Journal of Medicinal Plants Research Vol. 5(6), pp. 922-931, 18 March, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

An *in vitro* study of the antimicrobial and antioxidant efficacy of some nature essential oils

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Accepted 22 December 2010

The purpose of this study was to compare the antimicrobial efficacy of sodium hypochlorite (NaOCl) and different natural essential oils for root canal irrigation using two activity tests (contact and diffusion agar tests). Thirty-seven extracted human teeth were collected and incubated with *Staphylococcus aureus* for 48 h. The teeth were divided into three divisions' experimental groups, negative control and one positive control group. The canals were instrumented and irrigated with both 5.25 and 2.5% NaOCl as positive control and with essential oils as experimental groups. Bacterial samples were collected after instrumentation/irrigation and after additional canal enlargement. The data showed that essential oils were very effective for disinfection of *S. aureus* in root canals of patient's teeth in dentistry clinic without significant difference between NaOCl and tested extracts. Furthermore, the essential oils were subjected to screening for their possible antioxidant activity by two complementary test system, 2,2´-diphenylpicrylhydrazyl (DPPH) free radical scavenging and 2,2'- azino-bis [ethylbenzthiazoline-6- sulfonic acid] (ABTS) methods. Butylated hydroxyl anisole (BHA) was used as positive control in both systems. All the tested essential oils showed antioxidant activity against DPPH and ABTS radical more than 50% but less than butylated hydroxyl anisole as synthetic standard.

Key words: Essential oils, root canal irrigation; sodium hypochlorite, antioxidant activity, antimicrobial activity, *Staphylococcus aureus.*

INTRODUCTION

Elimination of microorganisms from the root canal system is one of the objectives of root canal treatment (Byström et al., 1987) and has a substantial effect on the outcome (Sundqvist et al. 1994). Unfortunately, microorganisms may remain after conventional canal preparation, either within the dentine tubules (Peters et al. 1995), embedded in the smear layer (Huque et al. 1998) or bound within the apical dentine plug (Abou-Rass and Bogen 1998). Primary endodontic infections are caused by oral microorganisms, which are usually pathogens that may invade a root canal and establish an infectious process (Siqueira et al., 2000). Intracanal irrigants have been used as an adjunct to enhance the antimicrobial effect of cleaning and shaping in endodontics. Although, instrumentation and the use of irrigating solutions with strong antimicrobial activity remove and kill the majority of the microbial cells in the root canal, it has been shown that a small part of the flora survives (Gomes et al. 1996). *Staphylococcus aureus* is one of the most common bacterial strains related to endodontic diseases and resistant to several antimicrobial agents as reported by Molander et al. (1998). Sodium hypochlorite has been largely used in endodontics as an irrigating solution since its first reported use by Walker (1936) is an effective antimicrobial agent and an excellent organic tissue solvent, but has a highly toxic effect on the periapical tissues, and therefore should be used in the lowest effective concentration and should not be forced beyond the apex. It has been reported that an increase in concentration will result in an increased antimicrobial effect. However, the irritant effects of NaOCl might be minimised owing to the short period that the solution remains in contact with the periapical tissue during instrumentation (Siqueira et al., 1999). Aromatics plants

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have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin et al., 1998). Most of their properties are due to essential oils produced by their secondary metabolism (Adam et al., 1998). Essential oils and extracts from several plant species are able to control microorganisms related to skin (Adam et al., 1998), dental caries (Cecanho et al., 1999), and food spoilage, including Gram-negative and Gram-positive bacteria (Galli et al., 1985).Essential oils exhibit various and variable antimicrobial activities, including antifungal, antiviral, antibacterial, insecticidal, and antioxidant properties (Prabuseenivasan et al., 2006).

One of the most important objectives of endodontic therapy is the complete elimination of microorganisms from the root canal system. The positive correlation between bacteria and endodontic disease has been established (Sundqvist, 1994).

Failure of root canal treatment is likely caused by the inability to eliminate the bacteria responsible for refractory endodontic infections.

