*Original Research Paper*

# Phytochemical compounds and bioactivity properties of the whole plant of maidenhair fern (*Adiantum capillus-veneris* L.) essential oil

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**Abstract:**

**Background:** The use of medicinal plants and their effective compounds as natural sources that have antioxidant and antibacterial properties, has been considered by researchers. In this study, chemical compounds of *Adiantum capillus-veneris* essential oil were used to evaluate its antioxidant and antimicrobial activity using water distillation and gas chromatography. **Methods:** The whole plant was dried at room temperature at 25 ° C and the distillation method with water with modifications was used using a Clevenger device for essential oil extraction. Gas chromatography-mass spectrometry (GC/MS) was used to identify the resulting essential oil compounds and the antioxidant and antibacterial activity of the essential oil was evaluated. **Results:** The most important chemical constituents of *Adiantum capillus-veneris* essential oil were carvone (33.00%), carvacrol (15.05%), hexadecanoic acid (7.02%), hexahydrofarnesyl acetone (4.25%) and n-nonanal (4.2%). The antibacterial activity of *Adiantum capillus-veneris* essential oils (mg/ml) was calculated and the highest antibacterial activity was determined by *Staphylococcus aureus* (8.23±0.68), *Streptococcus pyogenes* (12.46±0.31), and *Diphtheroid* (11.37±1.02) at a concentration of 100 mg/ml of essential oils. **Conclusion:** The results of the present study showed that the essential oil of *Adiantum capillus-veneris* prevented the growth of three pathogenic bacteria. This antibacterial property is due to the presence of flavonoid compounds in *Adiantum capillus-veneris* which has the capacity for therapeutic and medical uses.

**Keywords:** *Adiantum capillus-veneris* L., Chemical compounds, Antioxidant, Antimicrobial, Essential oil, DPPH.

## 1. Introduction

The use of medicinal plants to replace chemical and synthetic drugs is increasing. Contrary to some people's belief that traditional medicine is worthless and unusable in modern science. Research evidence on traditional medicines shows their astonishing therapeutic effects [1, 2]. From ancient times plants have been a rich source of effective and safe medicines [3]. Herbal medicines are the primary source of primary health care in many countries [4], and today about 80% of the world's population is still dependent on traditional medicines [5].

*Adiantum capillus-veneris* L. (figure 1) is a woody plant with a height of 35 cm that has a creeping rhizome [6, 7]. Its dried roots are used as medicine, and its leaves are covered with coats. The plant grows in southern Europe, the Alps, and the Atlantic coast, as well as Iran [8]. It grows mostly in humid areas rich in organic matter and along streams and rivers and is very similar to green coriander [9]. *Adiantum capillus-veneris* L. has various medicinal properties with various ingredients. The mucilage in this plant has the property of softening the chest and upper respiratory organs and making it easier to expel sputum [10]. In traditional medicine, *Adiantum capillus-veneris* L. has been used as an antitussive, antipyretic, expectorant, diuretic, and in the treatment of respiratory diseases in the form of tea and severe cough in the form of syrup [11].

Active constituents include 21-OH-adiantone, isoquercitrin, kaemferol, letuol, terpenoids, 3α-4α- poxyfilicane, flavones tannic acid, gallic acid, and essential oils were reported to be responsible for the potent medicinal values of this fern [12, 13]. According to the publications, *Adiantum* species are a rich source of triterpenes with various structural skeletons. Besides, flavonoids, phenylpropanoids, and sterols have been isolated from the genus Adiantum [14, 15]. Various extracts obtained from *Adiantum* had indicated potential antibacterial activities against *Staphylococcus aureus* [16], *Streptococcus pyogenes* [17], *Klebsiella pneumonia* [18], *Escherichia coli* [19], and antifungal activity [20] against *Candida albicans.* Some evidence indicates that the biological actions of these compounds are associated with their antioxidant activity [21]. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is a free radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical spectrophotometrically [22, 23]. Because the effective compounds of medicinal plants vary based on latitude, soil profile, climatic conditions, *etc*., this study was performed to determine the

chemical composition and antibacterial and antioxidant activity of *Adiantum capillus-veneris* essential oil using the Hydro distillation method.

