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Chemical Composition and Antimicrobial Activities of Cold-Pressed Oils Obtained FromNigellasativa and Prunus amygdales

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Abstract

The aim of this study is to put forward the antimicrobial activity of cold pressed oils obtained from seeds of Nigella sativa and Prunus amygdales. Oils of these seeds were analysed for their antibacterial and antifungal activities by the disk diffusion and MIC tests against fifteen microorganisms, Staphylococcus epidermidis DSMZ 20044, Staphylococcus aureus ATCC 25923, Salmonella typhimurium SL 1344, Salmonella kentucky, Salmonella infantis, Salmonella enteritidis, Pseudomonas fluorescens P1 ATCC 13075, Pseudomonas aeruginosa DSMZ 50071, Klebsiella pneumoniae, Escherichia coli ATCC 25922, Enterococcus faecium, Enterococcus faecalis ATCC 29212, Enterobacter aerogenes ATCC 13048, Candida albicans DSMZ 1386 and Bacillus subtilis DSMZ 1971. The results were compared against 11 standard antibiotics, which are cefazolin, clindamycin, chloramphenicol, ciprofloxacin, amoxicillin/clavulanic acid, sulfamethoxazole/trimethoprim, ceftriaxone, gentamicin, ampicillin, cephalothin, cefuroxime and vancomycin. The extracts

were also chemically analysed by using GC-MS. As a result, Prunus amygdales oil is observed to be active against all microorganisms except for C. albicanis,Staph. Aureus, B. subtilis, Staph.epidermis,Pseudomonasarginosa and E.coli, where Nigella sativa oil is no active against all microorganisms

Keywords:*Chemical Composition, Cold-Pressed Oil, Antimicrobial Activity, Prunus amygdales, Nigella sativa.*

INTRODUCTION

Our prophet Mohammed said: (Black cumin cures all malady except the death) [Al-Bukhari and Muslim] (Hussain, D. A., et al, 2016).

A lot of medicinal plants and their pure ingredients have been shown beneficial curative potentials. Seeds of Nigella sativa, a dicotyledon of the Ranunculaceae family, have been utilised for thousands of years as a flavouring and food preservative. The oil and seed ingredients, in particular, thymoquinone (TQ) (Salem, M. L, 2005) have shown medicinal characterises, in traditional medicine (Khan, M. A, 1999). As we noticed in the recent history there are a lot of findings provide clear evidence that both the oil and its active ingredients Importance, especially, thymoquinone, own reproducible anti-oxidant effects through augment the oxidant scavenger system, which as a consequence lead to antitoxic effects (Salem, M. L, 2005; Johnson-Ajinwo, 2014). The oil and thymoquinone of Nigella Sativa (ÇörekotuTohumu) content have effective anti-inflammatory effects on numerous inflammation-based including experimental of Encephalomyelitis, Colitis, Peritonitis,inhibition of Oedema and Arthritis into abolition of the inflammatory prostaglandins and leukotrien. The oil and active elements have given good properties for immunity system by raise the T cell and natural killer cell mediated immune responses and not only that the most important, in Nigella sativa both the oil and its active elements represent antimicrobial and anti-tumor characteristic against various microbes and cancers (Salem, M. L, 2005;Khanna, M., 1999).

Black cumin, (Nigella sativa) is widely grown in different parts of the world and the seed of black cumin (Nigella sativa) has been used to enhance health for countries in particular, in the Middle East and Southeast Asia (Kazemi, M., et al,2015). Black cumin seeds have been widely used in traditional medicine as diuretic and antihypertensive (Masson, R., et al, 2000), digestive and appetite stimulant (Gilani, A. U. H., et al, 2004), antidiarrheal(Gilani, A. H., et al, 2001),analgesic (Khanna, M., 1999), anthelmintic (Bhuiya, B. A, 1998)and antibacterial. Additionally, recent studies have shown black cumin to be antidiabetic (Meral, I., et al, 2004), anticancer, anti-inflammatory, spasmolytic and bronchodilatory ((Gilani, A. H., et al, 2001; Al-Ghamdi, M. S. 2001), hepatoprotective (Janbaz, K. H., et al 2003;Guler, T., et al 2006). Renal protective (Badary, O. A., et al 2001) and possessing antioxidant properties

