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Research Article

An in Vitro Detection of Antibacterial Inhibitory Effect of Aqueous Extract of Melissa Officinalis and Plantago Ovate

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Abstract:
Background: There is an increment in the public alarm about hygiene that stimulates many works and researches to inspect and improve different antimicrobial agents. However, the applications of various antimicrobial reagents must be taperedbecause of their possible harmful or toxic effects. Also, the usage of plants materials as an alternative of chemical or synthetic drugs is increased in last years, in order to reduce unwanted effects of these antibiotics. WHO informed that about 80% of population around the world still trusting on plants and their extracts as sources for principal health care, despite the fact traditional medication is the only available health source for more than 60% of the world'spopulation?
Aim: to evaluate the inhibitory effects of *Melissa officinalis* and *Plantago ovata* aqueous extracts, as growth inhibitors and /or inhibition of other physiological behaviors as surface adhesion and biofilm formation.
Material and Methods: Aqueous extracts of the mentioned plants were applied to examine their effects for inhibition of growth and some physiological parameters as adhesion and biofilm-formation against 19 bacterial isolates (both positive and negative clinical isolates). Agar wells-diffusion method, biofilm inhibition test by the using of tissue culture plate method and adherence inhibition assay were done for the estimation and evaluation the inhibitory effects.
Results:aquatic extract of*Melissa officinalis*expressing significant antibacterial inhibitory activity against Gram positive more than Gram negative bacteria, especially against *Strep. faecalis* with an inhibition zone 36mm**.**the aquatic extracts of *Melissa officinalis* gave high inhibitory effects for adhesion and biofilm formation against all the tested bacteria; in comparison with the inhibitory effect the extract of*Plantago ovata.*
Conclusion: Medicinal plants act as therapeutic agents that inhibit the growth of pathogens considered as potential antimicrobial compounds, as these compounds are cheap, available and broadly-effective against wide range of severe human pathogens, that causing dangerous diseases and complications.

Keywords:*Melissa officinalis*, *Plantago ovata*, inhibitory effect, biofilm, aqueous extracts.

1.Introduction:

Plants are considered as bulky reservoirs of various and vital secondary metabolites and they are reallyeffective sources of many pharmaceutical constituentsthatencompass active ingredient substances in one or some of their body parts[1]. Each plant extracthas multiple targets inside thecells, thus it is difficult to a considerthat their antibacterial effects are a result of single mechanism but it depends on the number of chemical compounds in every plant extracts. Moreover, these effective mechanisms are acted collectively not separately, sometimes some of these are affected positively or negatively by others [2].Most of the populations around the world are using herbal constituentsbecause of their robust antimicrobial activities and chief healthcare aids and the already-used antibiotic drugs can be substituted with biological materials as herbal extracts medicines with high antioxidant, antiviral, and antimicrobialactivities[3].

Plant-derived medicines are the principal medicine to treat various infections in manynations. These herbal materials extracts apply continuouslysignificantattempts to produce new biological compounds with major antibacterial effects. Variousworks hadrevealed that diverseplant-derivedmedicines are considered as sources of wide range of molecules, many of which exhibit radical scavenger against free radicals and antimicrobial featuresthat protect human against cellular oxidation reactions and pathogens [4].

Moreover, as a benefit of using naturally-producedmaterials as antibacterial compositesthat play a role in reducingeconomic losses and health hazards that may be produced by different pathogenic microorganisms, where various plant extracts are identified to be effective against a wide variety of pathogens, whetherGram-negative or even -positive bacteria [5].Thus, for these reasons different plants parts had been used to prepare natural extracts for emergingnovel antimicrobial drugs;since theyare effectiveagainst opportunistic pathogenic and pathogenic microbiota. Inhibitory antibacterial effects of the medicinal plants extracts is attributed to the composition and contentsof the plant essential oils, that consisting of large numbers of secondary metabolites, asflavonoids, tannins, and alkaloids [6,7].

