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Synergistic Antibacterial Activity of Hyssopus officinalis L. Extracts with Standard Antibiotics Against Multidrug-Resistant Bacteria: An In Vitro Study

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ABSTRACT: Background: Hyssopus officinalis L. (hyssop) is a traditional herb that already has established use, which implies inherent antimicrobial activity that would be applicable in the present crisis of antimicrobial resistance. Hypothesis: The purpose of the study was to identify the phytochemical composition, the antimicrobial action, and synergistic effect of the H. officinalis extracts in combination with standard antibiotics (Vancomycin and Erythromycin) to MDR clinical isolates (MRSA, E. coli and K. pneumoniae). Procedures: n-hexane, ethanol and ethyl acetate extracts were prepared. Initial phytochemical screening and total phenolic content (45 ug gallic acid equivalent/ml in the ethyl acetate fraction) was measured. Antibacterial activity was evaluated by broth micro dilution method (to find out the MIC) and the agar well diffusion method to evaluate the individual and combined treatments on the three bacterial strains. Findings: Polar extracts (ethanol and ethyl acetate) had great intrinsic activity with an MIC of 25 percent in all the isolates. The ethyl acetate extract as such had a significant inhibitory effect especially against MRSA (18 mm). More importantly, the extract had a synergistic effect with Vancomycin as it managed to regain its activity against a resistant MRSA and K. pneumoniae (producing up to 11 mm of inhibition zones). On the other hand, the extract was antagonistic in the presence of Erythromycin leading to total deactivation of the action of the drug on MRSA. Conclusion: Ethyl acetate extract of H. officinalis is an effective antimicrobial substance, and an effective antibiotic adjuvant. Its ability to revert the effects of Vancomycin on MDR pathogens justifies its possible use in combination therapy but additional mechanistic efforts are needed to better comprehend and overcome the observed antagonistic effects.

Keywords: Hyssopus officinalis L, MRSA, Escherichia coli

1. INTRODUCTION

The problem of antimicrobial resistance is a worldwide concern, and the issue concerning the methicillin-resistant Staphylococcus aureus (MRSA) is one of the most severe issues in clinical medicine. MRSA is associated with a mutation of the penicillin-binding protein in a chromosome which renders resistance to all b-lactam antibiotics and this includes flucloxacillin, oxacillin, cephalosporins and carbapenems. Bacteriophages can also mediate this resistance between strains of S. aureus. Vancomycin continues to be the first line of treatment of majority of MRSA infection in hospitalized patients, however, there has been rising evidence of diminished response and treatment restrictions leading to renewed interest in other or supplementary forms of treatment [1].

Over the last few years, medicinal plants have been given more scientific attention as a possible source of antibacterial agents that can be used to counter the resistant pathogens. Hyssopus officinalis L. (hyssop) is an ancient aromatic and medicinal herb, which has been used extensively in respiratory and digestive disorders and also topically where it has analgesic and wound-healing properties [2,3]. The most significant part of its biological activity is connected with its primary and secondary metabolites. The aerial parts of the plant produce monoterpenes (limonene, -isopinocamphone, g-pinene, and 1,8-cineole) that have been reported to be antibacterial, antiviral and expectorant [2]. A number of studies have established that the compounds have anti-bacterial activity on a wide spectrum of bacteria including Gram-negative species such as Pseudomonas aeruginosa, Escherichia coli, and Gram-positive species such as Bacillus subtilis and Staphylococcus aureus. Their antimicrobial action is said to be caused by interference with bacterial membranes and prevention of essential metabolic enzymes [4,5].

Besides its essential oil, H. officinalis also produces flavonoids including apigenin, luteolin and diosmin which explain its anti-inflammatory and anti-oxidant properties. The availability of phenolic acids, such as ferulic, caffeic, and rosmarinus acids, can also contribute to its capability of preventing oxidative stress and other associated pathological events [6]. The bioactive properties of H. officinalis offer it as a promising source of the development of plant-based antimicrobial agents.

