	$C_{16}H_{29}N$	(3E)-3-Hexadecenenitrile
27	29.50	5.73	267	$C_{15}H_9NS_2$	Benzothieno[2,3-c]quinoline-6(5h)-thione
28	30.17	7.82	612	$C_{24}H_{60}O_6Si_6$	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo-
29	30.56	0.83	456	$C_{31}H_{52}O_2$	3á-Acetoxystigmast-5-en
30	34.18	2.78	328	$C_{18}H_{20}N_2O_4$	1,3-Cyclohexanedione, 2-[2-(6,7-dimethoxy-4-quinazolinyl)ethyl]-
31	36.22	1.32	319	$C_{19}H_{17}N_3O_2$	1H-Benzimidazole, 1,3-diacetyl-2,3-dihydro-2-(1h-indol-3-yl)-
32	39.45	0.46	688	$C_{32}H_{48}N_2O_9Si_3$	5-(4-Hydroxyphenyl)-3-methyl-5-phenylhydantoin glucur-onide methyl ester tms ether
		90.73%			

Preservation of canned Mackerel

The four prepared extracts (ethanolic and water extracts of green and dry aerial parts of *O. majorana*) were added to the prepared Mackerel fish (Figure 11) with a concentration of 1mg/20ml. Results illustrated in Table 8 and presented in Figure (11and 12) revealed that the non-treated canned fish exhibited CFU values of $117x10^5$. On the other hand, ethanolic extracts of dry and green extracts of *O. majorana* exhibited a promising reduction in bacterial growth of treated canned Mackerel fish with reduction values of 92.31 ($9x10^5$ CFU) and 94.02% ($7x10^5$CFU), respectively. Aqueous extracts of green and dry *O. majorana* aerial parts exhibited CFU values of $30x10^5$ and $15x10^5$ CFU with a reduction percent of 74.36 and 84.61%, respectively. *O. majorana* L. ethanolic extract exhibited a promising effect on food deteriorating and poisoning microbes such as *S. typhimurium* and *L. monocytogenes*, G-ve and G+ve bacteria, respectively at 700 mg/l (Choi and Rhim, 2008).

Preparation of Packaging films for food preservation

The prepared PVA/PE were characterized for their antimicrobial, mechanical and FTIR studies.

Antimicrobial activity of PVA/PE films

Results in Figure (13) presented the antimicrobial sensitivity of both PVA and PVA/PE composites. It had been found that PVA (control) didn't has any antimicrobial toward any test microbe. On the other hand, all PVA/PE composites exhibited antimicrobial action toward all test microbes including *S. aureus*, *E. coli*, *C. albicans* as well as *S. typhimurium* and *L. momcytogenes*.

Table 8. The reduction of bacterial growth of canned Mackerel fish after treatment with different *O. majorana*

Makarel sample	Colony Forming Unit (CFU)	Reduction in growth (%)
Untreated	$117 x10^5$	-
Ethanol dry	9	92.31
Ethanol green	7	94.02
Water dry	30	74.36
Water green	18	84.61

Figure 11.The treatment of Mackerel with different extracts from *O. majorana*

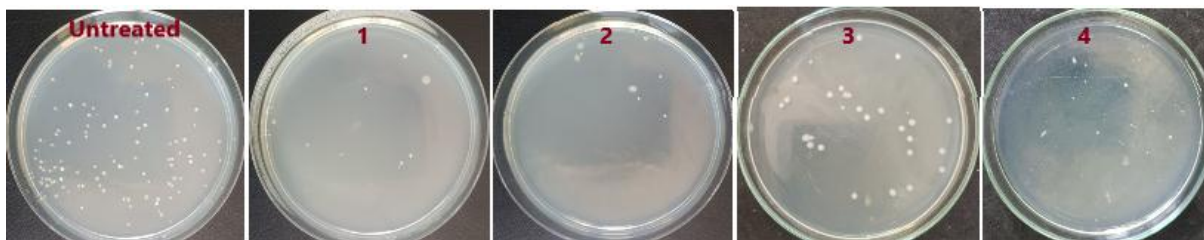

Figure 12. The reduction of bacterial growth of canned Mackerel fish after treatment with different *O. majorana* extracts