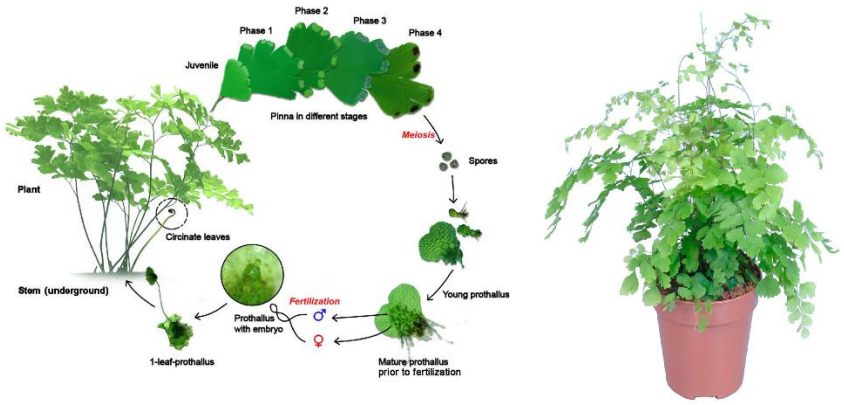


**Figure 1.** *Adiantum capillus-veneris* [24]

## 2. Materials and Methods

### *2.1 Plant collection and identification*

*Adiantum capillus-veneris* were collected at an altitude of 1170 m in Darab, Fars in 2019. All parts of the plant were dried at room temperature of 25 °C. The herbarium expert of Shiraz University of Medical Sciences was used to identify and confirm the scientific name of the collected samples. The collected plant, after cleaning and drying, was pulverized by a mill and kept in closed containers away from light and heat.

### *2.2 Essential oil extraction*

The hydro distillation method was employed with some modification using a Clevenger-type apparatus. Seventy grams of whole plant powder were mixed with 350 ml of distilled water and left for 3 hrs distillation [25]. The obtained oil was dried over anhydrous sodium sulfate and kept at 4–5 °C in the refrigerator till analysis [26].

### *2.3 GC and GC Mass*

Gas chromatography-mass spectrometry (GC/MS) was used to identify the essential oil compounds using ThermoQuest Trace GC 2000 (England). Its features were included capillary column DB5 with a length of 30 meters, the internal diameter of 250 micrometers, the thickness of the inner layer of 0.25 micrometers, a temperature program of 50 to 260 degrees Celsius, with an increase gradually 2.5 ° C/min. The column was kept at 265 ° C for 30 minutes. The injection chamber temperature was 250 ° C, and the helium gas flow rate was 1.5 ml/min. Also, mass spectrometry was performed by the ionization method (EI) of 70 electron volts and ionization source temperature of 250 ° C. Chemstation puls willey 7.1 was used to analyze the data processing [27].

### *2.4 Antimicrobial activity*

#### *2.4.1 Microorganisms*

Experiments were performed on three types of gram-positive bacteria including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *diphtheria*. All microorganisms were purchased from the Persian Type Culture Collection (Iranian Research Organization for Science and Technology).

#### *2.4.2 Antimicrobial Tests*

Disc diffusion method was used to determine the susceptibility of bacteria to plant essential oils. The bacterial strain was first prepared with microbial suspension equivalent to 0.5 McFarland, and then using sterile cotton swabs from the suspension prepared on the surface of Müller-Hinton agar [28], a uniform travel culture was performed. In the next step, the sterile blank discs (manufactured by Padten Teb) were immersed in concentrations (25, 50, and 100 mg/ml) and these discs were placed on the agar surface at regular intervals. The plates were incubated for 24 hours at 37 ° C and then the growth inhibition zones around the discs containing the essential oil were measured from the back of the plate with a ruler based on the millimeter [29]. The antibiotics results were measured with a standard table (CLSI2006) [30]. The experiments were repeated 3 times and reported as average.