Herbsantimicrobial activities depend on their contents and chemical composition, that is determined by the plant genotype and is influenced significantly by manyparameters, such as agronomical and environmental conditions and the geographical origin. Furthermore antibacterial activities depending on the type, compositions and concentrations of the herb or the active essential contents, also the type and concentrations of the herb or its effective essential oils, and the type and concentrations of the target pathogen, composition of the substrates; finally, the processing methods and the storage conditions [8, 9].

Bacterial adhesion to the body surfaces and medical device surfaces is the initial and limited step in the pathogenesis of many infectious diseases and implantation-related infections. As a traditional or alternative to methods that includebiocides or antibiotics that are released or infused, methods and new medical substances to be designed and to produce control material with criteria to decrease/control bacterial adhesion and thereby reduction of microbial infection are more attractive tactics[10]. Also, it is very important to develop new and safer antimicrobial agents able to prevent microbe's adhesion, proliferation and adhesion on materials surfaces, and reduce their negative effects. As biofilm allows bacteria to adhere irreversibly to a wide variety of surfaces whether living or non-living and make bacteria more resistant to many treatment and immunity agents leading to severely complicated infections[11].

1.1. *Melissa officinalis*

Melissa officinalis is one of the ancient and most traditional herbs. It is a memberof lamiaceae family and it is known as lemon balm. Lots ofresearches had been processed on this plant and its various properties were verified. The most public therapeutic applications are antiviral,antibacterial, anti-inflammatory, antispasmodic, antioxidant,sedative propertyand carminativecases [12].

Most of the pathogenic bacteria that involved in this study are considered as causative agents of many diseases, as UTI, burn, gastroenteritis, skin infections and other bacterial infections; the existence of antibiotic-resistant pathogens limited the availabilityof therapeutic choices for proper and effective treatment, additionally, bacterial resistance to antibiotic had led investigators to studyhow to replace effective antibiotics agents with lesser side effects instead of less effective antimicrobial agent with more side effects. Thus, the use of natural antimicrobial compounds, such as extracts of herbs, has become an interesting focus[13].

Mimica-Duki*et al*.[14]studiedboth the antibacterial and antioxidant activities of essential oil of lemon balm against thirteen bacterial types and six mold species. Also, he approved that affectivity andproper results of this substance were dose-dependent. Also, in 2005, Di pasqua and his collegues[15]showed that this plant is effective against *staphy.aureus*, pseudomonas,anthrococcus, andlactococcus.

1.2. *Plantago ovate*

*Plantago ovate*commonly isknown as Isabgol, it is a perennial herb, a member of family *Plantaginaceae*. It's extracts have a wide range of biological activities especially from its leaves, which includeanti-ulcerogenic, wound healing activity, immunomodulating, analgesic, antioxidant, weak antibiotic and anti-inflammatory activities[16], while Reddy *et al*. [17] showed that it is a weak anti-inflammatory and an effective antibacterial agent. Moreover, Karima *et al*.[18]revealed that it contains significant quantities of phenolic compounds and has a potential antibacterial and antioxidant activities with significant activities against multidrug–resistant microorganisms and can lead to new forms of treatment of infectious diseases.

Outer seed coat of plantagocontains 10-30% of hydrocolloid thatcan be separatedinto neutral and acidic polysaccharides and onhydrolysis can obtainD-galactose, L-arabinose, D-galacturonicacid, D-xylose and L-rhamnose.Plantago gum solution is thixotropic asits mucilage has a disintegrant action. Theouter seed coat is a white-rosy membranous cover of theseed, thatconsidered the drug part, that is natural product100%, and is used as asafe laxative, as in case ofchronic diarrhea, dysentery andhabitualconstipation;compose of soluble fibersto form gelin water. The usual dose of *Plantago ovata* is 7.5 g[19,20].

This work was aimed to evaluate the inhibitory effects of *Melissa officinalis* and *Plantago ovata* aqueous extracts, as growth inhibitors and /or inhibition of other physiological behaviors as surface adhesion and biofilm formation; since these parameters are important for bacterial pathogenicity and disease production.

2. Material and Methods

For preparation of aquatic extract from *Melissa officinalis*and *Plantago ovata*-related aquatic extracts according to [21,22].

