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Research Article

Isolation of carvacol from Origanum vulgare, synthesis of some organosilicon derivatives, and investigating of its antioxidant, antibacterial activities

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Abstract

Isolation of carvacol from *Organum* was done by hydro distillation using sodium bicarbonate to remove phenolic compounds and then it was extracted by Heptane. Carvacol was separated by chromatographic method, identified by HPLC and common spectroscopic methods. In order to study the mechanism of its action, some organosilicon derivatives were synthesized. In this way, antioxidant, and antibacterial activities were investigated. We proposed a mechanism for its action.

Keywords: Carvacol, Organum, silyl ethers, Antibacterial, antioxidant

INTRODUCTION

The genus *Origanum* belongs to the family of *Labiatae* and includes many species that are commonly found as wild plants in the Mediterranean areas, Euro-Siberian and Irano-Siberian. A total of thirty eight (38) *Origanum* species are recognized in the world. *Origanum vulgare* L. is the only species of the *Origanum* genus growing wild in Iran. *O.* regions *vulgare* L. is widely spread all over the country, particularly Gilan, Mazandaran and West Azarbaijan provinces.

Due to the variability in chemical and aroma characteristics, *Origanum* and cosmetic industries as a culinary herb, flavouring substances of food products, alcoholic beverages and perfumery for their spicy fragrance (Pirigharnaei et al., 2011).

Essential oils (EO) are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. Known for their antiseptic, i.e. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesicremedies. Up to the present day, these characteristics have not changed much except that more is now known about some of their mechanisms of action, particularly at the antimicrobial level (Bakkali, 2008).

The EO of oregano is composed of carvacrol and/or thymol as dominant components, followed by γ-terpinene, pcymene, linalool, terpinen-4-ol and sabinene hydrate (Pirigharnaei et al., 2011).

Studies on oregano plants in Greece showed that *O. vulgare* ssp. *hirtum*, contained a high amount of EO. The content of EO as high as 8% with carvacrol as dominant component (95%) was reported for this subspecies (Pirigharnaei et al., 2011).

Carvacrol (2-*p*-cymenol or 5-isopropyl-2-methylphenol)is one of the main components of the EO of some *Labiatae (Laminaceae)* members like oregano, thyme and savory, the content of which can reach up to 86% (Lagouri, 1993; Kustrak et al., 1996). The volatile oils of some conifers also contain carvacrol (Rudloff, 1980). It has been indicated that the antioxidant activity of the EO of the above mentioned herbs is due to the carvacrol, its isomer thymol and some other phenols (Lagouri, 1993; Deighton et al., 1993). They possess antibacterial activity and therefore find application in treating oral diseases (Didry et al., 1994). Their antifungal activity is used against phytopathogenic fungi (Mueller-Ribeau et al., 1995). Additionally, this EOs exhibit analgesic activity which is also related to the carvacrol content (Aydm et al., 1996). Antiviral activity has been observed for whole EOs containing carvacrol as the major component (Allahverdiyev et al., 2004; Garcia et al., 2003), but only a very low antiviral activity has been observed for carvacrol alone (So¨kmen et al., 2004).

Carvacrol, a phenolic compound, is considered one of the main components of certain EOs that exerts antimicrobial activity, not only because of its high abundance in some oils, (So¨kmen et al., 2004; Arrebola et al., 1994), but also because of its high specific activity as compared to other EO components (Dorman and Deans,2000; Friedman et al., 2002). Studies that have been performed on the antimicrobial activity of carvacrol have shown that it has a broad spectrum of antimicrobial activity against almost every Gram-positive and Gram-negative bacteria tested (Friedman et al., 2002).

Despite extensive research on carvacrol in recent years, not much is known about the mechanism of action of carvacrol against bacteria. The antibacterial activity of carvacrol has been attributed to its hydrophobic nature, the presence of a free hydroxyl

group and a delocalized electron system. Carvacrol acts on the cytoplasmic membrane, with considerable effects on the structural and functional properties of the membrane, which becomes increasingly permeable to protons and ions and loses its integrity. Carvacrol has also been shown to inhibit ATPase and to induce Hsp 60 in bacteria (Nostro et al., 2009).

Natural antioxidants are considered to be useful agents for the prevention of diseases (Duthie and Brown, 1998; Milner, 1998; Kutlu et al., 2011). Many studies have shown that phenolic compounds in plant essential oils display antioxidant activity as a result of their capacity to scavenge free radicals (Seyoum et al., 2006; Ozkan et al., 2010).

Their antioxidant effect was due to the presence of hydroxyl groups in their chemical structure. Also, a number of studies on antioxidant activities of EOs from various aromatic plants reported that the oregano essential oil, rich in thymol and carvacrol, has a considerable antioxidant effect on the process of the lardoxidation Yanishlieva and Marinova (1995) examined the antioxidant activity of hexane extracts of oregano grown in Bulgaria, as well as the mechanism of action of pure thymol and carvacrol (Kulisic et al., 2010).

In this study, we extracted carvacrol from the *Origanum vulgare* and the antioxidant properties of carvacrol and their derivatives were measured by two methods: FRAP and DPPH. And in other parts the antibacterial activity of carvacrol and its derivatives were investigated and effect of the hydroxyl functional group on antioxidant and antibacterial activity were investigated.