### *2.5 Antioxidant activities*

The use of stable radical of DPPH is widely used to study the antioxidant properties of plant essential oils. The movement of electrons throughout the molecule causes the free stable radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) to be dimers and produce a dark purple color at a wavelength of 515 to 517 nm. Therefore, to investigate the anti-radical activity of essential oil, stable radicals DPPH were used according to the method of Brand-Williams et al. Fifty microliters of essential oil at different concentrations were added to 2 ml of 0.004% methanol solution and kept at room temperature for 60 minutes. Then the optical absorption of the solution was read at 517 nm using the South Korea-made Shinko spectrometer. A sample containing 50 µl of methanol with 2 ml of solution DPPH was used as a control sample and the methanol solvent was used to reset the device. The experiment was performed in 3 replications [31]. The anti-radical amount of essential oil was calculated according to the following formula:

$$I\ (\%) = 100 \times (A_{blank} - A_{sample} / A_{blank})$$

A sample: The light absorption of the sample, A blank: The light absorption of the control, and I: Activity of radical reception (%)

$EC_{50}$ was used to better evaluate the anti-radical activity. This factor represents the percentage of essential oil that was

able to neutralize 50% of the initial free radical DPPH in the environment. To calculate this factor, a graph of the relationship between the values of essential oil concentration and the percentage of remaining DPPH was drawn, and based on the obtained diagram, the concentration of essential oil was determined to neutralize 50% of free DPPH radicals.

*2.6 Statistical analysis*

The data on antibacterial activity were analyzed using two-way analysis of variance *(*ANOVA*)* and mean values were compared by Duncan's multiple range method using SAS 9.1 software [32].

## 3. Results

*3.1 Chemical composition*

The composition of essential oil extracted from *Adiantum capillus-veneris* using hydro distillation technique was detected with gas chromatography-mass spectrometry. A total of 55 combinations of essential oil were presented in the table1. The most compounds in the essential oil of Adiantum capillus-veneris were carvone (33.00%), carvacrol (15.05%), hexadecanoic acid (7.02%), hexahydrofarnesyl acetone (4.25%) and n-nonanal (4.20%) (Table 1 and Figure 2).

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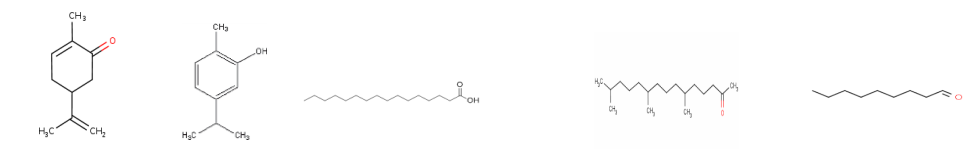
**Table 1**. The chemical compound of *Adiantum capillus-veneris* essential oil

| Number | Compounds | KI | HD (%) | Number | Compounds | KI | HD (%) |
|---|---|---|---|---|---|---|---|
| 1 | 4-octen-3one | 956 | 0.14 | 29 | Dodecanal | 1384 | 0.19 |
| 2 | 1- octen- 3ol | 961 | 0.08 | 30 | N-tetradecane | 1403 | 0.70 |
| 3 | N-octanal | 992 | 0.30 | 31 | Alpha- Ionone | 1407 | 0.22 |
| 4 | 1,8-cineol | 1028 | 0.22 | 32 | (Z)- beta-farnesene | 1450 | 0.21 |
| 5 | Alpha-limonene | 1032 | 0.04 | 33 | Alpha-santalene | 1461 | 0.15 |
| 6 | 2-octenal (E) | 1034 | 0.14 | 34 | Beta-ionone | 1462 | 0.92 |
| 7 | 2-octen-1-ol | 1045 | 0.16 | 35 | Alpha-curcumene | 1473 | 0.31 |
| 8 | 1-octanol | 1060 | 0.25 | 36 | E-2-tridecenal | 1477 | 0.12 |
| 9 | N-nonanal | 1082 | 4.20 | 37 | Alpha-murolene | 1499 | 0.26 |
| 10 | L- linalool | 1102 | 0.79 | 38 | N-pentadecane | 1502 | 2.20 |
| 11 | Camphor | 1126 | 0.22 | 39 | Calamenene | 1515 | 0.21 |
| 12 | Menthone | 1152 | 0.32 | 40 | Delta-cadinene | 1520 | 0.18 |
| 13 | Borneol | 1155 | 0.11 | 41 | Endo-1-bourbonanol | 1538 | 0.3 |
| 14 | Nonanol | 1156 | 0.17 | 42 | Caryophyllenyl alcohol | 1566 | 0.52 |
| 15 | Terpineol-4 | 1168 | 2.08 | 43 | Cubenol | 1623 | 0.10 |
| 16 | Myrtenal | 1168 | 0.19 | 44 | Beta- tumerone | 1634 | 0.12 |
| 17 | Cis-dihydrocarvone | 1206 | 0.91 | 45 | Cadalin | 1656 | 0.21 |
| 18 | Cuminic aldehyde | 1221 | 0.89 | 46 | Jatamansone | 1657 | 0.14 |
| 19 | Carvone | 1222 | 33.00 | 47 | 2-pentadecanone | 1674 | 0.10 |
| 20 | Nerol | 1226 | 0.14 | 48 | Hexadecanal | 1695 | 0.25 |
| 21 | Trans-anethol | 1261 | 2.85 | 49 | Heptadecane | 1702 | 0.70 |
| 22 | Thymol | 1268 | 2.95 | 50 | Octadecane | 1803 | 0.05 |
| 23 | Carvacrol | 1278 | 15.05 | 51 | Hexahydrofarnesyl acetone | 1834 | 4.25 |
| 24 | N-undecanal | 1282 | 0.12 | 52 | Hexadecanoic acid | 1919 | 7.02 |
| 25 | 2,4-decadienal | 1285 | 0.39 | 53 | Phytol | 2106 | 3.50 |
| 26 | Alpha-terpinyl acetate | 1333 | 0.41 | 54 | Linoleic acid | 2087 | 0.89 |
| 27 | Beta- bourbonene | 1380 | 0.30 | 55 | Oleic acid | 2096 | 0.91 |
| 28 | Delta-selinene | 1384 | 0.50 | | | | |