In the recent years, the antimicrobial and antioxidant actions have received much attention. This is so because of the increasing interest in human health and have been studied *in vitro* and *in vivo* by many researchers. The antioxidant may be useful in retarding oxidative deterioration of food materials especially those with high lipid content. The natural antimicrobial agents protect living organisms from damages resulting in the prevention of various diseases. There is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components as specific pharmaceutics (Abdoul-Latif et al., 2010). The aim of this present work was to determine the essential oil composition of five aromatic plants. These results will allow deduction of which components are likely to contribute to the antimicrobial activity against *S. aureus* and antioxidant activity.

MATERIALS AND METHODS

Materials

Chemicals and reagents

Butylated hydroxy anisole, Tween 20, 2, 2 diphenyl-1-picrylhydrazyl (DPPH) and scavenge 2,2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid (ABTS) were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Teeth samples

Thirty seven, freshly extracted, human, permanent, single rooted teeth were collected and stored in normal saline solution at room temperature.

Microorganism

Staphylococcus aureus (ATCC 25923): This strain is a pathogenic bacterial strain characterized as Gram-positive cocci cells, non-spore forming, clinical isolate from patients, catalase positive and facultative anaerobic. This strain was cultured on Trypticase Soy Agar +0.6% yeast extract slants at 37°C for 24 h and then maintained at 4°C till use.

Culture medium

Trypticase soy agar medium (APHA, 1978): It is a highly nutritious general purpose medium. It can be used either solid or broth for the cultivation of pathogenic bacterial strains. It composed of the following ingredients (g/l): Tryptone, 17.0; Soy peptone, 3.0; Sodium chloride, 5.0; di potassium phosphate, 2.5; Glucose, 2.5; Yeast extract, 6.0; Agar, 18.0 and pH = 7.0 ± 0.2. The medium can be sterilized by autoclaving at 121°C for 15 min.

Methods

Plant materials

The bulbs of *Allium cepa L.* (Onion) *family Liliaceae*, bulbs of *Allium sativum* (Garlic) *family Liliaceae*, seeds of Cuminum cyminum (Cumin) *family Apiaceae or Umbelliferae* and herb of *Petroselinum sativum* (Parsley) *family Apiaceae* were purchased from local market, while herb of *Corriandrum sativum* (Coriander) *family Apiaceae*, were purchased from experimental station of medicinal plants, Faculty of Pharmacy, Cairo University, Egypt.

Essential oil extraction

Plant samples (100 g) were hydro-distilled in Clevenger-type apparatus (Council of Europe, 1997). The essential oil samples were stored in the dark at 4°C. The amount of oil obtained from plant material was calculated as:

Oil (% v/w) = observed volume of oil (ml)/weight of sample (g) × 100

GC/MS analysis of essential oil

The essential oils were analyzed by GC-MS according to Adams (1989). GC/MS analysis was performed on a Thermoquest-Finnigan Trace GC-MS equipped with a DB-5 (5% phenyl) methylpolysiloxane column (60 m \ 0.25 mm i.d., film thickness 0.25 µm). The injection temperature was 220°C and the oven temperature was raised from 40ºC (3 min hold) to 250°C at a rate of 5°C/min, then held at 250°C for 2 min; transfer line temperature was 250ºC. 1 µl of sample was injected and helium was used as the carrier gas at a flow rate of 1.0 ml/min.

The mass spectrometer was scanned over the 40 to 500 m/z with an ionizing voltage of 70 eV and identification was based on standard mass library that National Institute of Standards and Technology (NIST Version 2.0) to detect the possibilities of essential oil components.

Disc diffusion method

Antimicrobial activity of essential oils can be determined using disk diffusion method. Test bacterial strain was inoculated in melted (at 50°C) trypticase soy agar + 0.6% yeast extract medium (APHA, 1978) with heavy inoculums.