(Masson, R., et al, 2000). Black cumin(Nigella sativa) its seeds are consist of a Volatile oil (0,5; 1,6%), a Fixed oil (35,6-41,6%), Protein (22,7%) (Guler, T., et al 2006) and Amino acids (Guler, T., et al 2006;Al-Gaby, A. M. A. 1998). In addition, the seeds of black cumin (N.sativa) consist fat, crude fibre, minerals; for instance Na, Cu, Zn, Ca, Fe, P and vitamins (Thiamine, niacin, pyridoxine, Ascorbic acid and Folic acid) (Guler, T., et al 2006;Takruri, H. R., &Dameh, M. A, 1998), Black cumin (Nigella sativa seeds) output esters of fatty acids, free sterols and sterol esters (Guler, T., et al 2006; Menounos, P., et al 1986).

Prunusamygdalus is referred to the Rosaceae family (Bombarely, A., et al, 2010). Almond core contains high level of unsaturated fatty acids, mainly mono-unsaturated fatty acids (MUFA) that perform a vital role in body diet, food (Balta, M. F. 2013).

Almond is an exporter of food and medicine, they extend from India to Persia, the tree had spread to east and occident of its region thousands of years. Almond is good sources of antioxidant nutrients. Almonds (Prunusamygdalus) involvement proteins, fiber, vitamin and certain minerals e.g. magnesium and calcium, potassium, low in saturated fatty acids and rich in unsaturated fatty acids (Agunbiade, S. O., &Olanlokun, J. O, 2006; Mangalagiri Mandal, G. D, 2012), for this reason it is reduce coronary heart disease risk factorsJenkins, (D. J. A., et al. 2003).

Almonds (Prunusamygdalus) are a useful food a cure for anaemia. It's beneficial in the remediation of constipation and various skin diseases like eczema, pimples. Almonds are helpful in heal gastroenteritis, kidney pains, diabetes, head lice, facial neuralgia and gastric ulcer and wound healing, skin cleaner, chapped lips and hand (Mangalagiri Mandal, G. D, 2012; Khan, I. A., &Abourashed, E. A. 2011).

Oil of Almond had used for the skin as a moisturiser which curbs the skin from drying and peeling skin, from old, Prunusamygdalus oil had used as the comforting cure for skin allergies, and to treat minor hurt. In addition, most widespread use of Prunusamygdalus oil is in massage because it is outstanding skin lotion (Khan, I. A., &Abourashed, E. A. 2011). Its properties make it popular with massage therapists' worldwide. Almond oil of seed does not have any oleaginous effect and will take a tiny bit of time before it is absorbed by the skin. utilise it for a massage makes a human body feel comfortable (Khan, I. A., &Abourashed, E. A, 2011;Pratima, N. A., &Shailee, T, 2012).

(PrunusAmygdalus L.) of the family Rosaceae was investigated for the oil seed characteristics. The physico-chemical properties and fatty acid composition of the seed oil were examined. Physicochemical properties of the oil were

performed according to AOAC procedures and fatty acids were determined by gas chromatography (GC)(Popa, V. M.,et al, 2013).

1. Materials and Methods

Plant samples

Nagillasativus and PrunusAmygdalus seeds were purchased from a local company in Turkey (ÖzşenLokmanHekim).

Oil extraction

The oil was obtained through a cold-press production (MP-001 Screw Press, Turkey (fig 1). One kilogram of each seed was pressed, filtered and allowed to stand overnight. After 24 hours the upper clear layer of oil was separated through a separation funnel. Obtained oils were kept in cold (4°C) and dark until used in test. The yield percentage for all plant samples were found to be 17% (w/w) for P. Amygdalus, 60% (w/w) for N. sativus (fig 2).

Figure 1: Pressing machine oil that use to extract oil.

Figure 2: One kilogram of P. Amygdalus got 60% of oil as shown in A, N. sativa got 17% of oil as in B.