2.1. Bacterial and Isolates

Collected clinical bacterial isolates (19 isolates) were used in thisstudy (listed at table (1)). Bacterial isolates were activated in three continual times on nutrient agar then stored at 4ºC as nutrient agar slant. The documentation of isolates were established by various biochemical tests [23].

Table 1: Gram-positive and-negative bacteria

Gram-negative bacteria	Gram-positivebacteria
Salmonella typhi	*Staphylococcus aureus*
Salmonellatyphimurum	*Staphylococcus epidermidis*
Pseudomonas aeroginosa	*Staphylococcus saprophyticus*
Pseudomonas flourscences	*Streptococcus pyogenes*
Proteus vulgaris	*Streptococcus pneumoniae*
Proteus merabilis	*Streptococcus mutanus*
Klebsiellapneumoniae	*Streptococcus feacalis*
Enterobacter aerugenes	*Streptococcusaglagtia*
Acinetobacter	
Escherichia coli	
Serratia spp.	

2.2. Antimicrobial activity test by Agar-well diffusion assay *(In vitro)*: [24, 22**].**
Antibacterial activity assay: According to Forbes [23], the antimicrobial activity was detected by agar-disc diffusion (the test were performed in triplicates).

2.3. Biofilm Formation Assay: Semi quantitative microtiter plate test or Tissue culture plate method assay (TCP) designated by Christensen *et al*. [25] was assumed as the gold standard method for detection of biofilm formation.

Table 2:Bacterial adherence and biofilm formation by method of TCP (Hindi *et al*[26]).

Mean of OD value at 630 nm	Adherence	Biofilm formation
0.120>	Non	Non
0.120-0.240	Moderate	Moderate
>0.240	Strong	High

2.4. Adherence test
Bacterial adherence to epithelial cell of the mouth is one of the chief and important virulence criterionof these bacteria and can be recognized using method designated by [27, 28].

2.5. Statistical analysis
Bonferroni test [29] was used to analyse data; as (P≤ 0.05) to show significant differencesbetween the types of extracts.

3. Result:

After completion of all work-experiments, the collected data were as following; regarding antibacterial effect of *Melissa officinalis* against Gram negative and positive bacteria; figure (1) showed thataquatic extract of*Melissa officinalis*expressing significant antibacterial inhibitory activity against Gram positive more than Gram negative bacteria, especially against *Strep. faecalis* with an inhibition zone 36mm, followed by inhibition zone of 35mm obtained by each of *Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus mutanus, Streptococcusaglagtia,* also obtained by *Proteus vulgaris, Acinetobacter* from Gram-negative bacteria. Moreover, lesser inhibition zone were obtained from *Proteus merabilis, Klebsiellapneumoniae,Salmonella typhi*and*Escherichia coli*with a range of 34-32mm progressively. In comparison to this, the lowest inhibition zone were achieved in relation to *Pseudomonas aeroginosa, Pseudomonas flourscences, Staphylococcus aureus* and *Serratia spp.* with inhibition zone of only 30mm for all. In spite of these ranged variable results that were accomplished by *Melissa officinalis*; Figure (2) showed antibacterial effects of *Plantago ovata* against the tested bacterial species but it was lower than that obtained by *Melissa officinalis*and with a range of only 17-25mm, as the *Pseudomonas aeroginosa*and *Strep. faecalis*were on the extremities respectively and the others were in between them.

Later on, only gram-negative bacteria were tested to examine the ability of the aquatic extract of the two plants in this study, and detect their ability to inhibit bacterial adhesion and biofilm formation. For that the results were of these tests were revealed in Table 3 and 4.