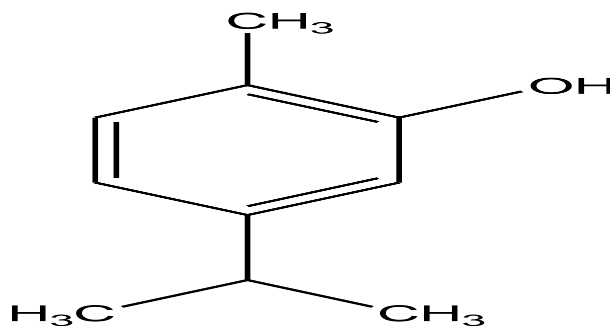

Figure 1. The Structure of carvacrol

MATERIALS AND METHODS

Instruments

FT-IR: FT-IR BRUKER Ps15
FT-HNMR: FT-HNMR BRUKER (400 MHz)
MS:
Instrument Specifications:
Manufacturer Company: Agilent Technology (HP)
1. MS Model: 5973 Network Mass Selective Detector
2. Ion source: Electron Impact (EI) 70eV
3. Ion source temperature: 230ºC
4. Analyzer: Quadrupole
5. Analyzer temperature: 230ºC
HPLC: A Jasco HPLC system, consisted of a PU-1580 isocratic pump, a Rheodyne 7725i injector with a 10–µL loop (Rheodyne, Cotati, CA, USA) and a UV-1575 spectrophotometric detector was used in the experiment.
UV-Vis: Double-beam spectrophotometer (UV-Vis T80) company made in England PG1100-190 wavelength range with deuterium lamp background correction equipped with a tungsten lamp as a radiation source.

Plant material

Origanum vulgare samples were collected during the flowering season from Arasbaran, Azarbaijan, Iran, in August 2012.Air-drying of oregano was performed in a shad place at room temperature for 10 days. The plant material was used for the isolation of the essential oil, immediately after drying.

Isolation procedure

The EO of *O. vulgare* was obtained by hydro distillation in 100 mL of H_2O for 3h with a Clevenger apparatus in the Chemistry Department of Azarbaijan shahid madani University. The essential oils were collected and dried over an hydroussodium sulphate and stored at 4–6°C.

Quantization of carvacrol by High-performance liquid chromatography (HPLC)

Ten milligrams of essential oil was added to a volumetric flask and diluted to 100 ml with acetonitrile(CAN):water(80:20) (100 µg/ml). Six samples were prepared and each one was injected three times.

A Jasco HPLC system, consisted of a PU-1580 isocratic pump, a Rheodyne 7725i injector with a 10–µL loop (Rheodyne, Cotati, CA, USA) and a UV-1575 spectrophotometric detector was used in the experiment. The chromatographic system was controlled by HSS-2000 provided by Jasco using the LC-Net II/ADC interface. The data were processed using BORWIN software (version 1.50). An analytical 250 mm × 4.6 mm ID, 5–µm particle, Perfect sil Target ODS–3 column (MZ–Analyse ntechnik, Germany) with a ODS–3 pre column (10×4.0 mm I.D., 5–µm), which was maintained at ambient temperature, was employed for separation.

The isocratic mobile phase consisted of acetonitrile–water in the ratio of 50:50 v/v, flowing through the column at a constant flow rate of 1 mL min^{-1}. The eluent was monitored using UV detection at a wavelength of 274 nm. The mobile phase was filtered through a 0.22 µm membrane–type GV filter (Millipore). A 40 kHz and 138 W ultrasonic water bath with temperature control (sonic bath model LBS2 – FALC instruments S.r.l TREVIGLIO, Italy) was applied to degassing the mobile phase.

Standard solution of carvacrol at a concentration of 500 mg $L^{-1}$was prepared in acetonitrile –water in the ratio of 80:20 stored at 4^0C.

Isolation of carvacrol

In order to obtain a fraction of carvacrol phenolic compound, 1g of the EO was dissolved in 5ml pentane and extracted with sodium hydroxide solution (20%) in water. In this manner, phenolic compounds were removed from the pentane layer. The aqueous phase, containing dissolved phenolic compounds sodium salts, was neutralized with hydrochloric acid solution (10%)in water. Finally, isolation of the carvacrol was performed by extraction with pentane (5 _ 5ml).
The effectiveness of this separation method was tested by Thin-layer chromatography (TLC) on silica gel plates (mobile phase: n-hexane: ethyl acetate 85:15 v/v) and a purity of the phenolic compound fraction was confirmed. These separation results were confirmed by FT-IR, Mass and FT-NMR analysis too.

Table 1. Direct Probe Temperature Program

Parameter	Step 1
Initial Temperature (ºC)	50
Initial Time (min)	0
Program Rate (ºC/min)	70
Final Temperature (ºC)	350
Final Time (min)	5

Carvacrol silicon derivatives Synthesis

Silicon derivatives synthesis was carried out as follows. Dry Tetrahydrofuran (THF) was the solvent used in the synthesis and tri-ethyl amine (ET_3N) as anbases. System refluxed for 24hour under argon gas at room temperature and The reaction process was controlled by TLC. The product by TLC, with solvent system n-hexane - ethyl acetate was isolated. The product was dried under vacuum and purified product.

chloro Tri-ethyl silane and chloro vinyl dimethyl silane were purchased from Merck Co and synthesis of two carvacrol derivatives (Tri-ethyl silyl carvacrol, vinyl dimethyl silyl carvacrol) were performed according to agenda. Spectrum FT-IR, FT-HNMR confirmed that the reaction products.