KI: Kovats index, HD: Hydrodistillation



Carvone Carvacrol Hexadecanoic acid Hexahydrofarnesyl acetone N-nonanal

**Figure 2.** The structure of most chemical compounds measured in *Adiantum capillus-veneris* essential oil

3.2 *Anti-microbial activity*

The antibacterial activity of essential oils of *Adiantum capillus-veneris* was calculated (mg/ml), and the total number of organisms tested *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Diphtheroid* were exhibited in Table 2 and Figure 3. The highest antibacterial activity was determined by *Staphylococcus aureus* (8.23±0.68), *Streptococcus pyogenes* (12.46±0.31), and *Diphtheroid* (11.37±1.02) at a concentration of 100 mg/ml of essential oils. With increasing the amount of essential oil, the antibacterial properties of the extracts had an increasing trend. The diameter of the inhibition zone of *Adiantum capillus-veneris* L. on *Streptococcus pyogenes* and *Diphtheroid* was highly significant (P<0.01). With the increase in essential oil consumption (100 mg/ml), the largest diameter of the inhibition zone was related to *Streptococcus pyogenes*, followed by *Diphtheroid* and *Staphylococcus aureus*. The effect of the extract on increasing the inhibition zone diameter grew with increasing the concentration of essential oil.

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**Table2.** Antibacterial activity of *Adiantum capillus-veneris* L. essential oils

| Microorganism | Essential oil levels (mg/ml) | | |
|---|---|---|---|
| | 25 | 50 | 100 |
| *Staphylococcus aureus* | * | $6.35\pm0.97^{cB(B)}$ | $8.23\pm0.68^{bA(B)}$ |
| *Streptococcus pyogenes* | $8.05\pm0.92^{bB(A)}$ | $9.11\pm0.85^{bB(A)}$ | $12.46\pm0.31^{aA(A)}$ |
| *Diphtheroid* | $6.17\pm0.20^{cC(B)}$ | $8.19\pm0.24^{bB(AB)}$ | $11.37\pm1.02^{aA(A)}$ |

[a-c]: Data with lowercase superscripts showing the comparison of all bacterial species in different doses of *Adiantum capillus-veneris* L. essential oil (P<0.01). [A-C]: Data with superscripts in capital letters showing the comparison of all different doses of *Adiantum capillus-veneris* L. essential oil in each row (P<0.01). [(A-B)]: Data with superscript capital letters in parentheses, expressing the comparison of all bacterial species in each column of *Adiantum capillus-veneris* L. essential oil (P<0.01).