Microorganisms

Several Gram positive and Gram negative microorganisms were selected to analyse the activity of the oils. The fifteen microorganisms used in this study are Staphylococcus epidermidis DSMZ 20044, Staphylococcus aureus ATCC 25923, Salmonella typhimurium SL 1344, Salmonella kentucky, Salmonella infantis, Salmonella enteritidis, Pseudomonas fluorescens P1 ATCC 13075, Pseudomonas aeruginosa DSMZ 50071, Klebsiella pneumoniae, Escherichia coli ATCC 25922, Enterococcus faecium, Enterococcus faecalis ATCC 29212, Enterobacter aerogenes ATCC 13048, Candida albicans DSMZ 1386 and Bacillus subtilis DSMZ 1971**.**

Inoculum

Microorganisms used in this study were cultured in line with their requirements as stated in some previous studies (Altuner and Çetin, 2009; Altuner and Canli, 2012; CanlıAltuner&Akata, 2015).

For inoculum, microorganisms were suspended in sterile physiological saline solution (Canlı, Altuner, Akata, Türkmen&Üzek, 2016; Canlı, Yetgin, Akata&Altuner, 2016a and b; Canlı, Yetgin, Akata&Altuner, 2017a) and to adjust equal the number of the colonies in the solution, 0.5 McFarland standard was used (Hammer, Carson & Riley, 1999; Altuner, Akata&Canli, 2012a and b).

Disk diffusion method

Diffusion method adopted according to Kavanagh. We have prepared a petri dish, then took anointed of the bacteria and fungi that prepared in a test tube by sterile swabs. Wipe swap on plate gently by spreading process, rotate the plate 60 degrees clockwise and again spread the bacteria going left to right, top to bottom After that, distributed the discs that contain extracts (oil) by a sterile needle with different concentration 15 µg/disc, 5 µg/disc and zero for control, plates were incubated at 37°C for 18 to 24 After the incubation, the plates were examined for inhibition zone. The inhibition zone was measured by using a ruler and recorded. The test was repeated three times to ensure reliability as shown in (fig 3).

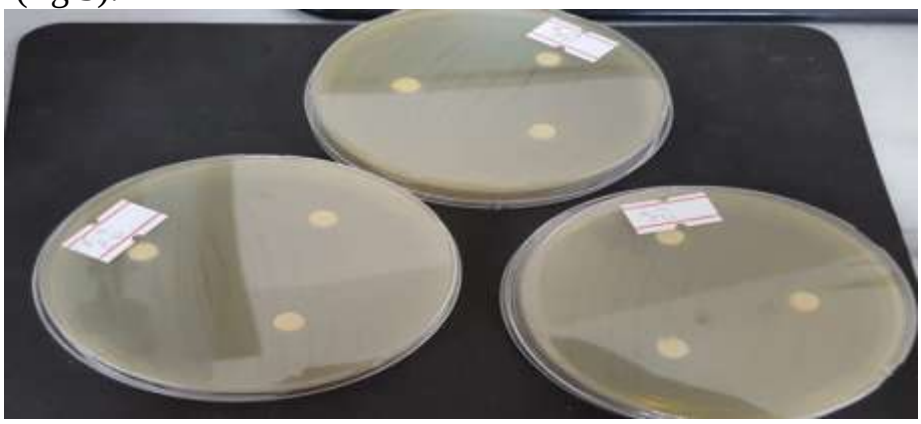

Figure 3: repeated three times to ensure reliability.

Determination of MIC

The MIC values for all oil samples were identified as stated previously (Balouiri, Sadiki &Ibnsouda, 2016). The concentration range was between 100 to 0.195 µg/mL.

Determination of chemical composition by GC-MS

For the identification of chemical components, each sample was analyzed by GCMS QP 2010 Ultra (Shimadzu) equipped with Rtx-5MS capillary column (30m·0.25 mm; coating thickness 0.25 μm). Analytical conditions were injector temperature, 250 ∘C; carrier gas Helium at 1 mL/min; injection mode: split, split ratio 1:10; volume injected: 1 μL of a solution in hexane of the oil; and oven temperature programmed from 40∘C to 240˚C at 4∘C/min, pressure:100kPa, purge flow:3 ml/min. The MS scan conditions used included a transfer line temperature of 250˚C, an interface temperature of 250˚C, an ion source temperature of 200˚C. Identification of the constituents was based on comparison of the retention times and on computer matching against Wiley Data library. When possible reference compounds were cochromatographed to confirm GC retention times.

