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Original Research Paper

Antibacterial and antioxidant activity different extract of Thyme (*Thymus vulgaris* L.) and Sage (*Salvia officinalis* L .)

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Abstract: The current study was conducted during (2024-2025) to determine antibacterial activity and antioxidant activity of different organic solvents extract of Salvia offcinalis L. (Sage) and Thymus vulgaris L.(Thyme). Five solvents were used (petroleum ether, Hexane, Acetone, Ethyl acetate and Ethanol) to extract dried leaves of Sage and Thyme individually using soxhlet apparatus at different concentrations.
Results: showed that S.aureus was more sensitive to plant extract than E.coli .Highest inhibition growth zone produced in both (ethyl acetate and petroleum ether. In general there is increase in growth inhibition zone with increase plant extract concentration compared to control. For antibiotic sensitivity test, both examined bacterial strains are resistant to most antibiotics. Antioxidant activity was studied using DPPH (2,2-diphenyl-1-picrylhydrazyl) method . Results showed that Sage proceed Thyme in its scavenging for free radicals .Ethanol extract gave highest value for antioxidant activity. There is increase in extract scavenging activity with increasing plant extract concentration.

Keywords: Salvia offcinalis, Thymus vulgaris , Antioxidant , Antibacterial , DPPH, organic solvents

1. Introduction

Salvia offcinalis L. (Sage) ,in Arabic it is Meramea) and *Thymus vulgaris* L.(Thyme) , in Arabic it is called "Za`atar"[1,2] , both belong to Labiatae (Lamiaceae) family ,it possess many classes of secondary metabolites including flavonoids , fatty derivatives , sterols , essential oils , etc ,thus ,they show an excellent antioxidant, cytotoxic, anti-inflammatory, antibacterial, antifungal, antiviral, analgesic, cardiovascular, hypoglycemic, hypolipidemic, antispasmodic, antiepileptic, anti-anxiety and anti-angiogenic activities [3].

Sage, is initially derived from the Latin salvere (to save), pointing out to its healing powers recording a long history of uses in nutritional, culinary, pharmacological and medicinal purposes due to compounds content reported in it which seem to be responsible for its antimicrobial, antioxidant and pharmacological properties, including cytotoxicity against human cancer cells in addition to food preparation as bio-preservative against Gram+ and Gram- bacteria [4].

Thyme , is initially derives from the Greek word ‘thymos’ which means courage or strength,its one of The most popular wild edible plants (WEPs) , having many benefits ,it is rich in phytonutrients, minerals, vitamins, flavonoids and antioxidants. In addition, the therapeutic effects against various diseases, this is attributed to its multi-pharmacological properties that include antioxidant, anti-inflammatory actions. Moreover, it has long been known for its antiviral, antibacterial, antifungal, and antiseptic activities [5].

Medicinal plants are a promising source of antibiotics with possible activity on multidrug-resistant microorganisms , the potential of plant extracts as alternative sources of novel antibiotics against pathogens [6], in addition , in the last decade, studies on antioxidant activities of medicinal plants have increased remarkably due to increased interest of the healthy food

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industry in their potential of being used as a rich and natural source of antioxidants [7].

2. Methodology

Leaves of Salvia offcinalis (Iranian) and Thymus vulgaris (Syrian) was provided from local markets ,dried leaves were used to prepare different organic solvent extract Plant samples (dried leaves illustrated in plant figures (1).

Fig.1: Dried leaves of 1- Iranian Salvia offcinalis (Sage) and Syrian Thymus vulgaris (Thyme)

Two bacterial species *Escherichia coli* (gram-negative) and *Staphylococcus aureus (gram -positive*) were used to conduct antibacterial activity.

Antibacterial activity

The bacterial suspension was prepared by culturing bacteria on nutrient agar and incubation for 24 h at 37 C°, colony were taken to test tube containing nutrient broth and incubation for 4-5 h in 37 C° , later comparing with McFarland tube to obtained suitable turbidity . The turbidity produced by growth culture was calibrated with sterilized broth to achieve an optical density comparable to the 0.5 McFarland requirements (1.5 X 108 cells/ml the equivalent).

Antibiotic sensitivity test

Testing sensitivity of bacteria to some antibiotic was done using disc diffusion method ,later measuring of inhibition zone to CLSI 2024 [8,9].

Plant extract activity test

Both different two plant extracts examined at four concentrations (0,2.5,5,10)mg /ml using well agar diffusion method by making equal well in Muller Hinton with diameter of 6mm by cork borer and adding of 0.1 ml from each extract , before this step , spreading of o.1 ml from bacterial suspension on surface of media ,after then incubation the plate over night in 37 C° and then measuring the diameter of inhibition zone to detect the activity of test plant extract on growth of bacteria using a ruller [10].