Since there is an increased need to find effective substitutes to traditional antibiotics, and especially to combat resistant pathogenic agents such as MRSA, Escherichia coli and Klebsiella pneumoniae, the scientific interest in exploring the antibacterial properties of H. officinalis is justified. This paper initially identifies the minimum concentration of two plant extracts (hexane and ethyl acetate) against MRSA, Escherichia coli and Klebsiella pneumoniae as the minimum inhibitory concentration (MIC) as a method of evaluating the intrinsic activity. It then considers the possibility of using these extracts together with standard antibiotics- vancomycin the first-line therapy that has significant side-effects and erythromycin that has low activity against MRSA, E. coli and Klebsiella pneumoniae to increase drug efficacy. The research aims at establishing whether H. officinalis is able to stimulate the effect of antibiotics and lower the dosage required, as an adjunctive method of treating MRSA, E. coli and Klebsiella pneumoniae infections.

2. MATERIAL AND METHODS.

2.1 PLANT EXTRACTION

We sourced the aerial portions of Hyssopus officinalis L. from north of Iraq at December/2024. The plant material was meticulously cleaned and removed of any foreign substances. Then, it was air-dried in a shaded area for two weeks to retain volatile chemicals and reduce phytochemical degradation. After drying the herb, it was mechanically milled until it was very finely ground and then weighed. The first step in extracting the non-polar components was to put 100 grams of the powdered plant material into a thimble and use 500 milliliters of n-hexane to subject it to Soxhlet extraction for three hours. A rotary evaporator was used to concentrate the resulting hexane extract. Then, in order to isolate bioactive chemicals with polar and semi-polar structures, 500 milliliters of 85% ethanol was used to re-extract the defatted plant residue for nine hours. The ethanolic extract was subjected to filtration and rotary evaporation for additional concentration. After adding 15 milliliters of distilled water to the concentrated extract, 50 milliliters of ethyl acetate was used to partition the mixture three times in a separatory funnel, allowing the flavonoid-rich fractions to be isolated. Following collection and drying, the top layers of ethyl acetate were analyzed for the presence of secondary metabolites and flavonoids [3].

2.2 PHYTOCHEMICAL SCREENING

- General preliminary test of H.officinalis extract [7]: Plant was extracted by decoction of about 1gm powdered materials with 10 ml of ethanol until the color of solvent turned into green, the extract was filtered and put 1ml in each test tube for the reagent to be added and the observation was noted. The control used was 1ml of plant extract without any addition.
- Test for flavonoids (Alkaline reagent test): Ethanolic extract (1ml) was mixed with 1 ml (10%) NaOH, the formation of intense yellow color indicates the presence of flavonoids.
- Test for tannins (Ferric chloride test): A few drops of 10% $FeCl_3$ solution were added to 1ml of the ethanolic plant extract. The presence of tannins is indicated when colors like green-blackish or blackish-blue are seen.
- Test for alkaloids (Mayer's and Wagner's reagents test): Ethanolic plant extract (1ml) was mixed with few drops of Mayer's and Wagner's reagents (potassium tetraiodomercurate(II) and iodo-potassium iodide solution), the formation of a white creamy and a reddish-brown precipitate, for each reagent respectively, indicates a positive result.
- Test for Cardiac glycosides (Baljet's test): Ethanolic plant extract (1 ml) was combined with Picric acid (2 drops). This step was followed by the careful addition of Sodium hydroxide (3 drops). The formation of pale brown color implies a positive result.

• Test for saponins (Froth test): ethanolic plant extract (5 ml) was combined with 5 mL of distilled water and shaken vigorously for 15 min. The formation of froth of about 1cm in height which is stable for 1 min reveals that saponins are present

• Test for terpenes/terpenoids (Salkowski's reagent test):1 ml of Chloroform was mixed with 1ml ethanolic plant extract. This step was followed by a care-full addition of concentrated sulfuric acid few drops, the formation of Reddish-brown color in the interphase indicates a positive outcome.

• Test for steroids ((Libermann-Burchard's test): Chloroform (1ml) and acetic anhydrous (2ml) were added to 1ml ethanolic plant extract. This step was followed by a care-full addition of concentrated sulfuric acid few drops, the formation of pink color that gradually turned to deep-green is positive.

