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Efficacy Determination of Antimicrobial Properties in *Mentha piperita* Plant Extracts

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ABSTRACT

Background and Objectives: Peppermint (*Mentha piperita*) is an herbaceous rhizomatous perennial plant that grows up to thirty-five inches tall, with smooth stems and square in cross-section. It is an aromatic herb that is used for health purposes, fragrances, and cosmetics. The study aimed to extract aqueous and non-aqueous plant extracts from *Mentha piperita*, determine the efficacy of these plant extracts against *Staphylococcus aureus* and *Escherichia coli* pathogens and compare the antimicrobial potency of the plant extracts.
Materials and Methods: The aqueous and non-aqueous plant extracts were subjected to the extraction process using solvent systems such as distilled water and methanol in the maceration technique. The crude extracts were obtained and further subjected for sample preparation using different concentrations of 20%, 40% and 50% respectively. Antibacterial activity assay was done to determine the sensitivity of *Staphylococcus aureus* and *Escherichia coli* using the concentrations.
Result: The peppermint aqueous and non-aqueous extracts showed the presence of antimicrobial activity on *Staphylococcus aureus* pathogens. The aqueous plant extracts had large and more distinct zones of inhibition between 15-20 mm for *Staphylococcus aureus.* The *Escherichia coli* pathogens showed resistance to all plant extracts.
Discussion: Based on the findings of the study, *Mentha piperita* plant extracts showed potential for antimicrobial efficacy against *Staphylococcus aureus* and not *Escherichia coli*. The antibacterial activity in the plant was attributed to the presence of polar and non-polar compounds in the plant. These compounds inhibited the growth of *Staphylococcus aureus* and not *Escherichia coli.* The extracts penetrated through the cell wall of *Staphylococcus aureus* inhibiting the growth of the pathogens.
Conclusion: Thus, the aqueous and non-aqueous plants extracts were more effective on *Staphylococcus aureus* pathogens than on *Escherichia coli* pathogens. Therefore, it was concluded that future work to focus on in vivo assays to be done for the establishment of right dosage development that could be used as a remedy for the treatment of *Staphylococcus aureus* infections.

Keywords: *Mentha piperita, Staphylococcus aureus, Escherichia coli*, Aqueous and Non-aqueous Extracts, Zones of inhibition, Microbiota, Diffusion

INTRODUCTION

In many parts of the world, medicinal plants are used for antibacterial, antifungal, and antiviral activities[2]. These plants are considered to contain biologically active compounds, many of which have been shown to have antimicrobial properties. There is considerable interest in the possible use of these compounds as a preventative to the growth of foodborne pathogens. Such pathogens include: *Escherichia coli* and

Staphylococcus aureus that are of great significance.

Staphylococcus aureus is a Gram-positive, round-shaped bacterium, a member of the Firmicutes, and is a usual member of the microbiota of the body. *Escherichia coli* is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of the human body. There is a collection of hydrophobic secondary metabolites that can be extracted from plants, in particular peppermint.

The plant consists of terpenes (phenolics in nature), which would explain their mode of action relating to those of other phenolic compounds. The antimicrobial actions may be due to the impairment of a variety of enzyme systems including those involved in energy production and structural component synthesis[4]. Tests for antimicrobial activity can be classified as diffusion, dilution, or bioautographic methods. The principles and practice of these methods are explained in the literature[1,8].

The plant is known to have antioxidant, antibacterial, antifungal and some other therapeutic activities. However, there are often large differences in the reported antimicrobial activity from the same plant[9]. In this study, the plant investigated was the *Mentha piperita* which is an aromatic herb. It is used for health purposes, fragrances, and cosmetics. Aqueous and non-aqueous plant extracts were extracted from the plant leaves and used for the microbiological analysis.

MATERIALS AND METHODS

A. Sample Collection

Fresh peppermint plant samples of the leaves were collected randomly from a garden at Cedar Grove Scheme in Mandeville, Jamaica. The samples were placed in sterilized plastic bags and transported to Agri-Research Lab at Northern Caribbean University. The samples were put on the lab counter on top of the newspapers to air dry for seven (7) consecutive days.