Antioxidant activity

Both different two plants extracts examined at five concentrations by dilution with methanol (0,0.25,0.55,1,2)mg/ml using DPPH (1,1 diphenyl-2-picrylhydrazyl) free radical scavenging assay . The DPPH radical cation method was performed according to .[11] . The DPPH reagent was DPPH (8 mg) dissolved in(methanol) MeOH (100 mL) for a solution concentration of 80 uL/mL. To determine the scavenging activity, 100 ml DPPH reagent was mixed with 100 ul of sample in a 96- well microplate and was incubated at room temperature for 30min. After incubation, the absorbance was measured 514 nm using an ELISA reader, and 100% methanol was used as a control. The DPPH scavenging effect was measured using the following formula:

Radical scavenging (%) = **[(*A*) control − (*A*) sample\ (*A*) control] ×100.** [12].

Statistical analysis

Data for both antibacterial and antioxidant activity was analyzed using SPSS ,version 22 .

3. Results and discussion

Results shown in table (1) indicates that examined bacterial strains are resistant to most antibiotics, thus it was ideal to examine plant extract against them .

Table 1: Antibiotic sensitivity test for bacterial strain , *S . aureus, E. coli, E.cloacae*

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Antibiotic	Bacteria strains	
	S.aureus	*E.coli*
CDR	R	R
Lev	R	S
TMP	R	R
NA	R	S
FOS	R	R
TE	R	R
NOR	R	S
F	S	R
CN	R	S
MEM	R	S
RA	S	R
PIT	S	S
CTX	R	R
CDR	R	R
P	R	
AMO	R	
CIP		R
IMP		R
MEM		R
ETP		R

Microorganisms can be either intrinsically resistant to an antibiotic or developed resistance following exposure to that antibiotic (acquired resistance), resistance can develop as a result of mutation or direct transfer of genes encoding a resistance mechanism [13]. Genetic material, including antibiotic resistance genes, can spread very effectively between bacteria, even those of unrelated species , antibiotic resistance can be considered to be an inevitable consequence of antibiotic use [14]. Resistance is a constant trait of a species, strain, or whole group of bacteria. A given microorganism is insensitive to an antibiotic due to its 'innate' resistance to certain groups of antibiotics. It may be linked to the absence of a receptor for the antibiotic, low affinity, cell wall impermeability, or enzyme production [15].The results of this systematic study have shown that antibiotic-resistant bacteria, or so-called (MDA), have become resistant to multiple drugs because they possess mechanisms and characteristics through which they can survive despite their exposure to antibiotics. These bacteria are primarily characterized by the properties of the plasma membrane of the bacterial cell. Most types of resistant bacteria prevent the antibiotic is unable to reach the bacterial cell due to the inability of the antibiotic to destroy or penetrate the plasma membrane of the bacterial cell in general. This may occur as a result of hereditary genetic changes resulting from mutations that lead to a change in the nature of the membrane. This change occurs continuously, which always leads to failure. After using the antibiotic successfully for a certain period of time against antibiotic-resistant bacteria [16].

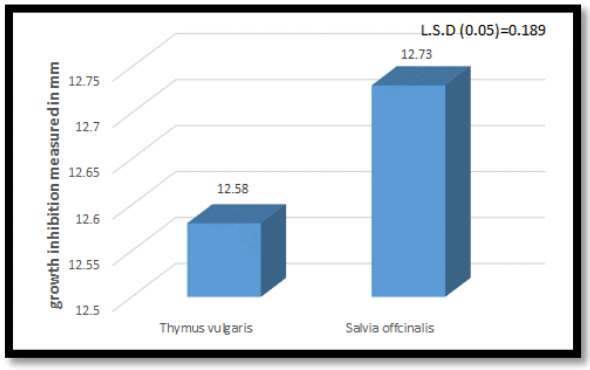

Fig 2. Effect of plant species (*T.vulgaris and S. offcinalis*) on bacterial growth inhibition measured in mm

Results indicates that there was no significant difference between T.vulgaris and S.. offcinalis in there on inhibition bacterial growth (12.58 and 12.73)mm respectively (figure 2). Thyme extracts were examined against a group of bacterial strains in which plant inhibit bacterial growth [17].

Another findings indicate the potential of thyme crude extract as a natural antibacterial agent with broad-spectrum activity against various pathogenic bacteria [18].