Then the inoculated medium was poured over a solid layer of uninoculated agar medium in sterilized Petri-dishes and left to solidify at 4°C (surface layer should be constant in volume and horizontally homogenous). Discs of Whatman No. 1 filter paper (6.0 mm in diameter) were sterilized by autoclaving at 121°C for 15 min. An accurate volume (10 µL) of undiluted essential oil was aseptically added to each disc and left to dry. Each disc was aseptically placed on the middle of agar surface (in triplicate) and left at 4°C for 1 h then plates were incubated at 37°C for 48 h. The antimicrobial activity of essential oils was evaluated by measuring the average of inhibition zone diameter against the test microorganism (Gillies and Dodds, 1984).

Preparation of the teeth samples

All teeth were scaled mechanically to remove any soft tissue debris, remaining bone or calculus. Canal potency was achieved and maintained with size 10 and 15 Flex –O file. The protaper rotary files, both shapers and finishers, were used.

Teeth grouping

Teeth were divided into experimental group (five essential oils), positive control group; NaOCl (5.25 and 2.5%) each group containing 5 teeth and negative control group of 2 teeth (distilled water).

Application of essential oils with teeth

Teeth were autoclaved at 121°C for 15 min. Broth culture of the *S. aureus* (ATCC 25923) was prepared in trypticase soy broth + 0.6% yeast extract medium (APHA, 1978) and the broth culture medium was inoculated with loop full of the bacterium and incubated at 37° C for 24 h, then count of cells in the broth culture was calibrated by dilution in sterilized culture medium to 5.0 x 10^4 CFU / ml. Twenty micro-liter of the bacterial culture were transferred to the canal lumen of the mechanically enlarged root canals using sterile micropipette and then stored at 37°C for 48 h. Initial count after storage and before treatments was estimated. The canal of each tooth was then washed by the essential oil using syringe and left for 15 min (NaOCl as positive control and saline as negative control were also applied). Thereafter, sterile paper points were introduced in the canal and maintained for 3 min for sample collection. The points were individually transferred to test tubes containing 2 ml of sterile physiological saline. Viable count of the bacterium in each tube was estimated using pour plate method on trypticase soy agar + 0.6% yeast extract medium (APHA, 1978). Plates were incubated at 37°C for 48 h (Eldeniz et al., 2007).

Antioxidants activity

DPPH radical method

The hydrogen atom-or-electron donating ability of the corresponding essential oils was measured from the bleaching of the purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical, 2,2´-diphenylpicrylhydrazyl (DPPH˙), as a reagent (Brand-Williams et al., 1995). Fifty microliters of various concentrations (100 and 200 µg/ml) of the essential oils in dimethyl sulphoxide (DMSO) were added to 5 ml of a 0.004% methanolic solution of DPPH˙. The reaction mixture was covered and left in the dark at room temperature. After one hour, the absorbance was read against blank at 517 nm, butylated hydroxyl anisole (BHA) was used as standard control. The antiradical scavenging activity of the two essential oils were evaluated in comparison with anisole the reference BHA as described above for extracts. The antioxidant capacity to scavenge the DPPH radical for the oils was calculated by the following equation:

$$\text{Scavenging effect (\%)} = ((A_{blank} - A_{sample}) / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of control reaction (containing each reagents except the sample), and A_{sample} is the absorbance of sample.

The percentage of scavenging activity was plotted against the essential oil concentration to obtain the effective concentration (EC_{50}), defined as the essential oil concentration necessary to cause 50% scavenging. Tests were carried out in triplicate.

ABTS method

This assay was based on the ability of different substances to scavenge 2,2'- azino-bis (ethylbenzthiazoline-6- sulfonic acid (ABTS.+) radical cation in comparison to a standard (BHA). The radical cation was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 h until the reaction was complete and the absorbance was stable. The ABTS.+ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734 nm for measurements. The photometric assay was conducted on 0.9 ml of ABTS $^{\cdot+}$ solution and 0.1 ml of tested samples (100 and 200 µg/ml) and mixed for 45 s, measurements were taken immediately at 734 nm after 1 min. The antioxidative activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations and using the following equation:

$$E = ((Ac - At)/Ac) \times 100,$$

Where, At and Ac are the respective absorbance of tested samples and ABTS.+, was expressed as µmol (Re et al., 1999).