B. Extraction of Plant Extracts

The dried peppermint leaves were placed into two (2) Erlenmeyer flasks which were labelled water (H_2O) and methanol. Water was added to one of the flasks while methanol was added to the other until fully submerged. The flasks were then covered with parafilm and then placed under a cupboard to decant for 24 hours. The flasks were then filtered into four labelled 250 ml beakers (Water and methanol) using a cheesecloth and residues were discarded. Flasks were then placed in the oven at 50 degrees Celsius for evaporation of the solvent. The methanol was evaporated within 24 hours whereas water flasks fully evaporated within 48 hours. They were then covered with parafilm and placed in the freezer for storage.

C. Saline Preparation

4.5 micro-gram of salt was weighed on an analytical balance in a petri dish, it was then placed in an Erlenmeyer flask. A small amount of water was added to the flask then swirled until salt dissolved. Water was then added until it reached the 500 ml point of the flask. It was repeated once, then both flasks were poured into 2 volumetric flasks. The flasks containing the solution were covered using autoclave tape and place in the Autoclave machine sterilization. After sterilization, the solutions were placed down on the lab counter to cool down then placed in the refrigerator.

D. Preparation of Stock Extracts

The pre-prepared methanol and water peppermint leave extracts were weighed in micrograms into Eppendorf tubes of specific weight (20mg, 40mg, and 50mg) using the analytical balance. 100 microliter of the prepared saline solution was added to all tubes and vortexed for two (2) minutes using a vortex machine.

E. Subculture of Microorganisms

Microorganisms: *Staphylococcus aureus* and *Escherichia coli* were sub-cultured in blood agar for usage in the next assays.

Also, the microorganisms were sub-cultured in trypticase soy broth to be stored in the refrigerator.

F. Minimum Inhibitory Concentration (MIC)

Saline solution was poured into four (4) vacutainer tubes. Then isolates from each bacterial plate was taken with clean cotton swabs and dipped into the tubes until turbidity/cloudiness was similar to that of the McFarland standard as they were compared against a blank paper to determine the turbidity of the inoculum. Two Microtiter plates were labelled H_2O and Methanol, 50 ul of each inoculum (5 tubes) were micropipette into wells containing *Escherichia coli*, and *Staphylococcus aureus*, respectively. 50 ul of both water and methanol extracts were micropipette into the wells (1, 2, 3, 4 & 5) representing the percentages 10, 20, 30, 40 and 50 of the extracts. The Microtiter plates were then placed in the incubator for 24 hours. Then, observation was made for minimum inhibitory concentration.

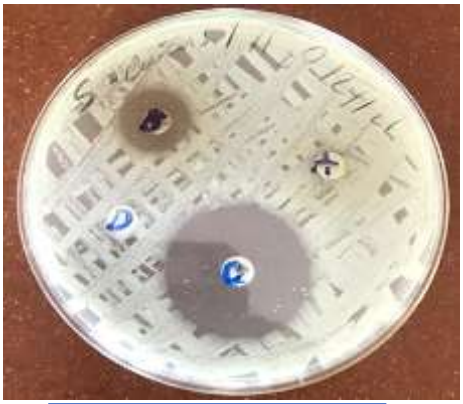
G. Kirby-Bauer Method/Disc Diffusion

Filter papers were punched and placed to make discs in a small beaker, then covered with autoclave tape. A clip was placed in another beaker and both beakers were then placed in the autoclave for 30 minutes, 121 degrees Celsius at 15 psi to be sterilized. Mueller Hinton agar plates were prepared to be used for the assay. The bacteria were inoculated into vacutainer tubes using saline and compared with McFarland standard to match their turbidity. The inoculum was then streaked on the agar plates using the continuous streaking method. 40% and 50% of the water (H_2O) peppermint extracts were used, as the discs were dipped and placed on the plates using the clips and labelled. Discs were also placed on the remaining agar plates of bacteria after being dipped in 20% and 40% methanol peppermint extracts marked and labelled accordingly. Positive control and negative control were used, being gentamicin and saline marked 'C' and 'D' respectively. The plates were then placed in the incubator for 18 hours. They were then read, and the zones of inhibition were recorded for each.