Controls

Empty SAD was used as negative controls for disk diffusion test and sterilized broth medium for MIC test. In addition, microorganisms were inoculated in Mueller Hinton broth in order to control the viability of each microorganism.
As positive controls eleven standard antibiotics, which are cefazolin, clindamycin, chloramphenicol, ciprofloxacin, amoxicillin / clavulanic acid, sulfamethoxazole / trimethoprim, ceftriaxone, gentamicin, ampicillin, cephalothin, cefuroxime and vancomycin are used.

statistical Analysis

ANOVA, Decriptive and Homogeneous were performed to test for defferencd in size of inhibitory zone formed by oil for Prunusamygdlus against ddifferent bacteria by IBM spss version 24.

1. Results and Discussion

The results obtained by GC-MS analyses of cold-pressed oils of Nigella sativa and Prunusamygdalus are presented in (Table 1, 2, 3 and 4). We chose the components more than 3 percent as the main components and other components have seen tables. Sixteen compounds were identified in fatty acid scanning of N. sativa. GC-MS analy¬ses revealed that N. sativa contained Hexadecanoic acid, methyl ester (5.03%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (23.44%), 9-Octadecenoic acid (Z)-, methyl ester (11.05%), (9E) -Octadecenoic acid (40.47%), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (4.09%), Methyl 5,11,14-eicosatrienoate (10.38%), Cyclohexanecarboxylic acid, decyl

ester (22.94%), Glycidol stearate (4.35%) as the major compounds as showen (Table1).

Table. (1). Nigella Sativa: Fatty Acid Scanning Results.

Peak	R,Time	Area	Area %	*Name*
1	3.660	298342	1.09	*2,5-cyclohexadiene-1,4-dione, 2-methyl-5-(1-ethyl)-(CAS)*
2	14.018	559892	0.22	*Meth tetradecanoate*
3	19.305	1376413	5.03	*Hexadecanoic acid, methyl eser*
4	23.489	6415955	23.44	*9.12-Octadecadienoic acid.(Z.Z)-*
5	23.616	3023630	11.05	*9-Octadecadienoic acid(Z,Z)-,methyl ester (CAS)*
6	23.743	281371	1.03	*9-Octadecadienoic acid(Z)-, methyl estr(CAS)*
7	24.206	498281	1.82	*Methyl stearate*
8	24.503	2865988	10.47	*9-Octadecadienoic acid, (E)*
9	28.065	1118474	4.09	*(R)-(-)-14-Methyl-8-hexadecya-1-ol*
10	28.701	46919	0.17	*Eicosanoic acid, methyl ester (CAS)*
11	31.963	2841221	10.38	*Methyl 5,11,14-eicosatrienoate*
12	32.047	6277096	22.94	*Cylclohexanecarboxylic acid, decl ester*
13	32.674	1189666	4.35	*Glycidol stearate*
14	38.424	380238	1.39	*9-Octadecadienoic acid, 1,2,3-propanetriyl ester, (E,E,E)-*
15	38.857	616019	2.25	*E,E,Z-1,2,12-NONADECATRIENE-5,14-DIOL*
16	41.184	76506 27366011	0.28 100.00	*1,3-Benzenaedic arboxylic acid, bis(2-ethylhexyl)ester*

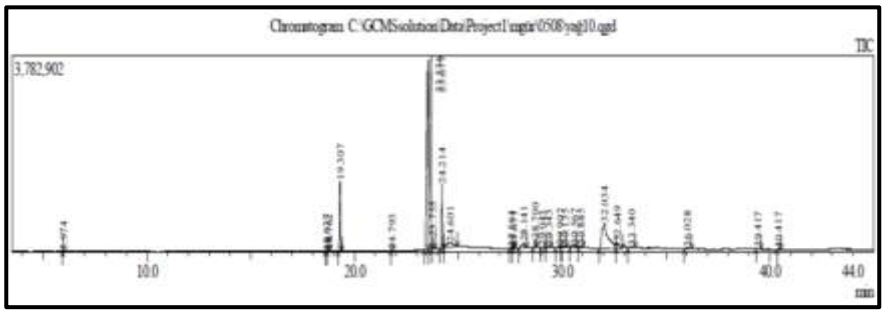

Figure 3: Nigila sativa fatty acid scanning results.