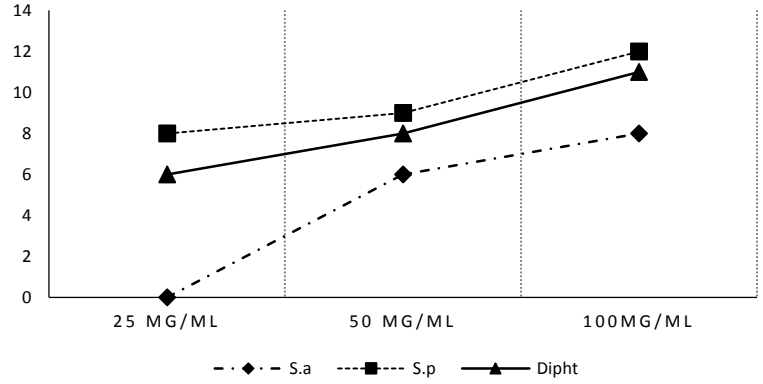


**Figure 3.** The antimicrobial activity (mg/ml) of *Adiantum capillus-veneris* L. essential oil

*S.a*: *Staphylococcus aureus, S.p: Streptococcus pyogenes, Dipht: Diphtheroid*

*[a-c]: Means with different superscript letters have significant differences (P<0.01).*

*Adiantum capillus-veneris* L. essential oil had the greatest effect on *Streptococcus pyogenes* in all doses, and the lowest effect of 25 and 50 mg/ml essential oil was recorded on *Diphtheroid* and *Staphylococcus aureus*, respectively.

*3.2 Antioxidant activity*

Compounds with antioxidant properties play an essential role in the body's defense system against reactive oxygen species (ROS). ROSs may cause early aging, cancer, and cardiovascular diseases. The antioxidant defense system plays a role in suppressing or dampening the effects of ROSs. When elements of the antioxidant defense system produced by the body cannot adequately reduce the effects of ROSs, supplementary natural antioxidants may be taken. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of many diseases. The increased intake of dietary antioxidants may help support the process of limiting antioxidant concentrates and also may support the normal functioning of various physiological systems. The antioxidant properties, of the essential oil of *Adiantum capillus-veneris* L., were investigated. The level of antioxidant activity of *Adiantum capillus-veneris* L. essential oil is shown in Table 3. In this experiment, the antioxidant activity of essential oil was estimated at 44.00±1.00 mg/ml.

**Table3.** Antioxidant activity of *Adiantum capillus-veneris* L. essential oil

| DPPH test, IC50 (mg/ml) | Hydro distillation method |
|---|---|
| *Adiantum capillus-veneris* | 44.00 ±1.00 |

## 4. Discussion

The latest studies conducted regarding the antimicrobial effects of medicinal plants show the increasing tendency of people to use these plants in terms of their low side effects compared to chemical drugs. Historical documents show that the use of plants to treat various diseases has been of interest to people since the distant past. The inhibitory effect of the whole extract of *Adiantum capillus-veneris* L. plant was shown in the form of a lack of growth, which was accurately measured with a caliper. According to the theory, the diameter of the inhibition zone of lack of growth is a function of the concentration of the effective substance in the plant. This phenomenon is a linear relationship between the size of the inhibition zone and the logarithm of the concentration of the substance under test. By measuring the diameter of the inhibition zone of non-growth and comparing it with a specific standard, the antimicrobial power of the substance under test is determined [16, 17, 33, and 34].

The results obtained from the drop plate method, which is an acceptable qualitative method, showed the inhibitory effects of the whole plant extract on bacteria so it was very helpful in the next repetitions and the use of quantitative methods [17]. Although the qualitative drop plate method in this study was a qualitative investigation of the antimicrobial effect of the whole *Adiantum capillus-veneris* L. extract, the results were considered a screening study for the experiments of the next stages. Various researchers in Iran and other countries have conducted much research on the bactericidal and bacteriostatic effects of *Adiantum capillus-veneris* L. and have proven the antibacterial effects of this plant. In 2002, the anti-bacterial effect of the active oils of the leaves of the *Adiantum capillus-veneris* L. on Klebsiella pneumoniae, species of *Pseudomonas, Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus pyogenes* was investigated using the cylinder plate method, and the most inhibitory effect of the leaves of the *Adiantum capillus-veneris* L. was respectively *Salmonlatifi*, *Pseudomonas*, *Klebsiella pneumoniae* and *Streptococcus pyogenes* species were obtained [35]. In another study conducted in 1989, the antibacterial effect of the methanolic extract of the aerial parts of *Adiantum capillus-veneris* L. on *Bacillus subtilis*, *Escherichia coli* (consistent with the findings of the upcoming study), *Staphylococcus aureus*, *Proteus vulgaris*, and *Candida albicans* was evaluated *in vitro* and the antimicrobial effect of this plant was proven. Despite the traditional uses of *Adiantum capillus-veneris* L., especially its positive effect as an antitussive and expectorant, *etc*., complete clinical research has not yet been done on this valuable plant. Therefore, it is necessary to investigate its various biological and therapeutic effects scientifically.