RESULT

The findings of the study showed potential in the antibacterial action of the plant extracts against *Staphylococcus aureus* and *Escherichia coli.* The figure 1x-y and Table 1 showed the zone of inhibition of *Staphylococcus aureus*. Figure 2x-y and Table 2: zone of inhibition of *Escherichia coli.* Table 3: standard deviation of the zones of inhibition and Figure 3: Aqueous vs non-Aqueous extracts concentrations and zones of inhibition.

A = 20% Methanol extract
B = 40% Methanol extract
C= Positive control (gentamicin)
D = Negative control (saline)



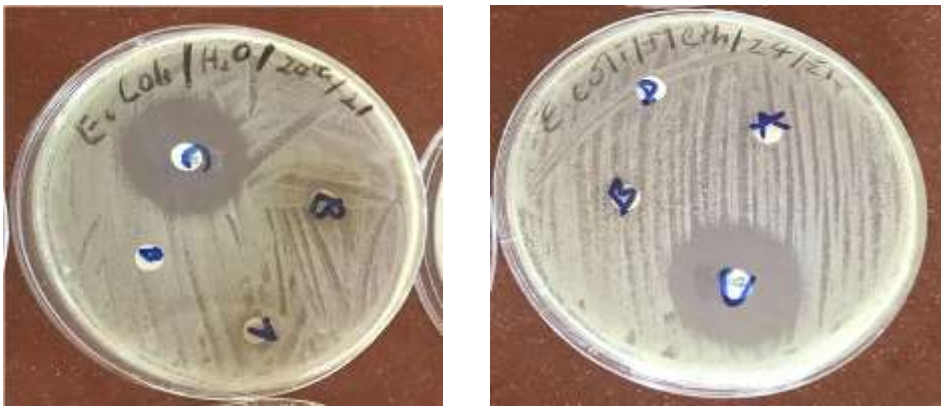
A = 40% H_2O extract
B = 50% H_20 extract
C= Positive control (gentamicin)
D = Negative control (saline)

Fig. 1x-y: Zones of Inhibition for *Staphylococcus aureus*

Table 1: Zones of Inhibition for *Staphylococcus aureus*

	Staphylococcus aureus (mm)	Positive Control (mm)	Negative Control (mm)
40% H2O	20 (S)	30 (S)	0 (R)
50% H2O	15 (S)	30 (S)	0 (R)
20% Methanol	10 (I)	28 (S)	0 (R)
40% Methanol	10 (I)	28 (S)	0 (R)

S = Susceptible R = Resistant I = Intermediate



A = 40% H_2O extract
B = 50% H_2O extract
C= Positive control (gentamicin)
D = Negative control (saline)

A = 20% Methanol extract
B = 40% Methanol extract
C= Positive control (gentamicin)
D = Negative control (saline)

Fig. 2x-y: Zones of Inhibition for *Escherichia coli*

Table 2: Zones of Inhibition for *Escherichia coli*

	Escherichia coli (mm)	Positive Control (mm)	Negative Control (mm)
40% H2O	0 (R)	32 (S)	0 (R)
50% H2O	0 (R)	26 (S)	0 (R)
20% Methanol	0 (R)	30 (S)	0 (R)
40% Methanol	0 (R)	28 (S)	0 (R)

S = Susceptible R = Resistant I = Intermediate

Table 3: Standard deviation of the zones of inhibition of *Staphylococcus aureus.*

X	$[x-\mu]$	$\Sigma[x-\mu]^2$
20	20 - 13.75	$(6.25)^2 = 39.06$
15	15 -13.75	$(1.25)^2 = 1.56$
10	10 -13.75	$(-3.75)^2 = 14.06$
10	10 -13.75	$(-3.75)^2 = 14.06$
Sum		68.74

$\frac{\Sigma[x-\mu]^2}{N}$	$\frac{68.74}{4} = 17.185$
$SD = \sqrt{\frac{\Sigma[x-\mu]^2}{N}}$	$\sqrt{17.185} = +/- 4.15$