Statistical analysis

Data were subjected to an analysis of variance, and the means were compared using the "Least Significant Difference (LSD)" test at 0.01 levels, as recommended by Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Essential oils composition

The hydro-distillation of *A. sativum*, *A. cepa*, *C. cyminum*, *C. sativum* and *P. sativum* yield 0.073, 0.051, 3.20, 0.31 and 0.45% (v/w) essential oil content respectively, as shown in (Table 1). The GC/MS analyses of essential oils of these plants are presented in (Tables 2-6) respectively. It seems that there were no similarities among chemical compositions of the five essential oils.

A total of 33 components constituting 99.47% of *A. cepa* (bulbs) essential oil were identified and the major components were diisopropyl trisulfide (20.69%), isopropyl dithio isopropane (18.10%) and 2-tridecanone (10.45%), in case of *A. sativum* (bulbs) Di-2proppenyl

Table 1. Essential oil concentration (% w/w) from aromatic plants studied, including the botanical name, family and traditional use.

Botanical name	Family	Traditional Use	Essential oil (% w/w)
Allium sativum(Garlic)	*Liliaceae*	As a seasoning or condiment	0.073±0.01
Allium cepa L. (Onion)	*Liliaceae*	As a food source for millennia	0.059±0.0
Cuminum cyminum (Cumin)	*Apiaceae or Umbelliferae*	Most popular spice	3.20±0.4
Corriandrum sativum (Coriander)	*Apiaceae*	Commonly used in cooking	0.31±0.06
Petroselinum sativum (Parsley)	*Apiaceae*	Used as a garnish.	0.45±0.07
LSD at 0.01			0.013

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.01 according to Duncan's multiple range test.

Table 2. Chemical composition of *Allium cepa* (bulbs) essential oil.

No.	Compound name	Relative conc (%)
1	Disulfide, 1-methylethyl propyl	0.98
2	2,4-dimethyl-thiophene	0.52
3	Methyl propyl disulfide	3.78
4	Dimethyl trisulfide	0.79
5	Isopropyldithioisopropane	18.10
6	Dipropyl disulfide	8.83
7	Methyl propyl trisulfide	8.10
8	Dimethyl tetrasulphide	0.19
9	3-Ethyl-5-methyl-1,2,4-trithiolane	1.60
10	Methyl-1-(methylthio) propyl disulfide	0.29
11	2-Undecanone	2.50
12	Diisopropyl trisulfide	20.69
13	*trans*-Propenyl propyl trisulfide	4.96
14	9-t-Butyl-9,10-dihydroanthracene	0.56
15	Ethyl-2-(formylamino)-4-methylthiazole-5-carboxylate	1.70
16	2-Methyl-2-methylthiopropanal	0.70
17	2-Hexyl-5-methyl-(2H)-furan-3-one	3.48
18	2-Tridecanone	10.45
19	4,4-Dimethoxy-2-butenoic acid	0.44
20	5-Methyl-2-octyl-(2H)-furan-3-one	2.21
21	Methyl 1,2,3,4-tetrahydro-1,1-dimethyl-2-naphthoate	0.70
22	Propyl-1-(propylthio)-ethyl- disulfide	2.90
23	Methyl- 2,6-anhydro-3,4,7-tridesoxy-1-erythro-hept-2-enulonate	0.66
24	6,10,14-trimethyl-2-Pentadecanone	0.08
25	1,3-bis (propylthio) – Propane	0.88
26	3,5-diethyl-1,2,4-Trithiolane	0.50
27	Hexadecanoic acid	0.34
28	Tricosane	0.08
29	Methyl- 2,6-anhydro-3,4,7-tridesoxy-1-erythro-hept-2-enulonate	0.66
30	6,10,14-trimethyl-2-Pentadecanone	0.08
31	1,3-bis (propylthio) – Propane	0.88
32	3,5-diethyl-1,2,4-Trithiolane	0.50
33	Hexadecanoic acid	0.34
LSD at 0.01		0.0124

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.01 according to Duncan's multiple range test

Table 3. Chemical composition of *Allium sativum* (bulbs) essential oil.