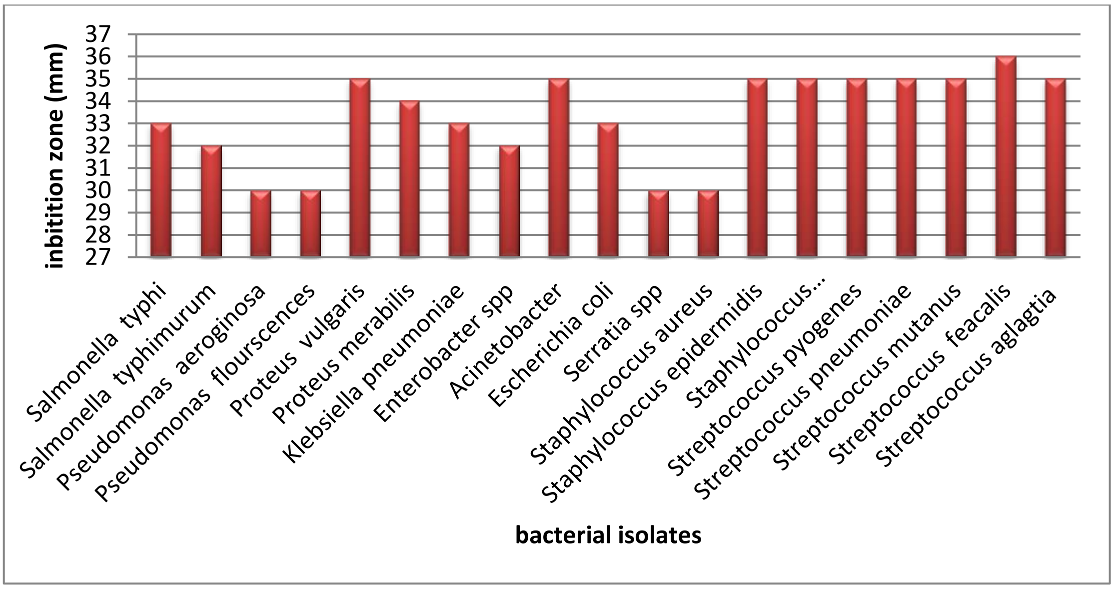

Figure 1 :Antibacterial effect of *Melissa officinalis* against Gram negative and positive bacteria by agar well method.

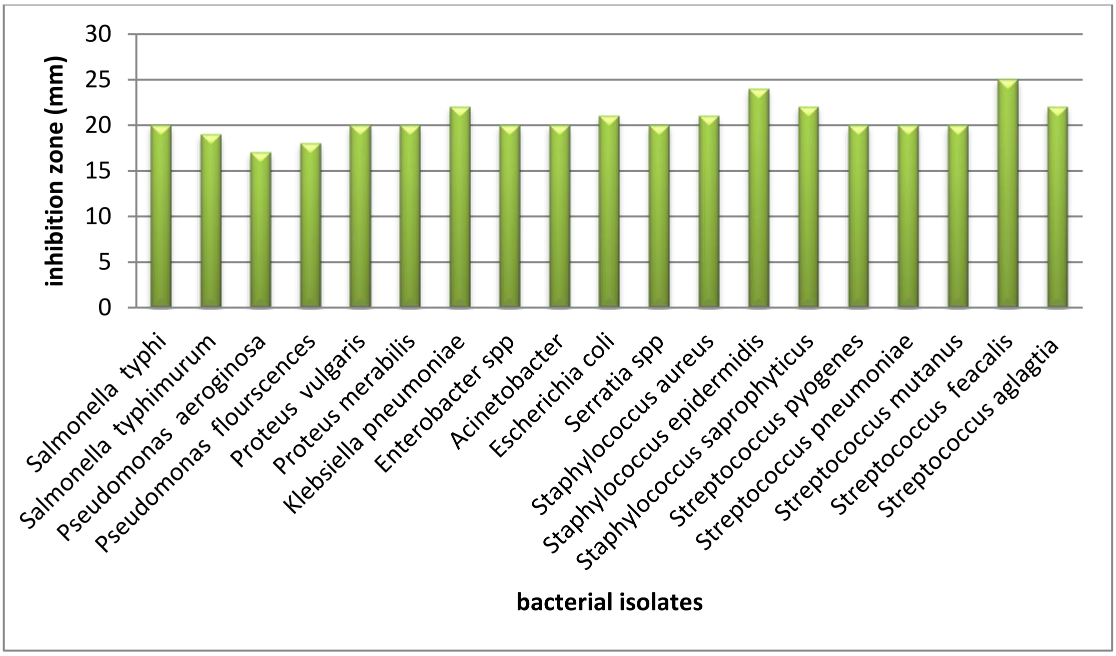

Figure 2: Antibacterial effect of*Plantago ovata* against Gram negative and positive bacteria by agar well method.