• Sugar test (Benedict's test): 1ml of benedict reagent was mixed with 1ml of ethanolic extract, and then this mixture was placed in a water bath for about few minutes until the development of red precipitates which is positive result for the presence of sugar.

• Determination of total phenolic content: Total phenolic content of the ethyl acetate plant extract was determined using Folin-Ciocaltu methods. 1ml of ethanol extract after dissolving in a suitable solvent was mixed with 1ml of Folin Ciocalteu (2 N), then 5 ml of distilled water was added. After 5 min 1ml of Na_2CO_3 (10%) was added to the previous mixture and incubated for 30 min at dark. The absorbance was measured using UV-Visible spectrometer. For calibration curve gallic acid standard was used at a five serially diluted solution in ethanol (100-50-25-12.5-6.25 µg/ml). Three replicates of sample (mean=3) were used and measurement was calculated as mean ± standard error.

2.3 THIN LAYER CHROMATOGRAPHY (TLC) OF H.OFFICINALIS EXTRACT

The Analysis of the Thin Layer Chromatography (TLC) was done to separate and identify compounds in three distinct extracts of Hyssopus officinalis L. which were mainly flavonoids. Silica gel 254 coated plates of aluminum in the fixed phase were employed (MERCK). About10 uL of all the extracts (crude ethanol, ethanol post-defatting and ethyl acetate) were placed on the plates. There were four different mobile phase systems (M1: Toluene-ethyl acetate-formic acid; M2: Chloroform-ethyl acetate; M3: Chloroform-acetone-formic acid; M4: Toluene-chloroform-acetone) whereby the chamber was saturated and the plate was then developed. After development and air-dry, the plates were examined under the short-wave (254 nm) and long-wave (365 nm) UV lamps to observe spots and the values of Retention factors were then obtained. [8].

2.4 INCLUSION CRITERIA OF THE BACTERIAL STRAINS.

Species Identification: The strains used were clinically important human pathogens that were specifically identified Methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, and Klebsiella pneumoniae.

Source of Acquisition: All the bacterial strains were obtained in the teaching hospital of Al-Kindy, Baghdad, Iraq, which is a certified microbiology laboratory.

Culture and Standardization: The strains that were included had to be viable and be able to grow successfully as per the protocol in order to produce a standardized bacterial inoculum that is comparable to the 0.5 McFarland standard.

Resistance Profile (Implied): Strains had an assumed or proven multidrug-resistant (MDR) phenotype, which is why the purpose of the study was to determine the synergy with organisms resistant to first-line agents such as Vancomycin and Erythromycin.

2.5 EXPERIMENTAL STUDY

The purpose of this in vitro experiment was to examine and contrast the antibacterial properties of the plant extract with those of antibiotics alone and in combination with one another. In this study utilized standard antibiotic of vancomycin (15 and 30 µg/ml) and erythromycin (10 µg/ml) either alone or in combination with the plant extract to find out if the two would have any synergistic or antagonistic effects.

2.6 MIC DETERMINATION USING THE WELL PLATE MICRODILUTION METHOD

The minimum inhibitory concentration (MIC) of Hyssopus officinalis L. extracts was determined using the broth microdilution method in 96-well microtiter plates, following the procedure described by [9]. Extracts were prepared using three different solvents: n-hexane, ethanol (85%), and ethyl acetate. Negative Controls As a precaution, we utilized sterile distilled water and ethanol as negative controls to be sure the antibacterial effects we saw were actually from the test materials and not the solvents. Each extract was serially from stock concentration of 100 mg\ml diluted in Mueller-Hinton broth to obtain concentrations of 100%, 50%, 25%, 12.5%, and 6.25%. A standardized bacterial inoculum equivalent to 0.5 McFarland standards was prepared and diluted to achieve approximately 1.5×108 CFU/mL. Three replicates of each bacterium were cultured in the wells, each well received 100 µL of the plant extract dilution and 100 µL of the bacterial suspension. Plates were incubated at 37°C for 24 hours. MIC values were determined as the lowest concentration of plant extract that completely inhibited visible bacterial growth, as observed by the absence of turbidity. Positive (growth) and negative (media-only) controls were included for accuracy.