Twelve compounds were identified in essential oil scanning of N. sativa. GC-MS analy¬ses revealed that N. sativa contained*9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)-, E.E.Z-1.3.12-Nonadecatriwne-5.14-diol and 9.12-Octadecadienoyl cloride.(Z.Z)-(*8.73%), (57.32%) and*(*33.72%), respectively as the major compounds as showen (Table 2) and (fig 2).

Table .(2). Nigella Sativa: Essential oil scanning.

Peak	R,Time	Area	Area %	*Name*
1	43.840	20398393	8.73	*9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)-*
2	49.665	22309808	57.32	*E.E.Z-1.3.12-Nonadecatriwne-5.14-diol*
3	50.810	100883	7.72	*9.12-Octadecadienoyl cloride.(Z.Z)-*
13	21.546	789694	0.34	*Thymoquine*
		76101798	100.00	

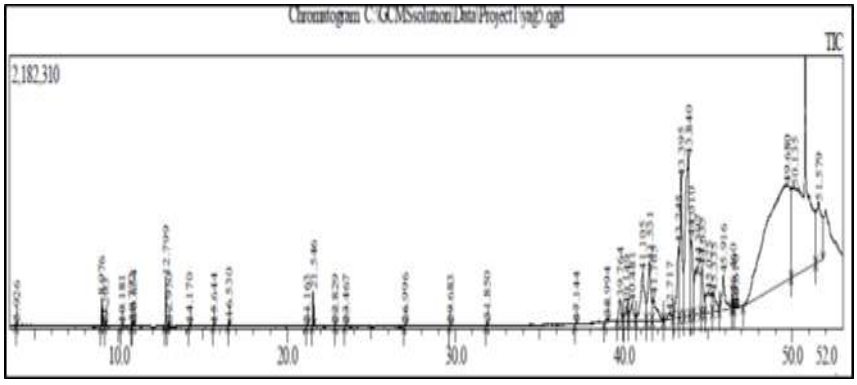

Figure 4: Nigella Sativa: Essential oil scanning.

Thirty-five compounds were identified in fatty acid scanning of PrunusAmygdalus. GC-MS analy¬ses revealed that P. amygdalus contained Hexadecanoic acid, methyl ester (6.23%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (16.52%), di-(9-octadecenoyl)-glycerol (12.03%: This compound has seen in five different retention times), Methyl stearate (3.17%), Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy- (15.83%), as the major compounds as showen (Table 3).

Table.(3). PrunusAmygdalus: Fatty Acid Scanning Results (GC-MS).

Peak	R,Time	Area	Area %	*Name*
1	14.005	32631	0.03	*Tetradecanoic acid, methyl ester (CAS)*
2	18.663	39793	0.03	*9-Hexadecanoic acid, methyl ester, (Z)- (CAS)*
3	18.782	779918	0.66	*9-Hexadecanoic acid, methyl*

				ester, (Z)-
4	19.387	7311680	6.23	*Hexadecanoic acid, methyl ester*
5	21,241	129476	0.11	*CIS-10-HEPIADECENOIC ACID ME*
6	21.818	66795	0.06	*Heptadecanoic acid, methyl ester(CAS)*
7	23.250	326367	0.28	*9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)-*
8	23.544	19392020	16.52	*9.12-Octadecenoic acid.(Z.Z), methyleeaster-*
9	23.771	4405015	37.52	*9.Octadecenoic acid, methyle ester, (E)-*
10	24.019	1056604	0.90	*DI-(9-OCTADENOYL)-GLYCEROL*
11	24.236	3726884	3.17	*Methylestearte*
12	24.531	988751	0.84	*9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)-*
13	24.655	765693	0.65	*9.12-Octadecenoic acid.(Z.Z), methyleeaster-*
14	24.840	785908	0.67	*DI-(9-OCTADENOYL)-GLYCEROL*
15	25.106	692234	0.59	*8-Hexadecenal,14- methyl ester, (Z)-*
16	25.364	417306	0.36	*DI-(9-OCTADENOYL)-GLYCEROL*
17	25.607	293997	0.25	*13-Octaddecenal, (Z)-*
18	25.898	130465	0.11	*9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)-*
19	26.071	120397	0.10	*Glycidol stearate*
20	26.277	198875	0.17	*Glycidol stearate*
21	26.602	45263	0.04	*Cycleohexanecarboxylie acid,undec-10-enyl ester*
22	26.915	111309	0.09	*DI-(9-OCTADENOYL)-GLYCEROL*
23	27.095	482935	0.41	*2-Methyl-Z-Z-3,13-octadecadienol*
24	27.293	319595	0.27	*13-Octaddecenal, (Z)-*
25	27.571	73454	0.06	*Opaneoctanic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cycleopropyl]methyl*
26	28.161	330319	0.28	*11-Escosenoic acid, methyl ester*
27	28.719	211484	0.18	*11-Escosenoic acid, methyl ester (CAS)*
28	29.855	32418	0.03	*Naphth[1,2-b]oxirne,decahydro-1a,7-dimethyl-*
29	31.391	18580341	15.83	*Tricle[20.8.0.0(7, 16)]triacontane, 1(22),7(16-diepoxy-*
30	32.060	11756612	10.01	*DI-(9-OCTADENOYL)-*