Table 3. Anti-adherence activity of aquatic extractsof *Melissa officinalis*, and*Plantago ovata* against Gram negative bacteria

Gram negative bacteria	*Melissa officinalis*	*Plantago ovata*
Salmonella typhi	High	Moderate
Salmonellatyphimurum	High	Moderate
Pseudomonas aeroginosa	High	Moderate
Pseudomonas flourscences	High	Moderate
Proteus vulgaris	High	Moderate
Proteus merabilis	High	Moderate
Klebsiellapneumoniae	High	Moderate
Enterobacter aerugenes	High	Moderate
Acinetobacter	High	Moderate
Escherichia coli	High	Moderate
Serratia spp.	High	Moderate

Table 4: Anti biofilm activity of aquatic extracts of *Melissa officinalis*and *Plantago ovata* against Gram negative bacteria.

Gram negative bacteria	*Melissa officinalis*	*Plantago ovata*
Salmonella typhi	High	Moderate
Salmonella typhimurum	High	Moderate
Pseudomonas aeroginosa	High	Moderate
Pseudomonas flourscences	High	Moderate
Proteus vulgaris	High	Moderate
Proteus merabilis	High	Moderate
Klebsiellapneumoniae	High	Moderate
Enterobacter aerugenes	High	Moderate
Acinetobacter	High	Moderate
Escherichia coli	High	Moderate
Serratia spp.	High	Moderate

Results showed that the aquatic extracts of *Melissa officinalis* gave high inhibitory effects for adhesion and biofilm formation against all the tested bacteria; in comparison with the inhibitory effect the extract of*Plantago ovata.*

4. Discussion:

Lemon balm (*M. officinalis*) is a rich source of phenolic componds such as thymol and carvacrol which are the potential reason for the antioxidant and antibacterial activity of lemon balm plant. The antimicrobial characteristics of lemon balm have been used against Gram-negative bacteria including *E. coli*,*Salmonella typhi*, *Pseudomonas aeruginosa*, Proteus, and Klebsiella and Gram-positive bacteria including*S. aureus*, *Sarcina lutea*, beta-hemolytic Streptococcus and *Bacillus cereus*. The antimicrobialactivities of lemon balm are mainly explained through the C15 and C10 terpenes with phenolic hydroxylgroups and aromatic rings, as well as other active terpenes, such as esters, aldehydes, and alcohols[13, 30].

The results of *in vitro* antibacterial activity of *M. officinalis* by agar disk diffusion and broth micro-well dilution assays. The examined gram negative bacteria (*E. coli* and S. *typhimurium*) were more resistant to the antibacterial activity of the extract than gram positive bacteria (*S. aureus* and *L. monocytogenes*)[31].

Hashemi *et al*., [32]established that highest sensitivity to *M. officinalis*was obtained by*S. aureus*. However, so manyworks about*M. officinalis* concerningits antibacterial effects were done on several Gram-negative and -positive bacteria, for example; *E. coli* and the multi-resistant strain of *Shigella sonei*haddisplayed the uppermost sensitivity to the antibacterial inhibitory effect of *M. officinalis*[33]. In another study, *M. officinalis* showna robust antimicrobial activity against *Salmonella enterica* and *E. coli* [34].On the other hand, weak action of lemon balm against Gram-negative bacteria was also observed in the study of Canadanović-Brunet *et al* [35]. Microbial inhibitory activities of various*Melissa officinalis* parts extracts were also examined by other researchers who showed different ranges of antimicrobial effects with their works[36,37, 38].

