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ANTIBACTERIAL EFFECT OF MINT LEAVES (MENTHA L) POLYPHENOLS EXTRACT: A STUDY

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***Abstract*:**

Natural antibacterial agents are attaining more importance now a days, as they are inexpensive, easily available, most of them are edible and mainly non-toxic even at the rate of milligram quantity. The aim was to study the efficacy of the anti-bacterial effect of Polyphenols enriched extract of Mint plant leaves (*Mentha L*) against staphylococcus aureus. The materials involved in this study include *Mint plant leaves*, micro-organism *staphylococcus aureus* in the bacterial type culture collection, agar, and blood-agar plates. At 10% concentration, of Polyphenols enriched the ethanol-water extract of *Mint leaves* had zero anti-bacterial activity while 10% and 20% concentrations revealed high activity against the bacteria. Thus, increased in the anti-bacterial activity was promising as the concentration augmented from 10 to 20%. The results acquired from this study points that polyphenol enriched extract of Mint leaves (*Mentha L*) had antibacterial property against *Staphylococcus aureus* when obtained to a necessary concentration.

***Keywords*:** Mint plant leaves, *Mentha L, Stephylococcus aureus,* Anti-bacterial effect, Polyphenols enriched extracts

I. INTRODUCTION

Enormous number of antimicrobial molecules, such as peptides, alkaloids, flavonoids, Polyphenols, proteins and other small molecular weight organic substances are present in plants, edible sources and herbs acting as host defense mechanisms (Lattanzio et al., 2006; Cheynier et al., 2013; Baetz & Mrtinoia, 2014). Many of these plant based components present are showing broad spectrum of inhibitory activity against pathogenic bacteria and fungi (Ruiu, 2013; Luplertlop et al., 2011; Erdem et al., 2015; Genskowsky et al., 2015). It was reported that, Mexican medicinal plants of 15 different families were showed promising results when analyzed their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Kakuko et al., 2005). It is reported that, some commonly used essential oils in micellar and aqueous extract on some of the most common pathogenic

bacteria. Frankincense, myrtle, thyme, lemon, oregano and lavender essential oils were tested against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and are showing inhibitory activity (Man et al., 2019). It was reported that, partially purified proteins from Turmeric rhizome showed antimicrobial activities. It was also reported by the same researchers that, 28kDa glycol protein shown antioxidant and antimicrobial activities (Dinesha et al., 2010; Dinesha Ramadas & Leela Srinivas, 2011). In a recent study, common flavonoids like flavones, flavonols, flavanones and some organic acids like aliphatic and aromatic acids are evaluated for their MIC against Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* than Gram-positive ones: *Enterococcus faecalis* and *Staphylococcus aureus* and most of them showed promising results towards inhibiting the growth of above said microbes (Ravishankar et al., 2013).

In this study, polyphenol enriched extract of Mint leaves is tested for antibacterial activity against human pathogenic bacteria like *S. aureus*.

II. MATERIALS AND METHODS

The present *in-vitro* study was piloted to study the antibacterial efficiency of different concentrations of polyphenols enriched extract of Mint plant leaves (*Mentha L*) against S. Aureus.

Leaves are washed thoroughly with water and rinsed in 0.5% $KMnO_4$ for five minutes and again washed in double distilled water to remove if any microbes present. Further, leaves were shade dried, powdered, sieved and stored in a dry glass container for further use. Mint leaves powder (25 g) was mixed with 250mL of methanol, followed with Soxhlet extractor for 72 h. Later, the excess methanol solvent was evaporated. In the same way, the extraction was done with other solvents like hexane, chloroform, ethyl acetate and butanol to obtain hexane, ethyl acetate, chloroform-butanol and residual methanol fractions, respectively. Finally, all crude extracts were mixed, filtered. The combined extracts were concentrated and dried by using rotary evaporator under reduced pressure (Hossain et al., 2014).

2.1 Proximate analysis:

The extract was subjected to phytochemical analysis to check the presence of bioactive compounds by using standard protocols (Dinesha & Leela Srinivas, 2010; Mylarappa et al., 2008; Dinesha & Leela Srinivas, 2010, Mohamed et al., 2015). The protein estimation was carried (Bradford, 1976) using BSA as standard and absorbance was read at 535nm. Total phenolics was determined according to the method of Folin Ciocalteu reaction (Kujala et al., 2000) using gallic acid as a standard and absorbance was read at 750 nm. Ascorbic acid estimation was carried out (Sadasivam &Manickam, 1997) and the absorbance was read against a reagent blank at 540nm. Total sugar estimation was done (Dubois et al, 1956) and the absorbance was read at 520 nm. Flavonoids estimation was done (Cheon et al., 2000) by using Quercetin as a standard and the absorbance was measured at 415 nm. In the above analysis, standard curve was used to compare.