				GLYCEROL
31	33.390	31711	0.03	*Methyle 20-methy-beneisanoate*
32	37.460	629054	1.54	*E,E,Z-1,3,12,3-Propanetriyl ester, (E,E,E)-*
33	37.058	1927061	1.64	*9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)-*
34	38.365	1410136	0.20	*Adipic acid, dec-4-enyl dodecyl ester*
35	40.877	147657	0.13	*Gamma.-TOCOPHEROL*
		3117395658	100.00	

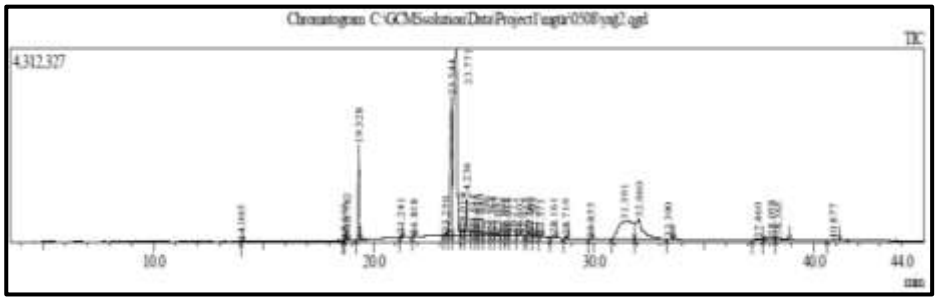

Figure 5: Prunusamygdalus fatty acid scanning results.

Nine compounds were identified in essential oil of PrunusAmygdalus. GC-MS analy¬ses revealed that P. amygdalus contained (51.10%) of 9.12-Octadecadienoyl cloride.(Z.Z)- ,(21.6%) 9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)- and (11.0%) Hexadecanoic acid, 2-(hydroxymethyl)-1,3-proparpnediyle ester (CAS)as major compound as shown (table 4) and (fig 6).

Table.(4). PrunusAmygdalus: essential oil Scanning Results (GC-MS).

Peak	R,Time	Area	Area %	*Name*
1	43,230	7424458	51.10	*9.12-Octadecadienoyl cloride.(Z.Z)-*
2	43.861	43455974	21.6	*9-OCTADENOIC acid, 1.2.3-propanetriyl ester. (E.E.E)-*
3	50.815	2329942	11.09	*Hexadecanoic acid, 2-(hydroxymethyl)-1,3-proparpnediyle ester (CAS)*

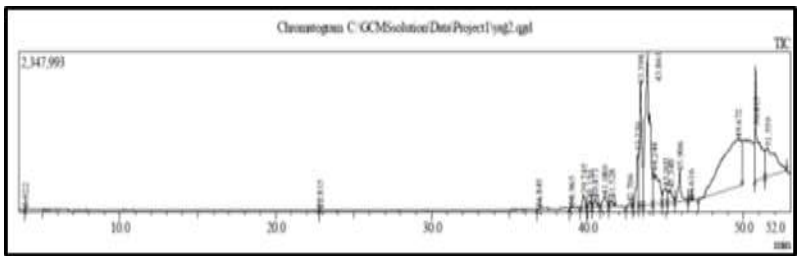

Figure 6: Prunusamygdalus essential oil results.