The comparison of the results obtained from the present study with the study conducted on the extract of *Adiantum capillus-veneris* L. in 1989 on *Escherichia coli* and *Staphylococcus* showed a completely similar effect. Carvone ($C_{10}H_{140}$) has a monoterpene group with a ketone functional group that is part of a family of chemicals called terpenoids. Based on a pre-clinical experimental study, this substance has a statistically significant sedative effect against increased excitement [13, 36, and 37]. Carvacrol is a monoterpene phenolic compound with a structure of 5-isopropyl -2-methyl phenol and a chemical formula of $C_{10}H_{14O}$ [38]. Carvacrol is an isomer of thymol and smells like them. These substances are present in the structure of edible oils of plants such as thyme, oregano, and savory, which are used as food seasoning [39, 40]. Researchers' studies showed that carvacrol was small, a lipophilic molecule that can easily cross the blood-brain barrier and exert its effects on different parts of the brain [41, 42]. The results of *in-vitro* and *in-vivo* studies also showed that carvacrol has various biological and medicinal properties such as antioxidant, antibacterial, antifungal, anti-cancer, anti-inflammatory, hepatoprotection, antispasmodic, and vasodilator [43]. Therefore, due to the biological and medicinal importance of carvacrol, the focus of this study is on evaluating the existing knowledge about the protective and medicinal effects of carvacrol as the main constituents of *Adiantum capillus-veneris* on various organs of the body in different disease models. Hexadecanoic acid or Palmitic acid with the chemical formula of $C_{16}H_{32}O_2$ is a saturated fatty acid [44]. This acid is the most common saturated fatty acid found in animals, plants, and microorganisms. Hexadecanoic acid is produced during the process of lipogenesis, or the process by which acetyl coenzyme A is converted to fat [45]. Palmitate is fed on acetyl-coenzyme A carboxylase. Extensive activity and non-specific action of fatty acids make them safe as antibacterial agents of interest for various applications in medicine, agriculture, and preservation of suitable foods, especially their use instead of conventional antibiotics is undesirable or prohibited [46]. Hexahydrofarnesyl acetone ($C_{18}H_{36}O$) is a cescaine terpene isolated from the essential oil of *Adiantum capillus-veneris* [47]. Hexahydrofarencyl acetone has antibacterial, analgesic, and anti-inflammatory activities [48]. The 5$^{th}$ compound and the highest amount of *Adiantum capillus-veneris* essential oil was n-nonanal. N-nonanal has a strong odor and at low dilution, it smells like roses and citrus. It may be synthesized by catalytic oxidation of the corresponding alcohol or by reduction of the corresponding acid [49].

According to these results, it seems that the antibacterial effects of *Adiantum capillus-veneris* L. are related to the presence of flavonoid active substances such as Rutin and Isocrates in the plant [50-52]. Therefore, purification of the extract and determination of active ingredients and different fractions of these plants is necessary and can be an effective step towards the supply of medicinal products that have antimicrobial properties. Although the present experiment has been performed *in-vitro* and on solid culture media, due to the acceptable results obtained, these findings seem to be a