2.2 Preparation of cultural media

Staphylococcus aureus bacteria obtained from a local Culture Collection and Gene Bank was added to a liquid infused with nutrient broth and incubated at 37°C for a period of 24 hours. The additive culture is then cultured on the nutrient agar plate, and it was passed through an incubation cycle at a temperature of 37°C for a time period of 24 hours.

2.3 Well plate method

The anti-bacterial efficiency of different concentrations of *Mentha L* leaves extract against *S. aureus* was tested with the help of well plate method. Wells were prepared in Petrie-dishes with the aid of a punch. The wells were packed with the equivalent quantity of *Mentha L* leaves extract. The entire process was repeated to test the four different concentrations of extract. The well plates were then incubated at 37°C for a period of 48 hours.

2.4 Study process

The wells were intended on the blood agar plates with the aid of a punch consisting of 3mm radius. An equal quantity of each of 10, 15 and 20 and 25% *Mentha L* extract was rested onto Petri dishes. The plates were kept at the normal temperature for a period of 1 hour which was then followed by incubation at 37°C for a period of 48 hours. The zone of inhibition was then examined and noted in millimeters.

2.5 Minimum inhibitory concentration (MIC)

By Serial dilution method in the nutrient agar, the minimum inhibitory concentration of isolated extract was determined, with concentrations like 10, 15, 20 and 25µg at a ratio of 1:10. Plates were incubated for 24 h at 37°C. MIC was recorded as the lowest extract concentration demonstrating no visible growth in the broth (Presscot et al., 1996).

III. RESULTS AND DISCUSSION

The Mint leaves extract was subjected to proximate analysis. It was noticed that, the extract rich with Polyphenols when compared to other phytochemicals where Gallic acid used as standard polyphenol. It contains other phytochemicals in a negligible amount.

Table 1 shows the effects of different concentrations of Polyphenol enriched Mint plant leaves extracts on *S. aureus*. There was nearly zero zone or negligible zone of inhibition detected with 10% Mint leaves extract. Zone of inhibition of 10.0 mm was witnessed with 15% extract and zone of inhibition of 15.0 mm was noticed at 20% and 25% extract

Concentration of Mint plant leaves extract (%)	Zone of inhibition (in mm)
10	0
15	10
20	15
25	15

The minimum inhibitory concentration of Mint plant leaves extract against staphylococcus was 15.5±0.5 µg at a ratio of 1:10 (w/v)

Medicinal / spice plants are known to harbor potential endophytic microbes, due to their bioactive components and also for their antioxidant activity (Gouda et al., 2016; Hareesha et al., 2010; Rajesh et al., 2010; Rajesh et al., 2010; Ningappa et al., 2010; Rajesh et al., 2010; Sivapriya et al., 2011). It was reported that, combination of cow urine and pepper extract enhance the Antibacterial Activity of *Azadirachta Indica* leaves (Pramod et al., 2016). Mint is known for its herbal remedy like easing queasy stomachs, calming stress, anxiety and promoting peaceful sleep. A animal model study was reported and it was conducted by the combination of seven different herbs along with a well known broad spectrum antibiotic Ciprofloxacin to enhance its activity (Vedamurthy et al., 2016). Mint is a common aromatic plant with lot of medicinal properties (Samarth et al., 2017). In our study, the polyphenol enriched extract of *Mentha L* was tested for its antibacterial activity against human pathogenic bacteria, it showed inhibition of bacterial growth. As shown in the results, the extract showed a very significant antibacterial activity by producing a clear zone of inhibition against *S. aureus* where streptomycin was used as positive control. In minimum inhibitory concentration (MIC), it was observed that it inhibited bacterial growth, with MIC value of 15.5±0.5 µg at a ratio of 1:10 (w/v). The MIC value of extract compared with standard antibiotics, which ranged from 11.2 to 20 µg (1:10 w/v). Thus the polyphenol enriched extract of Mint leaves is as potent as standard antibiotics in inhibiting the growth of *S.aures* strain.

Conclusion

The anti-bacterial activity of the Mint leaves polyphenol enriched extract was perceived with 10%, 15%, 20% and 25%. Anti-bacterial action augmented as the concentration amplified from 15 to 25%. With the results attained from the study, it can be determined that polyphenol enriched extract of Mint leaves have anti-microbial property against *S. Aureus*.

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Conflict of interest
The authors declare no conflict of interest.

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