Antioxidant activity

Antioxidant activity of carvacrol and vinyl dimethyl silane derivative was measured by DPPH and FRAPS methods.

2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) assay

The hydrogen atoms or the electron donation abilities of the corresponding samples were measured from the bleaching of a purple-colored methanol solution of DPPH. This assay was carried out following the same method as reported elsewhere (19, 20). Different volumes of samples dissolved in methanol were added to methanol solution of DPPH (2, 2-diphenyl-1 picrylhydrazyl, free radicals) so that the final volume was 1 ml. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The inhibition free radical DPPH, in percentages (I%), was calculated in the following way:

I% = (A control – A sample/A control) × 100,

where A control is the absorbance of the control reaction, containing all reagents except the test compound, and A sample is the absorbance of the test compound.

Different concentrations of the samples were prepared and DPPH assay were performed on them. (Table2). For the calculation of these values, Microsoft Excel was used.

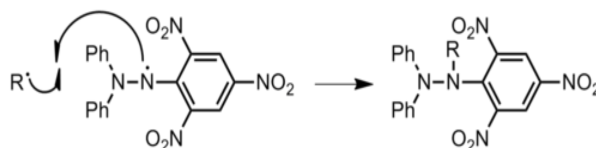

Figure 2. DPPH radical scavenging reaction

Ferric Reducing/Antioxidant Power (FRAP) assay

This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2, 3, 5-triphenyl-1,3,4- triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to the ferrous form at low pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer. The ferrous sulfate standard curve was used for calculation of frap values.

Antibacterial activity

Carvacrol natural compound extracted from the *Origanum vulgare* and vinyl dimethyl silyl carvacrol, Tri-ethyl silyl carvacrol were synthesized in the laboratory. Compounds were pure. Number of gram-positive and gram-negative bacteria (*S. aurous, E. coli, Proteus, Klebsiella, Pseudomonas)* were separately cultured in Eosin methylen-blue agar (EMB) medium. Antibacterial activity assays were carried out by disc diffusion method. Three discs (6mm in diameter) for each Petri dish were separately impregnated with carvacrol (P42), vinyl dimethyl silyl carvacrol (P41), Tri-ethyl silyl carvacrol (P40), and put in the middle of the inoculated plates. The bacterial cultures were incubated at37±2°C for 48 h, and then inhibition zones were measured in diameter (mm) around of the discs.

RESULTS AND DISCUSSION

Isolation procedure

The extractions were performed in a clevenger apparatus for 120 min (3 time) and the essential oils of Oregano aerial parts were obtained by hydro-distillation at yield of (0.80%).The essential oils were collected and dried over an hydroussodium sulphate and

Table 2. Different concentrations of samples (v/v) stored at 4–6 $^{\circ}$C

Volume of sample (µl)	Volume of DPPH solution (µl)	Concentration (V/V)
50	950	%5
70	930	%7
100	900	%10

Quantization of carvacrol by HPLC

The carvacrol, natural compound in the EO of *Origanum vulgare* L. was confirmed by HPLC analysis. In this analysis, at a wavelength of 274 nm the absorption peak intensity 22500 inhibitions at 20:5 shows that exactly matches the peak of the standard material (Figure 3).

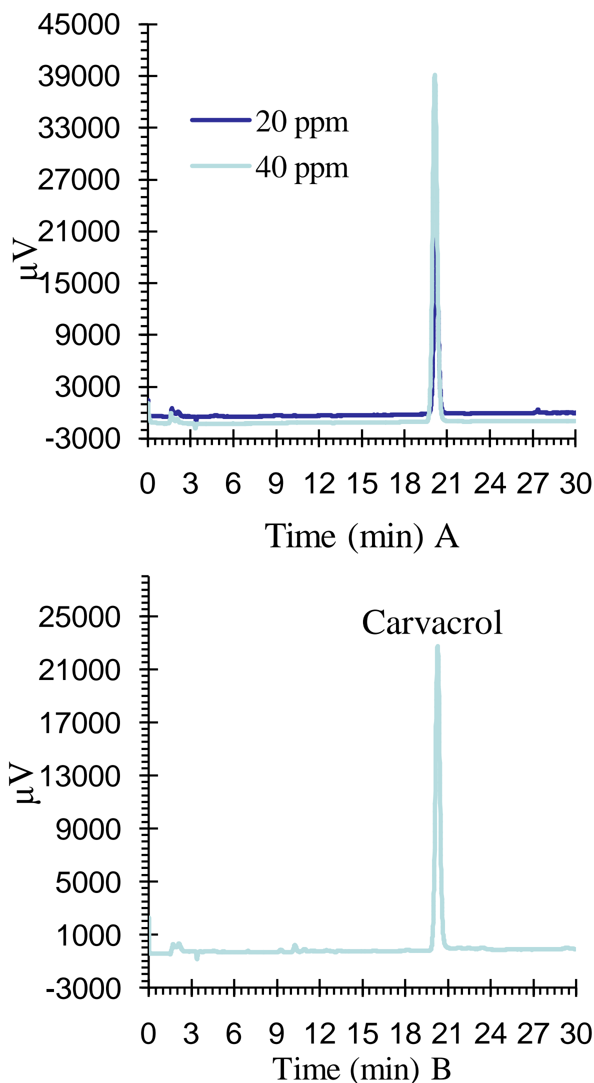

Figure 3. HPLC analysis. A: HPLC analysis of carvacrol standards, B: HPLC analysis of essential oil