Sage proved its antibacterial activity against number of gram positive bacteria and , approved plant ability as antibacterial agent .These pharmaceutical effect of two plants may related to their content of phenol and flavonoids . .[19,20] The most well-known members of Lamiaceae family including thyme and sage, are high content of volatile essential oils, nonvolatile bioactive metabolites [21]. A study conclude that these two plants can be considered as a suitable option for

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treating infections caused by pathogenic bacteria and helping to return the sensitivity of antibiotics [22].

Variation in activity between two plants may related to their various content of phytochemicals and their derivatives , sage rich in polyphenols including phenolics acids and flavonoids , essential oils , alkaloids, glycosidic ,derivatives , flavonoid glycosides, saponins, tannins, terpenes/terpenoids and waxes [23,24,25].

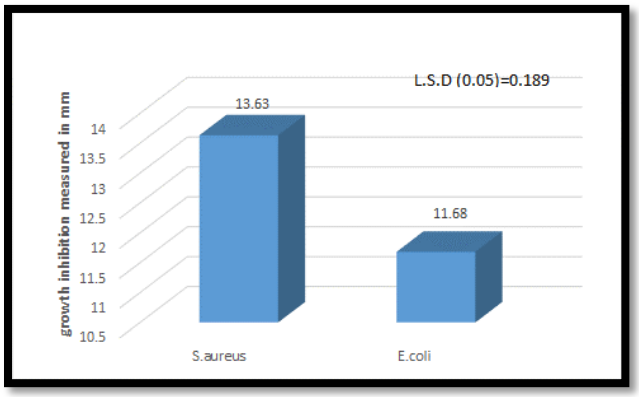

Fig 3. Effect of bacterial strain (*S.aureus and E.coli*) in bacterial growth inhibition measured in mm

Thyme major chemical constituent included, saponins , tannins, flavonoids, alkaloid , terpenoids , monoterpene phenolic 26 the major compounds were thymol and o-cymene, with the major components being oxygenated monoterpenes and monoterpene hydrocarbons [26,27].

Results illustrates that bacteria *S.aureus* was more sensitive to plant extract than E.coli since there growth inhibition 13.63 mm was more than E.coli 11.68mm (figure3) .Inhibitory concentrations of various plant extracts affect the virulence of both gram positive and gram negative bacteria [28,29].

Bacteria used efflux pumps as a mechanism to develop resistance to antibiotics so interfering with the functions of these efflux pumps would be an interesting therapy to control infectious diseases, besides inhibiting the function of efflux pumps, various plant extracts affect the virulence factors of bacteria with altering the quorum sensing (QS) gene expression [30].

Staphylococcus sp, possess lipophilic nature relative to the membrane and their planar structure, which to penetrating the cell wall, phytochemical compounds interact with the membrane, causing deformation and destruction of the basic structure of the bacterial membrane, thus allowing the entry of antibiotics into the bacterial cells,causing distortion or distortion of the bacterial DNA,this may be due to their combination with the nitrogenous bases that make up the bacterial DNA, phenolic acid in plant extract contain a number of natural acids that have clear activity against pathogenic bacteria , these acids lead to a change in the pH or hydrogen environment, in addition to the fact that these acids dissolve the phosphorylated fats in the wall of pathogenic bacteria, causing them to be inhibited and killed [31].

Results in figure (4) indicate that highest inhibition growth zone produced in both (ethyl acetate and petroleum ether (13.31 and 13.69/0mm (not different significantly between them). Lower value produced by ethanol ,hexane and acetone (11.75 ,12.13 and 13.69)mm (not differ significantly among each other).

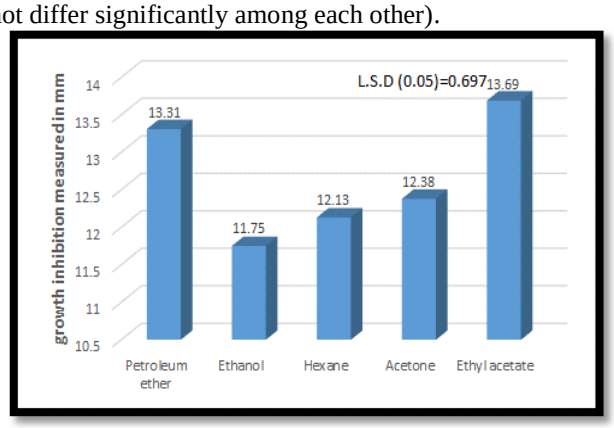

Fig 4. Effect of solvent type (Petroleum ether , Ethanol, Hexane, Acetone, Ethyl acetate) on bacterial growth inhibition measured in mm

All examined solvent extract are able to inhibit bacterial growth. Variation among solvents (polar and non polar) in antibacterial activity differ by differing of mechanism they follow in their effects , polar solvent as ethyl acetate is able to form a hydrogen bond with the phytochemical compounds; thus increasing solubility in it [32]. The extraction of active ingredient compounds from plant material depends on the type of solvent used in the extraction procedure which in turn affect its activity [33].