Hexadecanoic acid, methyl ester compound was seen in cold-pressed oil of N. Sativa and P. amygdalus plants as percentages of (5.03%), (6.23%), respectively. Octadecadienoic acid (Z,Z)-, methyl ester compound was determined in cold-pressed oil of N. Sativa, and P. amygdalus plants as percentages of 23.44, 16.52, respectively. 9.Octadecenoic acid, methyle ester, (E)-compound has higest determined in cold-pressed oil ofP. amygdalus plants as percentages of 37.52. 9.12-Octadecadienoic acid.(Z.Z)-compound has higest determined in cold-pressed oil ofN. Sativaplants as percentages of (23.44%).

The Prunusamygdalus fatty acid are with Gruia et al (2013) reported linoleic acid, oleic acid and Palmic acid 30.05%, 57.32%, 9.46% respectively (Popa, V. M., et al, 2013)higher than in the present study. However, with Youssef et al (2013), Standards et al (2004) and Rathee et al (1984) where reported in N. sativa was linoleic acid and oleic acid as major compounds Youssef, M. K. E., et al, 2013; Standards, N.C.f.C.L., 2004; Rathee, P. S., et al, 1982) this is agreement in the present study.

In this study agreement with Salem, M. L, (2005) that conduct the oil and seed of N. sativa ingredients, in particular, thymoquinone (TQ) whears its precente is 0.34% in this study

The results for disk diffusion testofN. sativa and P. amygdalusare given in Table 5 and the results for standard antibiotic disks are given in Table 6too.The MIC values observed N. sativa and P.amygdalusare given in Table 7.

According to the resultsP.amygdalusseed oil was observed to be active all bacteria except for *B. subtilis, Staph.epidermis, Pseudomonas arginosa, E.coli, Staph. Aureus* and fungi *Candida albicanis*the average of inhibition zone ranging (7-8mm) as shown in table 5 whears the highest antimicrobial activity against*P. flore in 15µ/disc* concentratiion *about 8,5±0,4 and on E. facium about 8,5±0,5*the MIC values were observed to be between 12.5 and 25 µg/mL.

N. sativaseed oil was observed to be active just for S. enteritis, E.aerogens and S. infantisand the MIC values were observed to be 100 µg/mL with same bacteria that affected in disc diffusion test.

Table .(5). Disk diffusion test results for 5 µL and 15 µL of cold pressoils obtained from seedsPrunusamygdulus and Nigella sativa (Inhibition zones in mm).

Microbial	***Prunus amygdalus* 5 µg/disc15µg/disc**	**Nigella sativa 5µg/disc15µg/disc**
S. enteritis	7.1 7.5	- 2
C. albicanis	-	-
Staph. aureus	-	-
E. faecium	7.5 8.5	-
E. faecalis	7.1 7.5	-
S. typhimirium	7.1 7.5	-
E.aerogens	7.1 7.5	- 6.8
S. infantis	7.1 7.5	- 6.8
S. Kentucky	6.7 7.5	-
Pseud.floverscans	- 8.5	-
Kleb.pneumonia	6.7 7.5	-
B. subtilis	-	-
Staph.epidermis	-	-
Pseudomonas arginosa	-	-
E.coli	-	-

"-": No activity

Table .(6). The results for standard antibiotic disks (Inhibition zones in mm).

	CFZ	**CLI**	**CAM**	**CPR**	**AMC**	**SXT**	**CRO**	**GEN**	**AMP**	**CEF**	**CXM**	**VAN**
B. subtilis	44	34	37	36	56	42	38	30	41	36	44	20
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-
E. aerogenes	14	-	26	30	9	24	21	23	-	-	16	-
E. faecalis	14	-	19	19	28	29	-	13	14	-	-	15
E. faecium	40	30	11	28	43	34	31	28	32	24	33	26
E. coli	18	-	22	-	16	12	-	20	6	6	6	6

K. pneumoniae	-	-	22	30	9	6	6	22	6	6	6	-
P. aeruginosa	-	-	9	28	-	-	-	15	-	-	-	-
P. fluorescence	10	8	22	19	26	26	-	12	14	-	-	16
S. enteritidis	23	-	28	36	28	31	27	24	16	-	16	-
S. infantis	22	-	28	24	26	24	26	24	14	-	17	-
S. kentucky	22	-	29	34	26	27	30	13	15	-	19	-
S. typhimurium	22	-	27	35	26	21	27	23	13	-	14	-
S. aureus	31	24	21	22	30	27	16	24	25	22	29	16
S. epidermidis	37	35	33	34	45	32	26	25	24	26	32	21