No.	Compound name	Relative conc (%)
1	3,3'-thiobis-1-Propene	3.27
2	Disulfide	4.60
3	Methyl-trans-propenyl-disulfide	0.20
4	cis-Propenyl methyl disulfide	0.47
5	Propanedioic acid	3.23
6	Dimethyl trisulfide	2.63
7	Limonene	0.14
8	Di-2-propenyl disulfide	25.18
9	Methyl-2-propenyl trisulfide	23.80
10	3-vinyl-[4H]-1,2-dithiin	1.30
11	2,4,5,6-Tetramethylpyrimidine	1.12
12	2-vinyl-[4H]-1,3-dithiin	1.85
13	Di-2-propenyl trisulfide	21.05
14	Isobutyl isothiocyanate	0.18
15	2,3-Dicarboxythiophene	1.45
16	Diallyl tetrasulphide	3.56
17	Diallyl pentasulfide	2.45
18	Diallyl hexasulfide	1.15
19	Methyl allyl pentasulfide	0.22
20	Methyl allyl hexasulfide	0.15
LSD at 0.01		0.0143

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.01 according to Duncan's multiple range test.

disulfide (25.18%), methyl-2-propenyl trisulfide (23.8%) and Di-2-propenyl trisulfide (21.05%) were identified as major components from 20 components constituting 98.0%, but cuminaldehyde (60.01%) presented as the major components in *C. cyminum* (seeds) from 20 components constituting 96.0%. In case of *C. sativum* essential oil components, linalool acts as the major essential oil (73.79%). In addition to, a total of 17 components constituting 96.94% of *P. sativum* essential oils were identified and the major components were myrisicin (25.2%), apiol (18.23%) and α-pinene (16.16%). All other components were present in amount lower than 10%.

Antibacterial screening

The test of sensitivity of the essential oils to *S. aureus* (ATCC 25923) pathogenic bacteria shows that, all essential oils have an inhibitory potential (Table 7). They gave a great antimicrobial activity and an inhibition with the diameters from 8 to 30 mm. the bacteria were most sensitive to the essential oil of *C. sativum* (30 mm); *A. sativum* (25 mm); *C. cyminum* (16 mm); *A. cepa* (13 mm) and *P. sativum* (8 mm).

Root canal irrigation

The most promising essential oils of *C. sativum; A. sativum* and *C. cyminum* were used for root canal irrigation, the results showed that, the growth of *S. aureus* (ATCC 25923) was significantly inhibited by the tested essential oils (Table 8) without significant difference between NaOCl as positive control (Stander) and tested extracts.In our study, the antimicrobial activity of the oil could be due to terpenoid derivatives (Tabanca et al., 2001; Vardar et al., 2003). The antibacterial activity of the essential oil of *C. sativum* is apparently related to its terpenes type components such as alpha and beta pinene, camphor, beta-myrcene, camphor and linolool (Table 5). These chemicals well-known having antimicrobial potentials (Dorman and Deans, 2000). The antimicrobial activity of the essential oil of *A. sativum* is apparently related to its sulfide derivatives as shown in Table 3. Furthermore, the antimicrobial activity of the essential oil of *C. cyminum* is apparently related to its terpenes types components such as alpha and beta pinene, beta-myrcene, p-cymene, camphor, geraniol, thymol and cuminaldehyde (Delaquis et al., 2002; Table 4) since there is a relationship between the chemical structures of the most abundant oils and their antimicrobial activities. Although, the mechanism of action

Table 4. Chemical composition of *Cuminum cyminum* (seeds) essential oil.