2.7 AGAR WELL DIFFUSION METHOD

The effectiveness against bacteria was established by means of the agar well diffusion technique. Bacterial suspensions were inoculated onto Mueller-Hinton agar plates at a concentration of 0.5 McFarland standard. A total of 100 µL of each treatment (plant extract, antibiotic, or combination) was added to 6 mm agar wells that were made with sterile cork borers. The plates were kept at 37°C for a full day of incubation. The plant extract (ethyl acetate fraction) was weight and re-dissolved in ethanol to get a concentration of 100mg/ml which was used to investigate the antibacterial activity either alone or in combination of antibiotic in equivalent volumes. Negative Controls were utilized also in this test using sterile distilled water and ethanol Table 1.

Table 1. - Summary of the agar well diffusion experiment setup showing the tested materials, their concentrations, and the bacterial strains used for antimicrobial assessment

Tested Material	Concentration	Tested Against Bacterial Strains
Plant extract (ethyl acetate fraction)	100mg/ml	K. pneumoniae, E. coli, MRSA
Vancomycin	30 µg/ml	K. pneumoniae, E. coli, MRSA
Vancomycin	15 µg/ml	K. pneumoniae, E. coli, MRSA
Vancomycin + Plant extract	15 µg/ml Vancomycin + Plant extract 100 mg/ml	K. pneumoniae, E. coli, MRSA
Vancomycin + Plant extract	30 µg/ml Vancomycin + Plant extract 100 mg/ml	K. pneumoniae, E. coli, MRSA
Erythromycin	10 µg/ml	K. pneumoniae, E. coli, MRSA
Erythromycin + Plant extract	10 µg/ml Erythromycin + Plant extract 100mg/ml	K. pneumoniae, E. coli, MRSA
Ethanol (negative control)	—	K. pneumoniae, E. coli, MRSA
Sterile water (negative control)	—	K. pneumoniae, E. coli, MRSA

2.8 MEASUREMENT OF INHIBITION ZONES

Following incubation, a transparent ruler was used to measure, in millimeters, the diameter of the inhibition zones surrounding each well. We recorded the mean value after triplicate testing of each test.

2.9 DATA VISUALIZATION

In order to compare the inhibition zones of each therapy against the three bacteria, a bar chart was constructed using the Matplotlib and Seaborn tools in Python. Patterns of effectiveness and possible interactions could be better seen in this way.

2.10 STATISTICAL ANALYSIS

Graphpad prism software applied one-way ANOVA to evaluate the significant differences among the diameter of the inhibition zones in the treatment groups. A p-value of 0.05$ was taken as statistically significant. In order to evaluate combined effects: Synergism was a significant and positive difference between an inhibitory effect and the additive effects (or an inhibitory effect that was even smaller than the effect of a strongest component alone).Antagonism was a significant and negative difference between an inhibitory effect and the additive effects (or an inhibitory effect that was even less than the effect of a strongest component alone). MIC data were produced as descriptive statistics and ANOVA was applied to determine whether there was a difference in variance among extracts as well as to determine the association that exists between solvent polarity and antimicrobial activity. Matplotlib and Seaborn were used in Python to carry out data visualization.