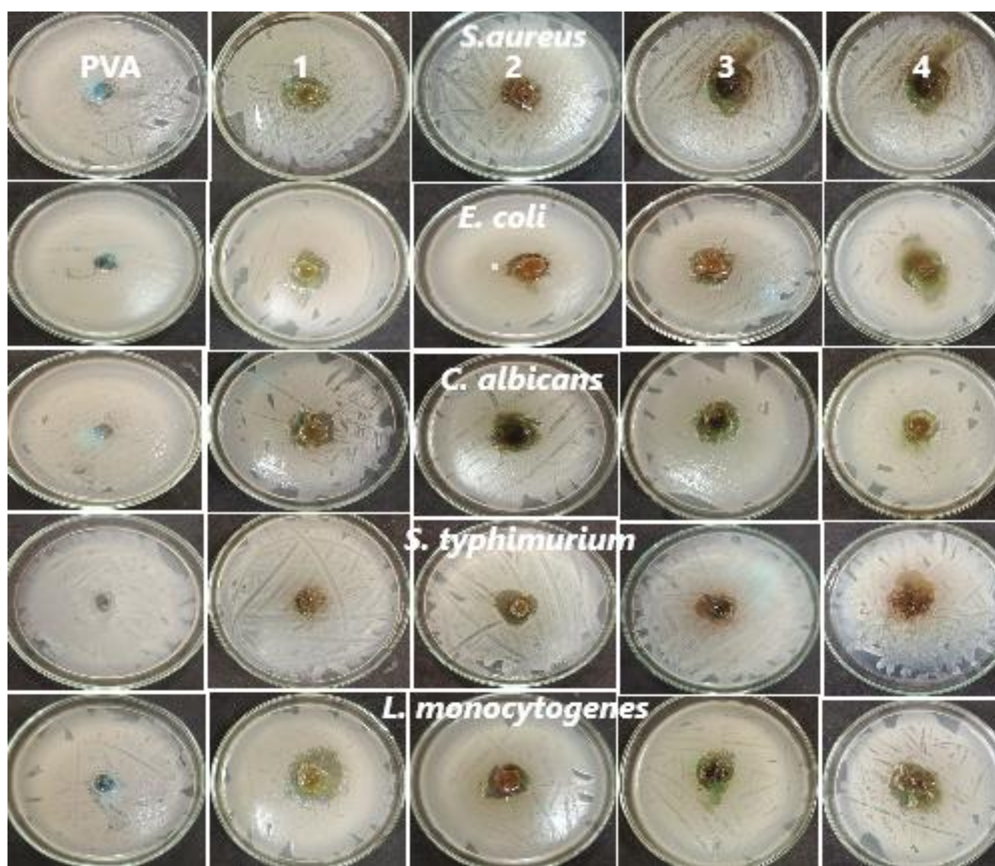

Figure 13. Antimicrobial effect of PVA and PVA/PE composite

Mechanical properties of polyvinyl alcohol/Plant extract PVA/PE composites.

Tensile strength (TS) and elongation (E) tests were utilized to designate the mechanical properties of the prepared PVA/PE composites containing varying types of plant extracts. Furthermore, the prepared PVA/PE composites displayed enhanced mechanical property. Table (9) shows the characteristic tensile strength, as the tensile strength (TS) of the PVA/PE1 composites increased from 12.26 MPa to 12.70 MPa, with the addition of 5% of PE1 into pure PVA matrix, whereas the elongation increased from 147.8 % to 550.5 % with the same addition. Moreover, the tensile strength (TS) of the PVA/PE2 composites increased from 12.26 MPa to 12.78 MPa, with the addition of 5% of PE2 into pure PVA matrix, while the elongation improved from 147.8 % to 510.8% with the same addition of PE2. Similarly, the tensile strength (TS) of the PVA/PE3 composites was improved from 12.26 MPa to 13.31 MPa, with the addition of 5% of PE3 inside the pristine PVA, although the great enhancement in the elongation which improved from 147.8 % to 806.2 % with the same addition of PE3. Furthermore, the tensile strength (TS) of the PVA/PE4 composites developed from 12.26MPa to 12.69 MPa, with the addition of 5% of PE4 into pure PVA matrix, although the elongation enhanced from 147.8 % to 493.2 % with the identical addition of PE4. The obtained results revealed that the significant enhancement of mechanical properties of the fabricated PVA/GPE composites.