Isolation of carvacrol

In order to obtain a fraction of carvacrol phenolic compound, the essential oil was analyzed and finally, carvacrol was extracted by thin-layer chromatography. These separation results were confirmed by FT-IR, Mass and FT-HNMR analysis.

FT-IR of carvacrol

Broad peak in the region of 3392 $cm^{-1}$is related to stretching vibrations of OH phenol. The aromatic CH stretching vibration peak about 3020 cm^{-1}, asymmetric stretching vibrations of aliphatic CH in 2927 cm^{-1}, symmetric stretching vibrations of aliphatic CH in 2869 $cm^{-1}$and as harp peak in 1620 cm^{-1} is related to aromatic C = C stretching vibrations (Figure 4)

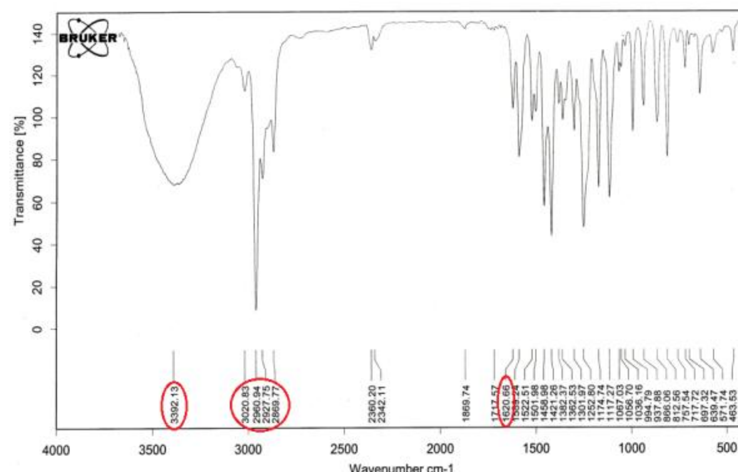

Figure 4. FT-IR of carvacrol

Mass of carvacrol

Molecular mass of carvacrol natural compound (A) is a 150.28. Fragment observed at m / z = 135 fragment of the molecule [A-CH3] and fragment observed at m / z = 107 is related to the fragment molecule [A-isopropyl group]. Fragment observed at m / z = 91fragment of the molecule tropylium and fragment observed at m / z = 77 is related to the fragment molecule Phenyl (Figure 5).

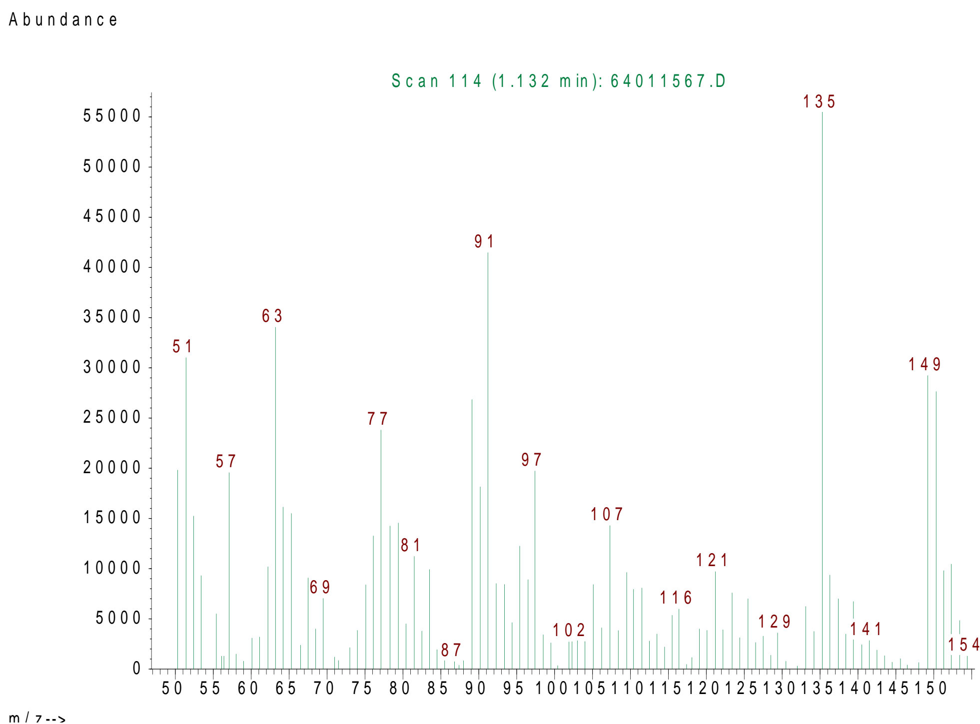

Figure 5. Mass analysis of carvacrol

FT-HNMR of carvacrol

Binary peak in the1.3 ppm related to 6 protons of the methyl group, isopropyl attached to the aromatic ring. Single branch peak in the 2.3 ppm related to3 protons of the methyl group attached to the aromatic ring. Peak in the 2.9 ppm related to 1proton of isopropyl group that by the 6 protons of the methyl group is split into seven branches. Single branches broad peak in the 5.8 ppm related to 1 proton of the hydroxyl group attached to the aromatic ring. Single branches peak