No.	Compound name	Relative conc (%)
1	α-pinene	2.14
2	Sabinene	1.01
3	β-pinene	4.89
4	β-myrcene	1.45
5	α-terpinene	0.84
6	p-cymene	1.77
7	Limonene	0.24
8	γ-terpinene	1.07
9	α-terpinolene	0.08
10	Camphor	0.12
11	Terpinen-4-ol	0.04
12	α-terpineol	2.47
13	Geraniol	0.07
14	geranyl acetate	4.11
15	β-caryophyllene	3.44
16	α-phellandrene	1.09
17	Cuminaldehyde	60.01
18	Thymol	2.04
19	β-Farnesene	3.01
20	Caryophyllene oxide	6.12
LSD at 0.01		0.0214

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.01$ according to Duncan's multiple range test.

Table 5. Chemical composition of *Corriandrum sativum* (herb) essential oil.

No.	Compound name	Relative conc (%)
1	α-thujene	1.43
2	α-pinene	2.05
3	camphene	0.75
4	sabinene	0.86
5	β-pinene	0.48
6	β-myrcene	0.77
7	α-terpinene	0.68
8	p-cymene	2.79
9	Limonene	3.59
10	cis-β-ocimene	0.08
11	trans beta ocimene	0.12
12	γ-terpinene	4.31
13	cis-linalool oxide	0.07
14	trans-linalool oxide	0.10
15	α-terpinolene	0.42
16	linalool	73.79
17	camphor	4.43
18	Terpinen-4-ol	0.01
19	α-terpineol	0.09
20	trans-Geraniol	0.21
21	geranyl acetate	1.27

Table 5. Continued.

22	β-caryophyllene	0.24
23	β-phellandrene	0.02
24	Linalyl propionate	0.20
25	cis-citral	0.02
LSD at 0.01		0.0022

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.01 according to Duncan's multiple range test.

Table 6. Chemical composition of *Petroselinum sativum* (herb) essential oil.

No.	Compound name	Relative conc (%)
1	Trans-Ocimene	1.99
2	l-Phellandrene	1.03
3	Limonene	3.23
4	Linalool oxide	0.24
5	α-pinene	16.16
6	γ-terpinene	0.43
7	Artemiseole	0.08
8	α-terpinolene	1.37
9	β-caryophyllene	2.68
10	L-Selinene	0.47
11	Caryophyllene oxide	0.14
12	β –pinene	11.16
13	Apiol	18.23
14	Elemicin	4.30

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.01 according to Duncan's multiple range test.

Table 7. Inhibition zone diameter (mm) of the essential oils against pathogenic bacterial strain (*Staphylococcus aureus).*

Essential oils	Inhibition zone diameter (mm)
Allium sativum (Garlic)	25±1.5
Allium cepa L. (Onion)	13±0.0
Cuminum cyminum (Cumin)	16±1.2
Corriandrum sativum (Coriander)	30±0.3
Petroselinum sativum (Parsley)	8±0.0
LSD at 0.01	0.285

Diameter of the filter paper disc = 6.0 mm; Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.01 according to Duncan's multiple range test.

of terpenes is not fully understood, it is thought to involve membrane disruption by the lipophilic compounds (Cowan, 1999). The essential oils containing terpenes are also reported to possess antimicrobial activity (Dorman and Deans, 2000), which are consistent with our present studies.

Previous studies indicated that, essential oils from garlic (Rees et al., 1993), cumin (Patrick, 1990) and coriander (Isao et al., 2004) were found to be most effective against *S. aureus*. The antibacterial activity of the essential oils was due to aldehyde constituents, there has been some evidence that alpha, beta unsaturated

Table 8. Counts of *Staphylococcus aureus* (CFU / paper point sample) in artificially inoculated root canals of teeth after the treatment with the effective essential oils.

Treatments	Replicates			Mean
	1	2	3	
Initial count before treatments	6.0 x 10^3	5.6 x 10^3	6.1 x 10^3	5.9 x 10^3
Saline (-ve control)	2.4 x 10^3	2.3 x 10^3	1.9 x 10^3	2.2 x 10^3
NaOCl 5.0% (+ve control)	ND	ND	ND	ND
NaOCl 2.5% (+ve control)	ND	ND	ND	ND
Allium sativum (Garlic) oil	ND	ND	ND	ND
Cuminum cyminum (Cumin) oil	ND	ND	ND	ND
Corriandrum sativum (Coriander) oil	ND	ND	ND	ND

ND: Not detected; Each value is presented as mean of triplet treatments.