Table 9.Mechanical properties of the prepared PVA/PE (plant extracts) composites

Samples	Plant extract (EP), %	Tensile strength, MPa	Elongation, %
PVA blank	0.0 %	12.26	147.8
PVA/PE (Ethanol dry) composites	5.0 %	12.70	550.5
PVA/PE (Ethanol green) composites	5.0 %	12.78	510.8
PVA/PE (Water dry) composites	5.0 %	13.31	806.2
PVA/PE (Water green) composites	5.0 %	12.69	493.2

FT-IR investigation of the synthesized PVA and PVA/PE (plant extract) composites

The prepared PVA/PE composites, each containing a different loading of 5 % of plant extract, all were examined via FT-IR (Figure 14). The FT-IR spectrum of pure PVA is also displayed in Figure (14a), where all main peaks related to the hydroxyl and acetate groups were found. Additionally, the stretching OH from the intramolecular and intermolecular hydrogen bonds is associated with the wide-ranging band perceived between 3720 and 3145 cm^{-1}. The stretching of C-H from alkyl groups is responsible for the vibrational band seen between 2894 and 2970 cm^{-1}, while the peaks between 1743 and 1700 cm^{-1} are related to the stretching of C-O and C-O from the acetate group from PVA (saponification action of polyvinyl acetate). Correspondingly, (Figure 14 b, c, d, and e) the FT-IR absorption bands of the fabricated PVA/PE composites The OH and CH groups do not changes and all the peaks are in the same wavenumbers A high peak was seen at about 3365 cm^{-1} for the PVA/PE composites film, which loading of 5% different plant extracts these overlapping absorption bands reflect O-H vibrations and OH groups of plant extract this enhanced the hydrogen bonding in the PVA/PE composite films, which may be the cause of the better mechanical properties of the fabricated PVA/PE composite films.

Sensory evaluation of canned mackerel with fresh and dry *O. majorana*.

The sensory characteristics of canned Mackerel were impacted by the addition of *O. majorana*, as indicated by results shown in Table (10) and Figure (15). It makes a difference between the controls and other groups by overall acceptability or appearance, Taste, texture, or color. The overall sensory characteristics score (which varied between 8.27 to 8.43) for Mackerel in Table (10) show a significant difference when compared with the other treatments. The Mackerel supplemented with 1% fresh *O. majorana* scored excellent (8.43) overall. Aubourg et al., (2002) indicated that storing Mackerel at low temperatures affects its shelf life, causing odor and discoloration, which consumers dislike. Our study confirmed a link between storage time and taste acceptance. Also, adding *O. majorana* improve the taste of canned Mackerel. These findings stress the need to mind ingredients to enhance canned mackerel's taste.