in the 6.7ppm related to 1proton attached to the aromatic ring. Peak in the 6.8ppm related to 1 proton attached to the aromatic ring that by a adjacent proton is a binary split. Peak in the 7.1 ppm related to 1 proton attached to the aromatic ring that by an adjacent proton is a binary split (Figure 6).

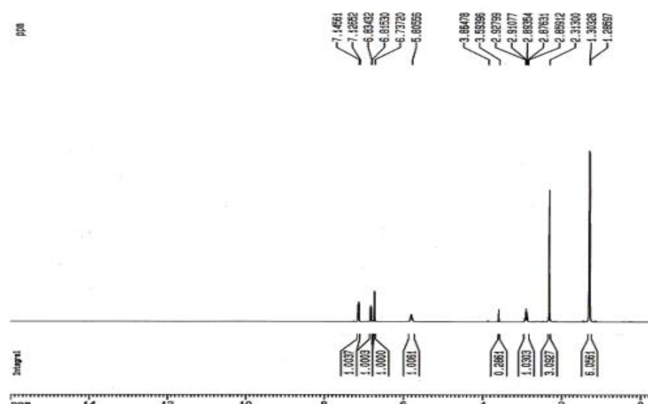

Figure 6. FT-HNMR of carvacrol

Carvacrol silicon derivatives Synthesis

Silyl ethers are a group of chemical compounds which contain a silicon atom covalently bonded to an alkoxy group. The general structure is $R^1R^2R^3Si{-}O{-}R^4$ where R^4 is an alkyl group or an aryl group. Silyl ethers are usually used as protecting groups for alcohols in organic synthesis. One of the main features of the silicon-containing material is a dramatic increase in their lipophilic properties.

There action in the synthesis of silyl ethers is as follows:

$$R_3SiCl + R'OH \rightarrow R_3SiOR'$$

Figure 7. Reactions of silyl ethers

Carvacrol with two compounds، chloro triethyl silane and chloro vinyl dimethyl silane as reacted according to the agenda. Synthesis of mentioned carvacrol derivatives were confirmed by FT-IR and FT-HNMR.