"-": No activity observed, CFZ: Cefazolin, CLI: Clindamycin, CAM: Chloramphenicol, CPR: Ciprofloxacin, AMC: Amoxicillin/Clavulanic acid, SXT: Sulfamethoxazole/Trimethoprim, CRO: Ceftriaxone, GEN: Gentamicin, AMP: Ampicillin, CEF: Cephalothin, CXM: Cefuroxime, VAN: Vancomycin

Table .(5). MIC values (µg/mL) for

Microorganisms	***Prunuusamygdalus***	***Nigella sativa***
S. enteritis	25	100
Candida albicanis	-	-
Staph. aureus	-	
E. faecium	12.5	-
E. faecalis	25	
S. typhimirium	25	-

E. aerogens	12.5	100
S. infantis	25	100
S. Kentucky	25	-
Pseudomonas floverscans	25	-
Klebsella pneumonia	25	-
B. subitis	-	-
Staph. epidermis	-	-
E.coli	-	-
Pseudomonas arginosa	-	-

"-" implies no effect.

As the current literature is concerned there are several studies for the antimicrobial activity of N. sativa, and P. amygdalus against several microorganisms. But only a minute amount of them are the antimicrobial activity of seed oils.

Neogi et al (2008) studied the fatty acid profile and the antimicrobial effect of P. amygdalus. Tested the antimicrobial of the P. amygdalus seeds oil against 7 microorganisms. They have found determined inhibition zone of well disc diffusion against S. typhimurium, E. coli, S. aureus and P. aeruginosa of bacteria 20±0.9mm, 14±0.5mm, 17±1.3mm and 17±0.6mm respectively. And Penicllium notaum, C. albicans and A. niger of fungi 20±0.8mm, 15±1.2mm and 18±0.7mm respectively. where in our study have the lowest determind inhibition zone of agar disc diffusion between 6.7±0.025mm and 8.5±0.4mm and MIC value 12.5%- 25% µg/mL. There are two reasons for this differences; (1) the method for extraction was different, so the oil composition may be different, Neogi et al (2008) extracted the oil from seeds by using Soxhlet apparatus, but we have extracted oils by direct cold pressing, (2) the test method was different Neogi et al (2008) tested by well disc diffusion, but we have agar disc diffusion.

Chaudhry et al (2008) studied the fatty acid profile and the antimicrobial effect of N. sativa seeds against 20 microorganisms of bacteria. They have found determined inhibition zone of Disc diffusion assay against Staphylococcus aureus, Streptococcus intermedius, Streptococcus morbillorium,

Streptococcus mutans, Streptococcus salivarius and Streptococcus sanguis 19.6 ± 1.8mm, 13.6 ± 1.5mm, 16.5 ± 4.9mm, 16.9 ± 3.9, 8.5 ± 0.8 and 14.6 ± 2.4 respectively. But no affected on pseud. aergenisa and klep. P. where in our study have abit affect against E. aerogenes, S. enteritidis and S. infantis There are two reasons for this differences; (1) the method for extraction was different, Chaudhry et al (2008) boiling 10g of N. sativa in 100ml distilled water. (2) Chaudhry et al (2008) seeds were purchased from the local market of Karachi, Pakistan and they boiling 10g of N. sativa seeds in 100ml distilled water.

Grasas et al (2005) studied the antimicrobial effect of N. sativa seeds oil against twenty-four affected of bacteria. They have found determined inhibition zone of Agar Disc diffusion between 7mm to 37mm. There are two reasons for this differences; (1) the method for extraction was different, (1) grasses et al (2005) extracted for 10 h in a Soxhlet extractor with 500 ml n-hexan. (2) seeds were grown in five different regions.

2. Conclusion

This study has revealed the P. amygdalus and N. sativa are a rich source of fatty acid as scannig by Gas chromatography–mass spectrometry (GC-MS) analysis and this medicinal plants can use them as Antimicrobial, but this depend on the ways that have use, in this study had use oil that extract from seeds with 5µg/disc and 15µg/disc concentratiom may if increase rate of concentration it will giving more well affect or by use leaves or cruch seeds. In addation P. amygdalus oil it very useful for skin wheares can use for skin therapy

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