very good basis for further *in-vivo* studies. Carvone, Carvacrol and Hexahydrofarnesyl acetone in the essential oil have antibacterial properties [13, 42, and 46]. Carvacrol employs a wide range of antimicrobial activity against gram-positive and gram-negative bacteria. Carvacrol is distributed in the membranes to make them permeable, thereby disrupting the ion gradient. In fact, measuring the mean phase transition temperature of bacterial lipids demonstrated that the membranes immediately become more fluid in the presence of carvacrol. The presence of a free hydroxyl group and a non-local electron system is vital for antibacterial activity. It has been suggested that the carvacrol structure of this compound allows it to act as a membrane carrier of monovalent cations by exchanging its hydroxyl proton for potassium ions, thereby lessening the slope of the cytoplasmic membrane. Accordingly, carvacrol has been shown to decrease cytoplasmic membrane potential, lower intracellular pH, inhibit ATP synthesis and stimulate potassium ($K^+$) leakage. $K^+$ acts as a cytoplasmic signaling molecule, activating and generating enzymes and transport systems that allow the cell to adapt to high osmolarity. Disruption of the proton drive and depletion of the ATP reservoir eventually leads to bacterial cell death [52, 53].

Plant extracts due to their phenolic compounds have antioxidant activity and a high capacity to donate hydrogen atoms or electrons and free electrons. The use of DPPH is one of the valid, accurate, easy, and cost-effective methods with high reproducibility that is used to investigate the antioxidant properties of essential oils and plant extracts *in-vitro*. As the concentration or degree of hydroxylation of phenolic compounds increases, the free radical scavenger activity of DPPH increases, which is considered as antioxidant activity [53]. Therefore, in this test, the antioxidant activity of the extracts is expressed in terms of the percentage of reduced light absorption of DPPH solutions in the presence of phenolic extracts compared to the solution without extract. Studies showed that high phenolic compounds can be a major reason for the high antioxidant activity of some extracts, including polar extracts [54-57]. Phenolic compounds, which are widely found in plants and have high antioxidant activity, can be extracted mostly through their plant extracts. The solubility of phenolic compounds varies with other compounds in plant tissues depending on the type of solvent, the degree of polymerization, and their interaction. The use of water as the extraction solvent creates a highly polar environment in which some low-polarity phenolic compounds are extracted to a lesser extent. Extraction of plant essential oil due to the use of water solvent produces less phenolic compounds compared to ethanol and methanol. Carvacrol was found to have influential antioxidant activity parallel to Trolox. However, in incubations where $V_{79}$ cells were exposed to $H_2O_2$, carvacrol was less efficient in sequestering ROS at lower concentrations (1–25 μM) and even improved ROS production at the highest 100 μM concentration [52, 58].

## 5. Conclusion

The use of antibiotics has created bacterial resistance that has threatened the lives of living organisms. So the search for a new alternative is inevitable. One of the current problems in treating bacterial infections is increasing their resistance to antibiotics. Antibiotic-resistant bacteria cause significant mortality compared to non-resistant bacteria. Among the gram-negative bacteria resistant to antibiotics that cause nosocomial infections are *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter*, and among the gram-positive bacteria are *Staphylococcus*, *Streptococcus*, and *Enterococcus* [59-62]. *Adiantum* species have been the subject of several pharmacological studies, selected for their various ethnomedicinal uses. Research demonstrates that these species perform a wide range of biological functions; they can be used in wound healing and to reduce fertility, and they exhibit antimicrobial, antidiabetic, nephroprotective, and hepatoprotective properties. In a study using extracts of lavender, eucalyptus, thistle, and *Adiantum capillus-veneris*, significant antibacterial properties were observed against bacteria. The inhibitory effect of the complete extract of *Adiantum capillus-veneris* is in the form of a disk of inadequate growth, which is accurately measured with a caliper. Theoretically, the diameter of the growth inhibition zone is a reaction to the concentration of the active substance in the plant. This phenomenon is a linear relationship between the disk size and the logarithm of the concentration of the test substance, which is determined by measuring the diameter of the non-growth halo and comparing it with a specific standard, the antimicrobial power of the test substance [54].

Like regular medicines, herbal remedies can affect the body and can be harmful if not used properly. Therefore, they must be used carefully and sensitively, similar to conventional drugs. Other medications may cause problems with these medications, and sometimes herbal remedies may reduce or increase the effects of the medication, including potential side effects. Herbal products are usually intended for conditions such as coughs, colds, or general aches and pains that can only be used for self-medication and do not require medical supervision. However, due to the lack of antibiotic resistance in bacteria and their lower cost, study and research on them are recommended for further understanding.

## Conflicts of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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