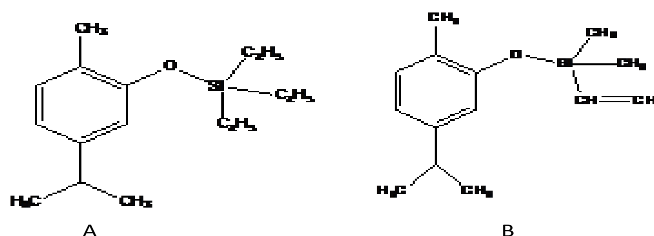

Figure 8. A: triethyl silyl carvacrol. B: vinyle dimethyl silyl carvacrol

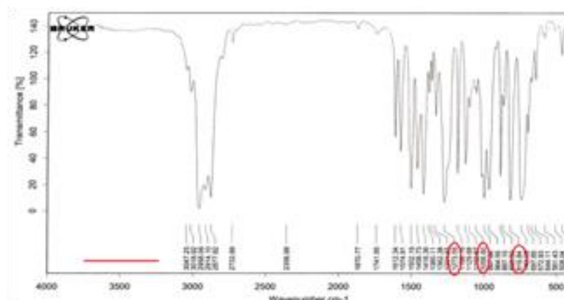

Figure 9. FT-IR of triethyl silyl carvacrol

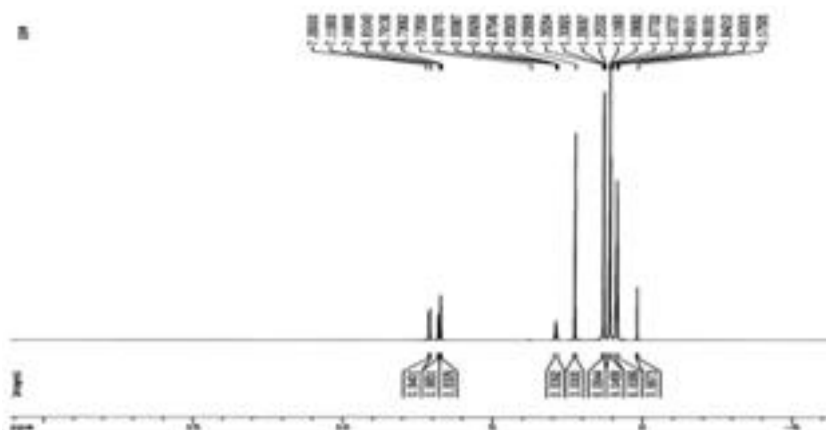

Figure 10. FT-HNMR of triethyl silyl carvacrol

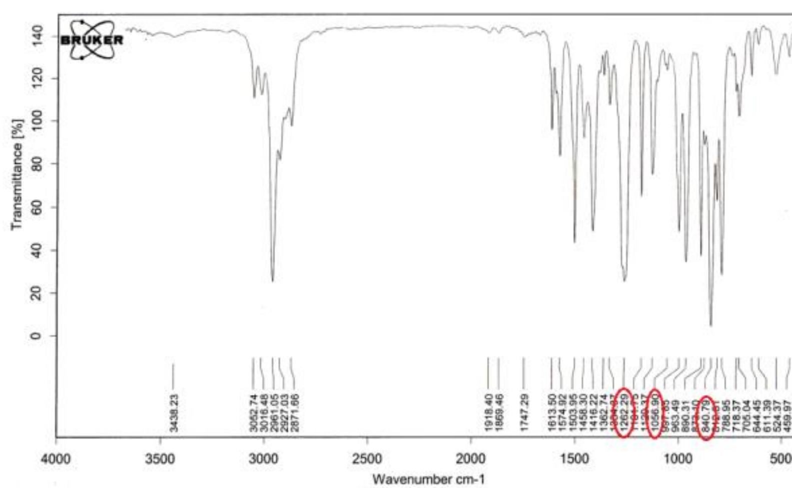

Figure 11. FT-IR of vinyle dimethyl silyl carvacrol

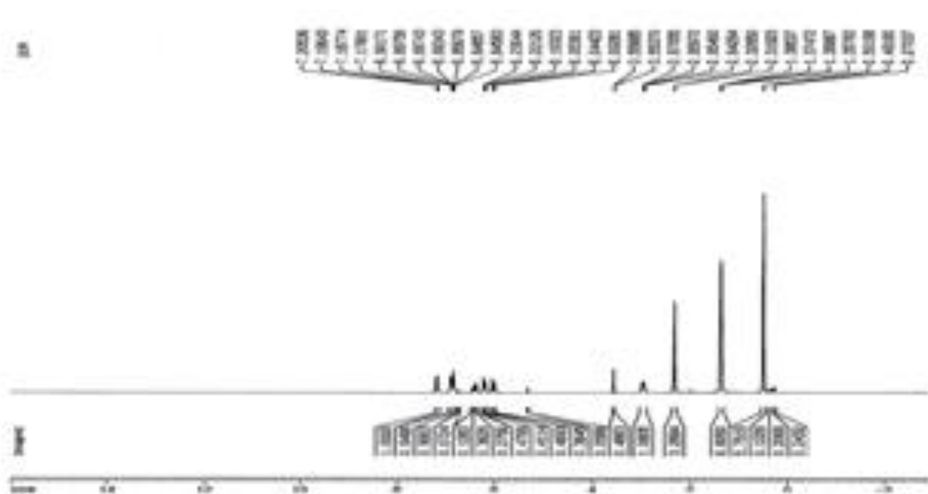

Figure 12. FT-HNMR of vinyle dimethyl silyl carvacrol

Antioxidant activity

Reactive oxygen species such as super oxide radicals, hydroxyl, Proxyl are produced continuously during the reaction in the body. These radicals play an important role in the pathogenesis of diseases associated with oxidative stress. Today,

it is well known that oxidative damage caused by the activity of this molecule causes and progression of chronic diseases such as heart disease-cardiovascular diseases, atherosclerosis, cancer, Alzheimer's, Parkinson's, cataracts and inflammation (Parejo et al., 2002; Kay and Holub, 2002; Morton et al., 2000)

Flavonoids are compounds found in fruits, vegetables, and certain beverages that have diverse beneficial biochemical and antioxidant effects. Their dietary intake is quite high compared to other dietary antioxidants like vitamins C and E. The antioxidant activity of flavonoids depends on their molecular structure, and structural characteristics of certain flavonoids found in hops and beer confer surprisingly potent antioxidant activity exceeding that of red wine, tea, or soy. The antioxidant activity of phenolic compounds and their beneficial role in coronary heart disease, cancer and age-related degenerative disease of the brain have been studied. Antioxidant effect of these compounds is due to the it's reducing effect. Reducing the hydrogen donor and chelating metals act (Jamshidi et al., 2010; Shariatifar, 2011; Henry and Heppel, 2002)

In this study the antioxidant activity of carvacrol (P42), vinyl dimethyl silyl carvacrol (P41) were evaluated using the DPPH method and FRAP method.

DPPH radical scavenging method

Relatively stable organic radical DPPH$^{\cdot}$ has been widely used in the determination of the antioxidant activity of single compounds as well as the different plant extracts. The method is based on the reduction of alcoholic DPPH$^{\cdot}$ solutions in the presence of an hydrogen donating antioxidant. DPPH$^{\cdot}$ solutions show a strong absorption band at 517 nm appearing a deep violet colour. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH·, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant.