3. RESULTS

3.1 GENERAL PRELIMINARY TESTS, TOTAL PHENOLS, AND TLC OF H.OFFICINALIS

General preliminary test of crude ethanolic plant extract revealed the presence of steroids, flavonoids, tannins, alkaloids, cardiac glycosides, terpenes, and sugars, while saponins were absent, Table 2, Fig.1. The total phenolic content of H.officinals was 45 µg gallic acid equivalent/ml plant extract.The plant extract from H.officinalis were submitted to TLC analysis to emphasize the presence of flavonoids. TLC chromatogram of H.officinalis extracts is shown in Fig.1, Table3. Depending on their structure, flavonoids can give differents colors like yellow-green, orange, orange-yellow, etc.… The Retention factor (Rf) value of the flavonoid's spots was also analyzed by TLC. In Fig.1a) the right picture, three plant extract were applied on TLC in four solvent systems, crude ethanolic plant extract (F1 and F2) prepared by boiling the plant materials with ethanol followed by filtration step was used to give indication of the presence of flavonoids since they appear as fluorescent spots under UV 365 nm (the circled zone). The ethanol extract after defatting (F3, and F4) was also applied and the results were close to the ethyl acetate fraction (F5, F6, and F7) since the ethyl acetate fraction can give clear and good separated zone since it enriched with flavonoids because of polarity. The flavonoids identified were (salvigenin S1) and (chrysoeriol S2), their structures are shown in Fig.2. These flavonoids are (trimethoxyflavone) and appeared as yellow spots in TLC. The chromatogram also separated another yellow fluorescent zone S3 which appeared clearly under UV lamp; however, it is not identified. Among the mobile phases used, M3 give the best separation, since in the rest three solvents the spots were overlapped. Fig1.b) showing the TLC of the ethyl acetate fraction in the four mobile phases detected under UV 254 nm.

Table 2. - General preliminary tests of H.officinalis

Test	Positive indicator	Results
Flavonoid Test (alkaline reagent test)	Yellow to orange color	+ ve
Tannins Test (FeCl3 test)	Dark green	+ ve
Alkaloids test (Mayer's test)	White-creamy color	+ve
Alkaloid Test (Wagner's test)	Reddish-brown precipitate	+ ve
Cardiac glycosides Test (Baljet's test)	Reddish-brown precipitate	+ ve
Saponin Test (froth test)	No Foam observed	-ve
Terpens test (Salkowski's test)	Reddish-brown color in the interphase	+ ve
Steroids test (Libermann-Burchard 's test)	Pink color developed into deep green	+ ve
Sugar test (Benedict's test)	Red color	+ ve

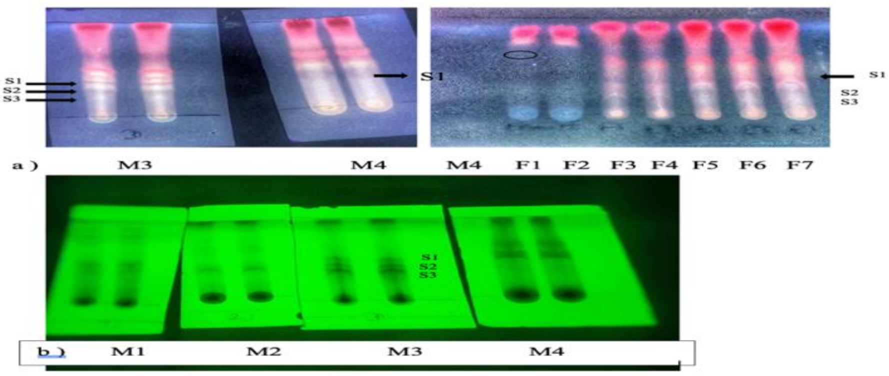
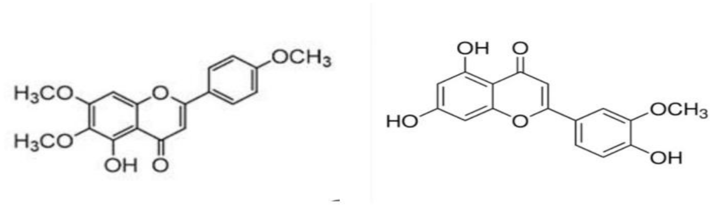


FIGURE 1. - Flavonoids separated in TLC of H.officinalis extracts, a) to the left, ethyl acetate extract in mobile phase M3 separated three flavonoids appeared as yellow fluorescent zones under UV-365 nm (S1 salvagenin, S2 chrysoeriol) and spot S3, while in M4 only S1 zone appeared clear. To the right in M4 crude ethanol extract (F1, F2), ethanol extract (F3, F4), and ethyl acetate extract (F5, F6, F7), b) ethyl acetate extract in M1, M2, M3, and M4 under UV-254nm



S1 S2

FIGURE 2. - Flavonoids identified in TLC of H.officinalis extract Salvigenin S1, and Chrysoeriol S2

3.2 MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC was measured using the broth micro-dilution method in 96-well microtiter plates for three extracts of plant (hexane, ethanol, and ethyl acetate). For hexane extract the MIC was 50% [not effective at lower concentration] for both MRSA and E. coli, while For Klebsiella pneumonia the MIC was 25%. For Ethanol and ethyl acetate extracts MIC was at the concentration of 25% for all isolates. MRSA and E. coli When tested at a concentration of 50%, the hexane plant extract inhibited the growth of both MRSA and E. coli, as measured by the minimum inhibitory concentration (MIC). for ethanol and ethyl acetate, they produced MIC results at 25% concentration for all isolates.