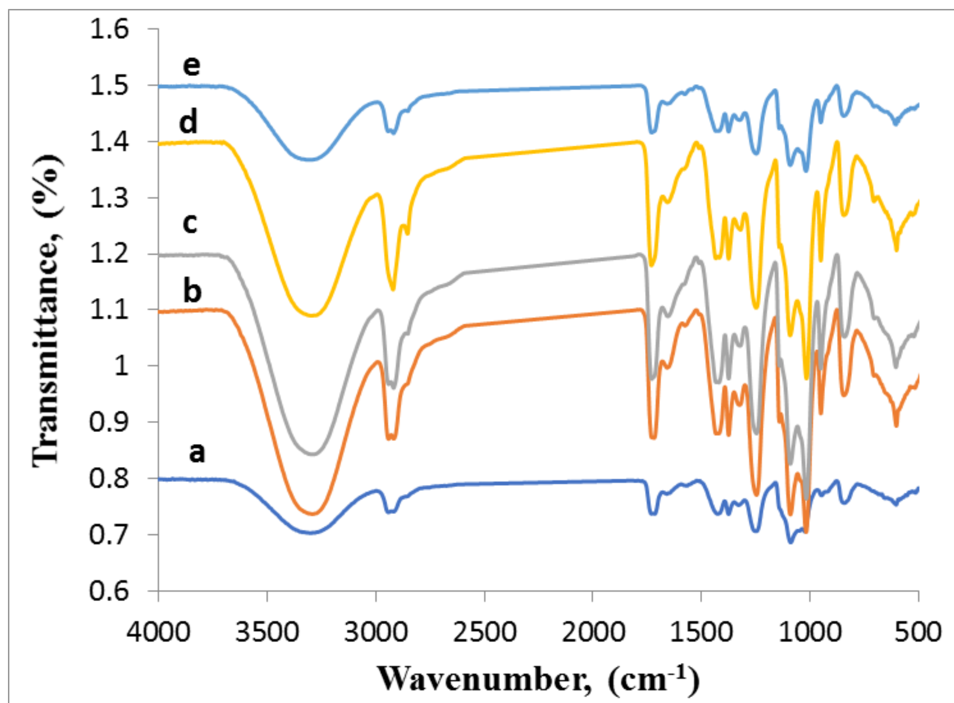

Figure 14. FTIR of a) Pure PVA, as well as PVA/PE composites containing different 5 % of different plant extracts, b) PVA/PE1 composites, c) PVA/PE2 composites, d) PVA/PE3 composites, e) PVA/PE4 composites

Table 10. Sensory evaluation of canned mackerel (n = 30)

Sample	Appearance	Taste	Texture	Color	odor	Acceptability
C.C.M	8.13^{a}±0.97	8.10^{a} ±0.71	8.20^{a} ±0.81	8.40^{a} ±0.86	8.20^{a} ±0.81	8.27^{a} ±0.87
C.M.F.M 1%	8.30^{a} ±0.79	8.33^{a} ±0.55	8.33^{a} ±0.66	8.53^{a} ±0.78	8.23^{a} ±0.90	8.43^{a} ±0.86
C.M.D.M 1%	7.10^{b} ±0.80	7.27^{b} ±0.78	8.00^{a} ±0.46	7.30^{b} ±0.70	7.17^{b} ±0.59	7.43^{b} ±0.57
(F)	17.11	19.90	1.96	22.57	18.34	14.24
(P)	0.001	0.001	0.147	0.001	0.001	0.001

*C.C.M = Control canned mackerel C.M. F.M = canned mackerel with fresh *O.majorana* extract C.M. D. M = canned mackerel with dried *O. majorana* extract ,*Values are expressed as means ± SE Mean values and significantly different ($p < 0.05$)

(1) C.C.M **(2) C.M. F.M** **(3) C.M. D. M**

Figure 15. Sensory evaluation of canned mackerel

*C.C.M = Control canned mackerel, C.M. F.M = canned mackerel with fresh majorana extract, C.M. D. M = canned mackerel with dried majorana extract

CONCLUSION

Origanum majorana L (sweet marjoram) is widely cultivated worldwide. This herb exhibited many medical purposes for treating diarrhea, cough, etc. Ethanolic and aqueous extracts from *O. majorana* exhibited promising antimicrobial, antioxidant and antibiofilm activities. *O. majorana* possessed high phenolic contents especially: Rosmarinus acid > chlorogenic acid > kaempferol > catechin and gallic acid as evaluated by HPLC fingerprint. The extracts were considered as safe source for the preservation of packaged food especially canned Mackerel as they had low cytotoxic activity against normal cells with IC50 more than 200µg/ml. The authors recommend incorporating these extracts into polyvinyl alcohol (PVA) films used to preserve foods from bacterial infections, as well as using these extracts as food additives .