Table3. The results of DPPH method

Samples concentration	%5	%7	%10
Carvacrol (P4)	%30.68	%36.41	%53.13
Vinyl dimethyl silane carvacrol (P41)	%20.62	%23.29	%31.23

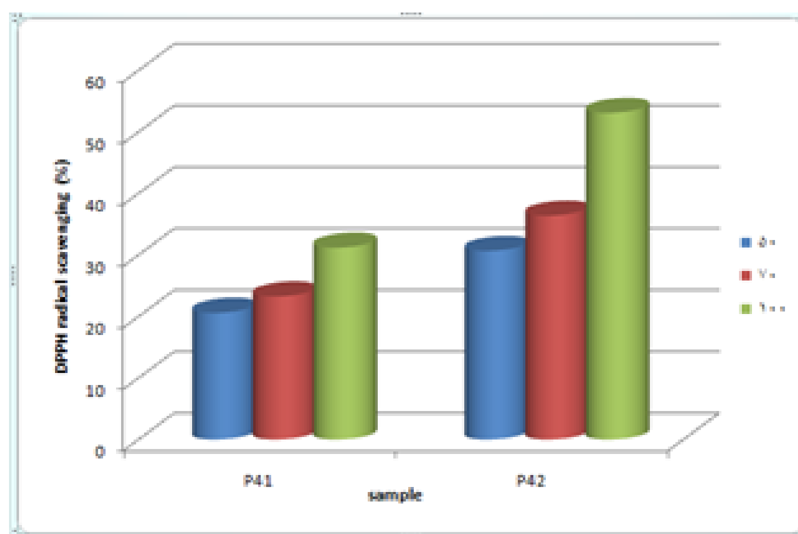

Figure 13. The results of DPPH method (carvacrol (P42), vinyl dimethyl silyl carvacrol (P41)

Different concentrations of each sample were prepared (Table 2) and antioxidant activity of the samples was measured by DPPH method. With according to results free radical scavenging ability of carvacrol is more than vinyl dimethyl silyl carvacrol. The structurally only difference between carvacrol and vinyl dimethyl silyl carvacrol is hydroxyl group of carvacrol that has been replaced with vinyldimethyl silane. These data showed that the antioxidant activity of carvacrol largely reduced by replacing a hydroxyl group with a hydrophobic group (vinyl dimethyl silane carvacrol). Added groups (vinyl dimethyl silane carvacrol) are not biologically active and it is used only to protect the hydroxyl group of caracole. The results strongly confirmed that the antioxidant property of carvacrol is mainly dependent on the hydroxyl functional group that in this study is protected by vinyl dimethyl silane carvacrol.

Ferric Reducing/Antioxidant Power (FRAP) assay

FRAP method to measure the antioxidant activity of samples was used. This method is based on the ability of samples to reducing the ferric ions (Fe^{3+}) to ferro ions (Fe^{2+}) Isusing TPTZ reagent. In the presence of antioxidants, ferric ions (Fe^{3+}) to Ferro (Fe^{2+}) are reduced and in the presence of reagents TPTZ, the solution comes in a purple color. Than the amount of reduced power of samples, absorbed dose-dependently increases. With this change in absorption we can measure the antioxidant activity of different compounds.

Table 4. The results of FRAP method

Compound	FRAP value(µM Fe2+)(25µl)
P41	1027.667 ± 101.933
P42	2222 ± 164.1097

Data from the FRAP assay also showed that the antioxidant activity of carvacrol is stronger than its derivatives‹and showed that the antioxidant activity of carvacrol largely dependent hydroxyl functional group. Results from the two methods, DPPH and FRAP showed antioxidant activity of carvacrol stronger in comparison with the P41. And we can say that the antioxidant activity of carvacrol is owed to the hydroxyl functional group.

Antibacterial activity

In addition to carvacrol, two carvacrol derivative compounds: triethyl silyl carvacrol and vinyl dimethyl silyl carvacrol, (containing a triethyl silane and a vinyl dimethyl silane group ,respectively instead of the hydroxyl group of carvacrol) also were tested. In this study‹the culture plate (Plating)was used for culturing bacteria. Variety of Gram-positive and gram-negative bacteria include Escherichia coli, Staphylococcusaureus, Pseudomonas, Klebsiella and Proteuswerea were cultured in the laboratory on a separate plates. Antibacterial activity assays were carried out by disc diffusion method. Three discs contain carvacrol (P42), vinyl dimethyl silyl carvacrol (P41) and triethyl silyl caracole (P40) was used for investigation of anti bacterial activity.

After loading the discs‹plates incubated for 24hat37 °C. Then‹the diameter of the inhibition zone around the discs as the power inhibitory substances was measured. Inhibition zone of compounds were measured and were given in Table 5.

Commonly silicon is used as a protective group for the functional group of alcohols and phenols. In this research, the silicone was used to protect the hydroxyl group of carvacrol. Results indicate that the inhibition zone diameter of disks containing native carvacrol in all cultures (including gram-positive and gram-negative bacteria) is larger than the inhibition zone diameter of carvacrol derivatives (Table5).

Penicillin (P10) was used as control disc for evaluation of inhibitory capacity of carvacrol. The result indicate that inhibition zone of carvacrol disk is equal to a penicillin antibiotic disc.