Certain work showed that *M. officinalis* was described by its compositionwhich including four dominating components: β-caryophyllene, Citral, Thymol andCitronellal [31].Other study [39]quantified high concentrations of total phenolics in its watery extract. These high total amounts of phenol contentswere also determined by Dastmalchi*et al*[40]; the water-ethanol extracthad 268.9 mgGAE/g of extract and by Trendafilova*et al*.[41],water extract had 1126.5 mg/L GAE/g of extract.

Many studies were carried to show the valuable role of the biologically active plant extracts and the isolated pure componentsforenhancing the *in vitro* efficiency of frequently used antibiotics against variouspathogenic microorganisms [42, 43,38]. Thus for utilizing these beneficial actions, and employing the fact that plant extracts can act in synergism with different antibiotics could be a new tactic to resolve the problem of bacterial antibiotic-resistance and bacteria with less susceptibility. Especially withthe broad-spectrum antimicrobial activity of lemon balm extracts.

Regarding *Plantago ovata* many studies showed that their extracts have shown antibacterial inhibitory activities against various bacteria suchas *Bacillus subtilis*, *Staphylococcus aureus*, methicillin-resistant *Staph*. *aureus* and *Escherichia coli*. The precise mechanism of effect is not exactlyassumed, but its antibacterial inhibitory activity can be strongly attributed to a caffeic acid derivedsubstance called plantamajoside.AlsoPlantamajoside is identified to have anti-inflammatory activity through its inhibitory effect on arachidonic acid metabolism [17].

Karami*et al* [44] and Jabbar *et al*. [45] documented that antimicrobial properties of seed extracts of *Plantago ovata* by utilizing disc diffusion method showed high and moderate inhibitory effect of *Plantago ovata* was reported against *Bacillus saphaericus*and *pseudomonas aeruginosa*. Moreover, *P. ovata* is one of the commonly used, traditional medicinal plants which had been used since ancient years for so many reasons in Asian countries and it has high antibacterial properties. Its effect on various bacteria, including, *S. pyogenes*,*Bordetella bronchiseptica*and *Staphylococcus aureus*[46]. In comparison to sharma*et al*. [47], the aqueous extracts of *plantago ovata* had no activity while ethanolic extract showed strong inhibition activity against *E.coli*, *K.pneumoniae*, *P. aeruginosa* and *K. oxytoca*.

Also, Karima *et al*.[18]shown that the aqueous and ethyl acetate fractions of *P. ovata* were more effective against Gram-positive than -negative one. Where outcomes were gotten with these fractions against three bacterial types (*Acinetobacter*,*Staphylococcus aureus* and*Pseudomonas aeroginosa*. On the other hand, no inhibitory effects of the same fractions were found on *Citrobacter freundii*, *Enterobacter faecalis*, *Serratia sp*, *Listeria monocytogenes* and *Candida albicans*.

The mechanism of action behind the activity of plant compounds is that they prevent cell growth by disrupting bacterial cell wall, resulting in separation of components of the cell wall and exposure of its contents causing cell death. Thus antibacterial effects of *P. ovata* extracts may be related to their secondary metabolites [48].

El-Bashiti *et al*. [11] showed that *Melissa officinalis* aquatic extract produced high antibiofilm inhibitory effect against different bacterial pathogens with efficacy higher than 85%; thus this plant has an antimicrobial inhibitory effect against seriously pathogenic bacteria. Moreover, these results also agreed with results of Adwan and Mhanna[49] who approved that *Pseudomonas aeroginosa* isolates were more susceptible to antibiofilm activity of *Melissa officinalis* extract,when compared to *Staphylococcus aureus*. These suggesting that this plants may contain potential sources of natural antimicrobial compounds that may be of great use in the development of new therapies against many pathogens, with the importance antibiofilm activity of this plant in controlling different infectious diseases.

5. Conclusion: Medicinal plants act as therapeutic agents that inhibit the growth of pathogens considered as potential antimicrobial compounds, as these compounds are cheap, available and broadly-effective against wide range of severe human pathogens, that causing dangerous diseases and complications.

6. References:

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