Acetone thyme extract approved as antibacterial agent since reported its high content of total phenolics and total flavonoids , polyphenols may exhibit an important

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antimicrobial activity, the mechanisms of which have not been completely recognized yet [34]. The known mechanisms include the capability for modifying cell membranes permeability, the changes in several intracellular functions caused by the phenolics to enzymes binding or by the loss of the cell wall integrity due to various interactions with the cell membrane [23]. Petroleum ether as non polar solvent can dissolve alkaloids, glycosides and steroids, which all reported as powerful antibacterial agent [35].

A study reported that varying solvent result in varying type and quantity of phytochemicals [36].

The inhibition action of alcohol extract is due to it contained Thymol, flavonoids, tannins, and include some of phenolic compounds which have a biological influence on many bacteria races due to the presence of hydroxyl groups (-OH-), as it have the ability to form hydrogen bonds between hydroxyl group in these compounds and water molecules in bacterial cell, were water is (90%) of weight and that will disables dynamic actions in bacterial cell . These compounds as phenolic compounds have the ability to coagulation the bacterial cell proteins and destroy enzymes involved in the manufacture of necessary amino acids to increase cell division [37].

Results in figure (5) indicate that in general there is increase in growth inhibition zone with increase plant extract concentration compared to control , highest value was 20.5mm in 10mg/ml and lowest value was 13.1mmin 2.5mg/ml . Inhibitory concentrations of various plant extracts affect the virulence of both gram positive and gram negative bacteria [28,29]. Bacteria used efflux pumps as a mechanism to develop resistance to antibiotics so interfering with the functions of these efflux pumps would be an interesting therapy to control infectious diseases, besides inhibiting the function of efflux pumps, various plant extracts affect the virulence factors of bacteria with altering the quorum sensing (QS) gene expression [30].

Increasing inhibition diameter of plant extract against examined pathogens with increasing extract concentration is varying due to rate of active materials in different concentration [37].

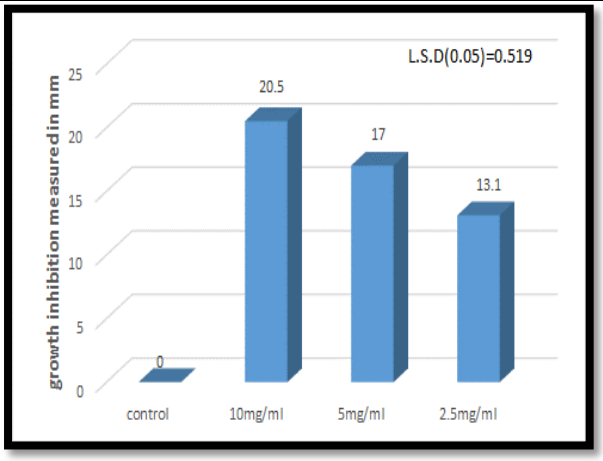

Fig 5: effect plant extract concentration (0,2.5,5,10) mg/ml on bacterial growth inhibition measured in mm

Antioxidant activity using DPPH method

Results in figure (6) showed that Sage proceed Thyme in its scavenging for free radicals reached 72.414 and 71.072 respectively .

Generaly, plant antioxidant activity is related to extract ability in preventing an autoxidation process of other compounds or neutralize free radicals .[38].Different mechanisms suggested for antioxidant activity ,it may be due to, such as prevention of chain initiation, peroxide decomposition, prevention of continuous hydrogen abstraction, free radical trapping, reduction capacity and binding of transition metal ion catalysts [39]. It was established Sage and Thyme as powerful antioxidant agents due to their high content of phenolics [21,40]. Efficiency of Sage as powerful antioxidant proved by their results related strongly to its content of phenols and flavonoids [41].

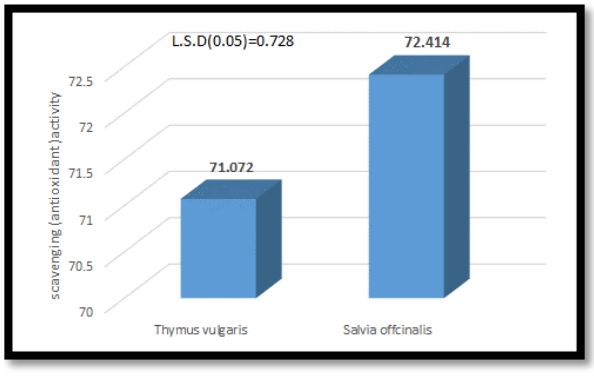

Fig 6. Effect of plant species (*Thymus vulgaris* and *Salvia offcinalis*) on antioxidant (scavenging activity)using DPPH method

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Results in figure (7) showed that ethanol extract gave highest value for antioxidant activity 68.425 while lowest value produced by hexane 53.341.