Looking at the structure of the three compounds will be reveal the only difference between carvacrol and its derivatives is hydroxyl group of carvacrol. Results down with different types of bacteria showed that the antibacterial activity of carvacrol greatly depends on hydroxyl functional group. Anti-bacterial activity will be greatly reduced if functional group is eliminated.

Table 5. Inhibition zone of compounds (carvacrol (P42), vinyl dimethyl silyl carvacrol(P41), triethyl silyl carvacrol(P40))in bacterial culture

Bacteria	Inhibition zone of P42 (mm)	Inhibition zone of P41 (mm)	Inhibition zone of P40(mm)
E.Coli	24	2	2
Proteus	40	2	4
Klebsiella	36	11	9
Pseudomonas	12	-	-
S.Aureus	40 P10 40	20	28

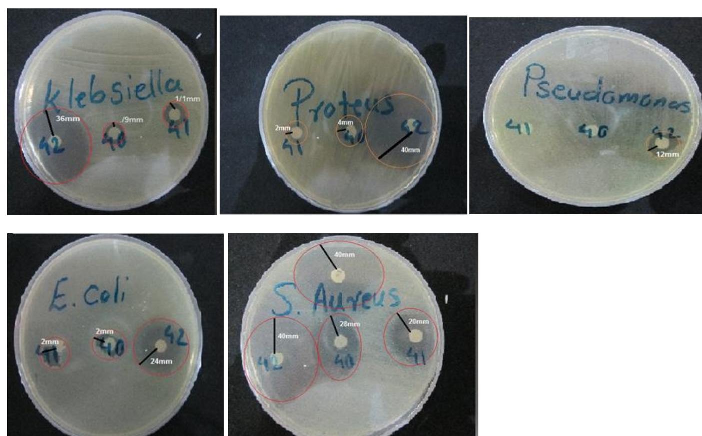

Figure 14. Bacteria culture and show inhibition zone around the disk

Bacterial sensitivity against carvacrol (P42):S .Aureus = Proteus>Klebsiella>E. coli>Pseudomonas.

Bacterial sensitivity against Triethyl siloxane Carvacrol (P40):S. Aureus> Proteus> E. coli> Klebsiella> Pseudomonas.

Bacterial sensitivity against Vinyl dimethyl siloxane carvacrol(P41):S. Aureus> E. coli =Proteus > Klebsiella> Pseudomonas.

One of the main features of the silicon-containing material is a dramatic increase in their lipophilic properties. The cell membrane is made of phospholipids and has lipophilic properties. Silicon compounds can easily pass through the cell membrane and hydrolyzed to the original state of the drugs that have pharmacological properties. However, in this study silicon compounds showed reduced antibacterial activity.

Despite extensive research on carvacrol in recent years, not much is known about the mechanism of action of carvacrol against bacteria. Carvacrolan example of natural phenolic compounds, which is due to hydroxyl groups in its molecule structure is hydrophilic properties. The replacement of the hydroxyl group of carvacrol with silicon, addition to eliminate the possibility of hydrogen bond formation in aqueous environment، reduced their hydrophilic properties. The hydro phobicity of the carvacrol indicates that the bacterial membrane is likely to be the initial target of the compound. In addition, carvacrol has previously been shown to disrupt the bacterial membrane, affect the proton motive force and disrupt both the pH gradient and the electron flow across the membrane (Helander et al., 1998; Ultee et al., 2002).

Carvacrol causes inhibition ATPase activity and increased are non-specific permeability of the cell membranes of bacteria.(Helander et al., 1998; Gill and Holley, 2006) Gill and colleagues observed that carvacrol destroyed is bacterial membrane and inhibited is ATPase activity (Gill and Holley, 2006). Helender and colleagues observed that carvacrol destroys cell membrane and thus reduces intracellular Stored ATP and increased extracellular Stored ATP (Helander et al., 1998; Ultee et al., 2002). At least two amino acid residues are essential for the

activity of this enzyme, namely, aspartic acid, which is phosphory lated by ATP, and lysine, which is labeled by fluoresce in 5'-isothiocyanate (FITC)(Post and Kume, 1973; Walderhaug et al., 1985; Hesse et al., 1984; Farley et al., 1984; Kirley et al., 1984; Ohta et al., 1985).

The results of this work showed that the antibacterial activity of carvacrol largely depends on it hydroxyl group and silicon compounds synthesized showed little antibacterial activity. A variety of gram-positive and gram-negative bacteria showed sensitivity to carvacrol. Given these data and the results reported in antibacterial activity of carvacrol, is quite evident that there is a hydroxyl functional group of carvacrol is essential for action. Carvacrol impact on bacteria through the effectiveness of the external membrane of bacteria and bacterial cell membrane is impaired.

According to these results, carvacrol causes disruption of the ATPase enzyme function and the hydroxyl functional group of carvacrol is the main cause of anti-bacterial activity. This possibility can be raised that anti-bacterial activity of carvacrol due to the reaction between the hydroxyl functional group of carvacrol and the two amino acids in the active site of ATPase (asparticacid and lysine). Another possibility is that with replacement of the hydroxyl functional group with silane, carvacrol could not enter ATPase active site and antibacterial activity of carvacrol reduced.

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