Table 9. Antioxidant activity of different plant essential oils against DPPH and ABTS radical at various concentrations (100 and 200 µg/ml).

Treatments	Conc. (µg/ml)	% Scavenging activity	
		DPPH	ABTS
Allium sativum (Garlic) oil	100	46.5±3.15	55.8±2.41
	200	85.6±4.20	89.3±4.87
Allium cepa L. (Onion) oil	100	60.5±4.31	66.7±3.58
	200	88.6±3.65	91.5±4.27
Cuminum cyminum (Cumin) oil	100	63.5±2.68	60.47±2.64
	200	80.0±4.05	80.9±3.78
Corriandrum sativum (Coriander) herb oil	100	50.8±3.65	60.8±1.99
Petroselinum sativum (Parsley) herb oil	200	66.5±2.65	82.3±3.05
	100	60.2±1.85	42.5±2.45
BHA	200	80.2±3.21	85.6±3.66
	100	86.3±4.75	87.8±4.63
	200	92.8±4.98	96.6±3.12
LSD at 0.01		0.9373	0.6470

Each value is presented as mean of triplet treatments, LSD: Least significant difference at P ≤ 0.01 according to Duncan’s multiple range test.

aliphatic aldehydes possess growth inhibitory and bactericidal activity, this property depends on the length of the aliphatic carbon chain and the group of microorganism (Pauli, 2001).

Antioxidant activity

The DPPH and ABTS free radical scavenging activities of 7 essential oils extracted from different plants at two different concentrations (100 and 200 µg/ml) were determined and compared with that of the stander antioxidant BHA (Table 9). All the tested samples showed lower DPPH radical scavenging activity when compared with ABTS radical scavenging activity and in both methods the samples extracts give activity less than synthetic standard (BHA). The highest antioxidant scavenging effect (%) was obtained with BHA (92.8, 96.6% against DPPH and ABTS radicals at 200 µg/ml, respectively). The essential oils of different plants

reduced the concentration of DPPH and ABTS with an efficacy near to that of stander antioxidant. Different plant essential oils were able to reduce the stable purple colored radical DPPH into yellow-colored DPPH reaching 50% of reduction with EC_{50} values as follow: EC_{50} (Garlic oil) = 115 µg/ml; EC_{50} (Onion oil) = 80 µg/ml; EC_{50} (*Petitgrain mardari*n) = 78 µg/ml; EC_{50} (Cumin seed oil)= 81.5 µg/ml; EC_{50} (Coriander herb oil) = 101.5 µg/ml; EC_{50} (Parsly H oil) = 82.1 µg/ml; EC_{50} (*Matricania chamomilla*) = 76.56 µg/ml; EC_{50} (Mixture) = 63.5 µg/ml and EC_{50} (BHA) = 56.8 µg/ml.

The antiradical scavenging activity of oils might be attributed to the replacement of hydroxyl groups in the aromatic ring systems of the phenolic compounds as a result of their hydrogen donating ability (Brand-Williams et al, 1995; Prabussenivasan et al., 2006).

Analysis of garlic essential oil showed propene derivatives as the major constituents (Table 3). Also, gamma-terpinene, linalool, alpha-pinene were the major constituents in *C. sativum* (Table 5) but alpha-pinene, beta-pinene, alpha-terpineol, gerneyl and cuminaldehyde were the major constituents in *C. cyminum* (Table 4). Also, α-pinene, β-pinene and apiol were the major constituents in *P. sativum* essential oil (Table 6). These compounds are known to possess antioxidant properties (Fayed, 2009; Emami et al., 2007).

Conclusion

The irrigation regimen based on the use of natural sources example essential oils seems to be a promising endodontic tool because it promoted the elimination of root canal *S. aureus* throughout the experimental period and there is positive correlation between antibacterial and antioxidant activity of tested essential oils.

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