3.3 ANTIBACTERIAL EVALUATION USING AGAR- WELL DIFFUSION METHODS

The test was used to test the antibacterial action of ethyl acetate plant extract, vancomycin and combinations of these antibiotic against three bacterial types K. pneumoniae, E. coli MRSA (Table 3, Fig.3) and the most important observations were as follows: The plant extract alone demonstrated a significant antibacterial activity against all the bacteria since the plant extract had an inhibition zone of 16 mm K. pneumoniae, 12 mm E. coli MRSA and 18 mm MRSA. By comparing the mean inhibitory zone of each bacterial strain to the various treatment groups with the one-way (ANOVA) method, the observed agar well diffusion method antimicrobial activity of each bacterial strain was compared. A p-value of less than 0.05 was statistically significant, indicating that inhibitory zones were significantly higher and bigger in the case of using the ethyl acetate extract of Hyssopus officinalis alone as compared to antibiotics alone (vancomycin or erythromycin).

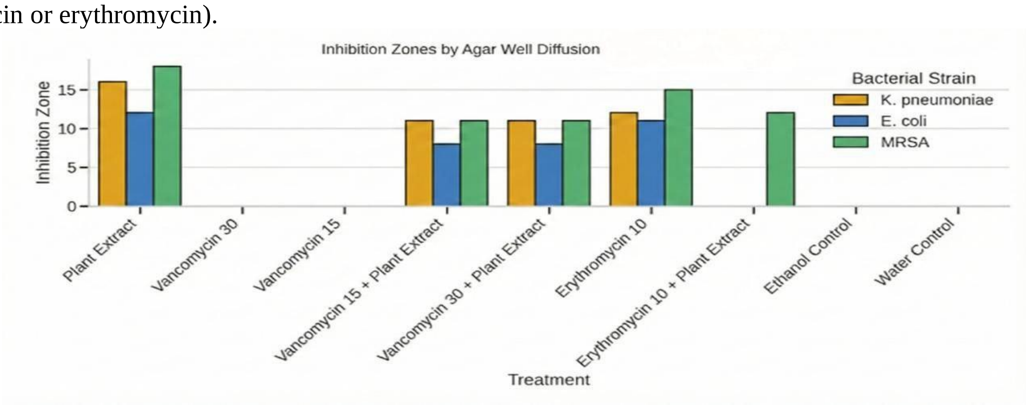


FIGURE 3. - Antibacterial activity of H.officinalis and antibiotics. PT: ethyl acetate plant extract 100mg/ml, ERT 10: erythromycin 10 µg/ml, VAC15: vancomycin 15 µg/ml, VAC30: vancomycin 30 µg /ml.

Table 3. -. Antibacterial activity of ethyl acetate extract of *H.officinalis* alone and in combination with two antibiotics (vancomycin and erythromycin) against *K.pneumonia, E.coli,* and *MRSA*

K. pneumonia (inhibition zone in millimeter)	E. coli (inhibition zone in millimeter)	MRSA (inhibition zone in millimeter)	Ethyl acetate plant extract 100 mg/ml/antibiotics in µg/ml
16 mm	12 mm	18 mm	Plant extract
0	0	0	Vancomycin 30
0	0	0	Vancomycin 15
11 mm	0	11 mm	Vancomycin 15+plant extract
11 mm	0	11 mm	Vancomycin 30+plant extract
0	11	15 mm	Erythromycin 10
0	0	12 mm	Erythromycin 10+ plant extract
0	0	0	Ethanol (negative control)
0	0	0	Water (negative control)