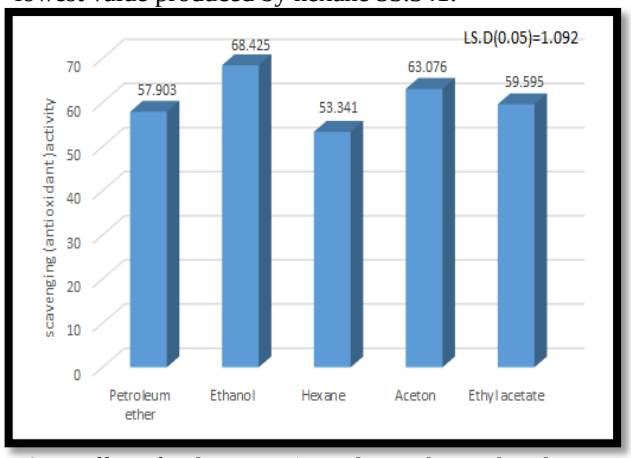

Fig 7. Effect of solvent type(Petroleum ether, Ethanol , Hexan , Aceton and Ethyl acetate) on extract antioxidant (scavenging activity)using DPPH method

A study conducted indicated that increasing solvent polarity positively increase its ability to dissolve phenols which consider powerful antioxidant and their results agreed with our results in proceeding ethanol over hexane in anti oxidant activity [42].

The Phenolic chemicals from plants are also well known to perform a significant function in stabilizing lipids metabolism against peroxidation and inhibiting various types of oxidizing enzymes. The differences in the structure and function relationship of flavonoid structures and their substitutions influence the phenoxyl radical stability, thus affecting the antioxidant properties of the flavonoids [35]. Hydrogen peroxide naturally occurs at low concentrations in the human body, air, water, plants, food and microorganisms .It is promptly decomposed into water and oxygen, which may then produce hydroxyl radicals (OH), capable of initiating lipid peroxidation and DNA damage. Therefore, the observed effect of the ethanol efficiently scavenging hydrogen peroxide may be attributed to the presence of phenolic groups that donate electrons to hydrogen peroxide, thereby neutralizing it into water [43].

It was reported reported that varying solvent result in varying type and quantity of phytochemicals [36].

A study proved ethanol as solvent examined for antioxidant activity due to its ability to dissolve polyphenols and flavonoids [21].

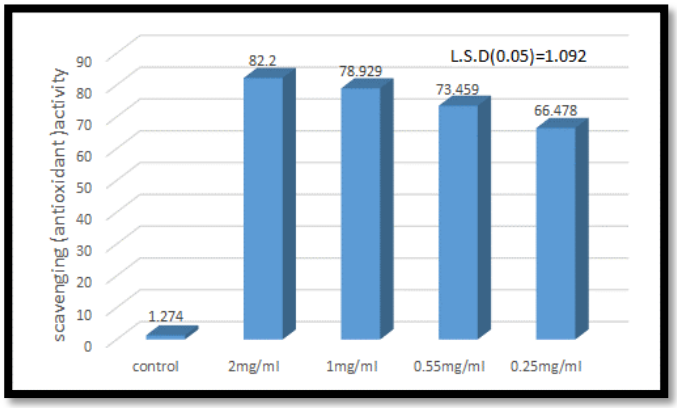

Fig 8. Effect of extract concentrations (0,2,1,0.55,0.25)mg/ml on antioxidant (scavenging activity)using DPPH method

In general , in figure (8) there is increase in extract scavenging activity with increasing concentration , highest value was 82.2 at concentration of 2mg/ml and lowest value was 66.478 at concentration of 0.25mg/ml compared to control treatment 1.274.

Since phenols and flavonoids are major antioxidant constituent [44], their concentration will increase as plant extract concentration increases , previous study established that the highest concentration of phenolic compounds in the extracts especially due to using solvents of high polarity result in high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity [45]. Concentration dependent activity established by established by [46].

Conclusion

Antioxidant activity was studied using DPPH (2,2-diphenyl-1-picrylhydrazyl) method . Results showed that Sage proceed Thyme in its scavenging for free radicals .Ethanol extract gave highest value for antioxidant activity . There is increase in extract scavenging activity with increasing plant extract concentration.

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