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الملخص العربي

تقييم تأثير مستخلصات البردقوش كمضاد للميكروبات في سمك الماكريل المعلب: دراسة على السلامة الغذائية والتخزين

نيفين الوردانى، مريم عبد القادر، إسلام الصاوي، محمد عبد العزيز

البردقوش هو عضو في العائلة الشفوية، ويزرع في مناطق مختلفة بما في ذلك منطقة البحر الأبيض المتوسط ومصر أيضًا. تم استخدام مستخلصات البرقوش كعامل مثبط ضد ميكروبات التسمم الغذائي. تم استخلاص الأجزاء الهوائية (الخضراء والجافة) من البردقوش باستخدام الإيثانول (٩٦٪) والماء. تم اختبار المستخلصات لنشاطها المضاد للميكروبات ضد المكورات العنقودية الذهبية والاشريكية القولونية، خمائر الكانديدا وكذلك البكتيريا المنقولة بواسطة الغذاء الليستيريا والسالمونيلا. وقد لوحظ أن جميع المستخلصات لها أنشطة واعدة مضادة للميكروبات تجاه جميع الميكروبات التي تم اختبارها بما في ذلك الميكروبات المنقولة بواسطة الغذاء. أظهرت المستخلصات المحضرة فعالية مضادة للأكسدة خاصة المستخلصات الإيثانولية (١٠٤٠,٠٦ و١٠٥٥,٤٤ مكافئ حمض الاسكوربيك/ جم). بالإضافة إلى ذلك، أظهرت المستخلصات محتويات فينولية إجمالية واعدة (٥٣٢,٩٤ و٩٠٠,٥٢ مليجرام مكافئ لحمض الجاليك/جرام). تم إجراء كشف تحليل كروماتوغرافيا الغاز – مطياف الكتلة للمستخلصات الأربعة وكشفت النتائج أن تيربينين-٤-أول (١٢,٨٢%)، تراي سيلاين (١١,٠٨%)، ٩-أوكتاديسين،١،١-[١،٢-إيثانيديلبيس(أوكسي)]-، (٤,٧٤%)،-ميثوكسي-٦ حمض ميثيل بنزويك (٢١,٠٤%)، دوديكان، ٢،٢،٤،٩،١١،١١-هيكسامثيل (١٣,٦٦%) وإيثيل-٩،١٢ ١٥-أوكتاديكاترينوات (٧,٥٧%) هي المركبات الرئيسية الممثلة. أكدت نتائج الفينولات الكلية ومضادات الأكسدة وكشفت عن خمس مركبات بوليفينولية موجودة في جميع المستخلصات وهي حمض الروزمارينيك > حمض الكلوروجينيك > الكيمبفيرول > الكاتكين وحمض الجاليك. تم إجراء اختبار السمية الخلوية للمستخلصات وأظهرت النتائج أن جميع المستخلصات آمنة للاستخدام كمضافات غذائية (تركيز المادة الموافق للتثبيط النصفي أكثر من ٢٠٠ ميكروجرام/مل). لذلك ننصح بإدراج هذه المستخلصات في أفلام كحول البولي فينيل المستخدمة لحفظ الأطعمة من الالتهابات البكتيرية، وكذلك استخدام هذه المستخلصات كإضافات غذائية خاصة مع الماكريل لحفظها لمدة لا تقل عن ٣٠ يومً وقد نالت الخصائص الحسية قبولاً ممتازاً عند إضافة البردقوش الطازج متفوقاً على البردقوش المجفف إلى الماكريل المعلب.

الكلمات المفتاحية: البردقوش، الماكريل، مضادات الميكروبات، مضادات الأكسدة، البوليفينول، المضادات الحيوية، كحول البولي فينيل.