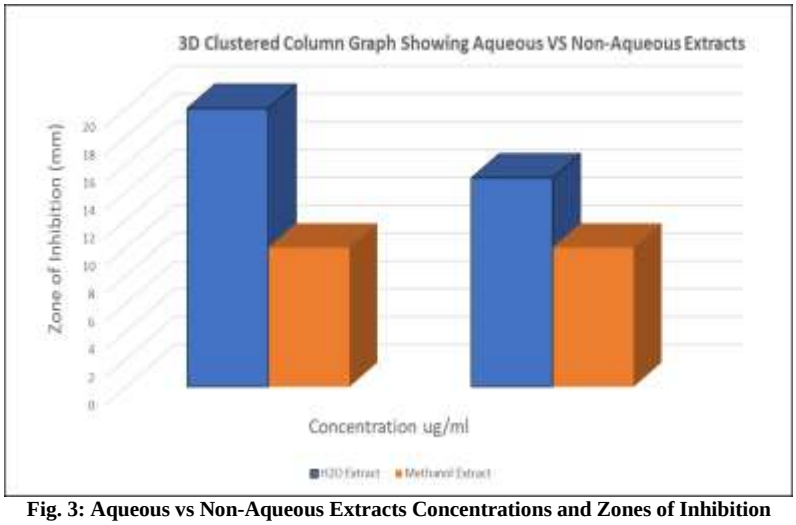


Fig. 3: Aqueous vs Non-Aqueous Extracts Concentrations and Zones of Inhibition

DISCUSSION

The study provided an insight and additional knowledge about the potential effect of peppermint extracts on bacterial strains. Based on the findings, *Staphylococcus aureus* was susceptible to the concentrations 40% & 50% when tested except the negative control as seen in Fig. 1x-y. *Escherichia coli* was resistant to peppermint extracts at the minimum inhibitory rates of concentration of 20%, 40% in non-aqueous extract and 40%, 50% in the aqueous extract as seen in Fig. 2x-y.

S. aureus was susceptible to peppermint plant extract properties and *E. coli* was resistant to such properties. The properties include the alkaloids found in peppermints such as menthol, menthone, methyl acetate and Menth furan. This was because *E. coli* was Gram-negative and *S. aureus* was a Gram-positive bacterium. *E. coli* as a gram-negative bacterium was resistant to the extracts due to the difference in its outer membrane layer on the cell wall compared to the Gram-positive bacteria, *S. aureus* which doesn't have any lipopolysaccharide on the outer membrane layer. The outer membrane consists of a lipopolysaccharide peptidoglycan layer which provides a barrier against both aqueous and non-aqueous solutions.

Also, from the results, the highest antibacterial properties were observed in aqueous solution (H_2O extracts) against *S. aureus* where the inhibition zones varied at a range of 15 - 20 mm. The non-aqueous solution (Methanol extracts) showed a constant inhibition zone of 10 mm for both concentrations. The activity f inhibition was expressed in terms of the diameter of the inhibition zone >12 mm: sensitive, 9-12, Intermediate, <9 mm: resistance. Generally, gram-negative bacteria were more resistant than gram-positive bacteria as they are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide.

Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan with approximately 90% - 95% of the cell wall consisting of peptidoglycan[7]. Hence, it seems that the antibacterial action of the plant extracts was more effective on Gram-positive than on Gram-negative bacteria and these findings correlate with the observations of previous screenings of medicinal plants for antimicrobial activity, where most of the active ingredients of the plants showed activity against Gram-positive strains, only a few were active against gram-negative bacteria[7].

CONCLUSION

In conclusion, edible plants could be a potential source of inhibitory substances for bacterial pathogens. Natural substances extracted from have applications in controlling pathogens[5]. This study tested the antibacterial activity from *Mentha piperita*

plant extracts employing the disc diffusion method and the determination of minimum inhibitory concentration. *S. aureus* had a higher susceptible rate than *E. coli*. Results reported in this study showed that *S. aureus* is more sensitive than *E. coli* to peppermint extracts. The confirmation of the results demonstrates that *Mentha piperita* has potential in medicinal properties. These herbal plant extracts could represent a new source of antimicrobial agents, less expensive and alternative medicine than the imported drugs[10].

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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