4. DISCUSSION

Phytochemical screening of Hyssopus officinalis L. confirmed the existence of a few dominant groups of secondary metabolites, such as sugars, flavonoids, alkaloids, tannins, terpenes, cardiac glycosides, and steroids, and saponins were not found. Such results are in line with earlier studies that show that H. officinalis harbours a broad array of bioactive compounds that serve to give it its pharmacological effects [5,10]. The overall phenomenon of phenols (45 ug GAE/mL) also lends weight to the possible antioxidant activity of the plant in question, and the thin-layer chromatography revealed three clear flavonoids, in particular, salvigenin and chrysoeriol, which were more concentrated in the ethyl acetate extract. Considering the established antibacterial and anti-inflammatory effects of flavonoids [11] these phytochemicals tend to be the ones attributed to the observed antimicrobial behaviour in the study.

Minimum inhibitory concentration (MIC)-assays revealed that the ethanol and ethyl acetate extracts were better than the hexane extract. The polar extracts inhibited all the bacteria tested with 25 percent and the hexane extract inhibited with concentrations of 50 percent in the case of MRSA and E. coli. Such distinctions can be explained by biology, polar solvents are usually more effective at extracting phenolic acids and flavonoids than non-polar solvents. This is consistent with previous reports stating that the solvent polarity of plant extracts has a strong effect on antibacterial activity and polar fractions are often more potent [6,12].

The findings of the agar diffusion were in support of the MIC results. Ethyl acetate extract showed clear inhibition zones against the three bacterial strains with the greatest activity on MRSA (18 mm) and K. pneumoniae (16 mm) and E. coli (12 mm). This has been in accordance with earlier studies whereby essential oils and extracts of H. officinalis exhibited antibacterial activity against Gram-positive and Gram-negative bacteria [6]. High sensitivity of MRSA can indicate that particular compounds of ethyl acetate extract can disrupt membrane integrity or vital metabolic processes of Gram-positive bacteria.

The plant extract was found to have both synergistic and neutral interaction with antibiotics. Whereas vancomycin alone (15 ug and 30 ug) did not give any inhibition zones with the tested isolates, it reacted with the plant extract yielding observable zones (11 mm) with MRSA and K. pneumoniae. This implies that there could be a possible synergistic effect, which could be mediated by an enhanced membrane permeability or resistance mechanism interference, which would permit vancomycin to re-enter partial activity [13]. The results of such findings indicate that the H. officinalis extracts could serve as adjuvants that could increase the effectiveness of some antibiotics.

Conversely, the use of the plant extract alone together with erythromycin led to a decrease of activity on the MRSA and E. coli, which were antagonistic. This could be due to the competitive effects of the constituents of the plant and erythromycin, decreased stability of the antibiotic or changed bioavailability of the extract. Toxic relationships between plant-derived and antibiotic compounds have already been documented and the necessity to cautiously consider the use of plant-drug interactions before use [14].

As a whole, the results indicate H. officinalis and its ethyl acetate fraction, in particular, is inherently antimicrobial in nature and has potential to enhance the effects of the chosen antibiotics, specifically, vancomycin. Nevertheless, the antagonism that is observed with erythromycin highlights the importance of caution. More molecular, mechanistic, and in vivo research are needed to understand under what circumstances H. officinalis is synergistic and antagonistic as well as when it can be used as a complementary mode of therapy [15].

5. CONCLUSION

The results of this paper have shown that ethyl acetate extract of Hyssopus officinalis L. has a significant antibacterial effect on Gram-positive and Gram-negative bacteria, including MRSA, which is significantly high. This activity is probably due to its phytochemical profile that is rich in phenolics and flavonoids. The extract was also capable of improving the activity of vancomycin against some strains of bacteria which may indicate that it could be used as an antibiotic adjuvant. Even though these findings suggest that H. officinalis L.can be a promising natural antimicrobial agent, additional research needs to be conducted. Mechanistic experiments should be done in detail to understand the interactions between the extract and bacterial cells, and in vivo tests are necessary to test the safety, effectiveness, and clinical significance. All in all, the plant is a worthy potential in future development of supplementary antimicrobial therapy.

None

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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