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(RESEARCH ARTICLE)

Antibacterial effect of polyphenols enriched drumstick plant leaves (*Moringa oleifera*) extract: A research study

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Abstract

Microorganisms are raising resistance against available antibiotics due to usage of antibiotics in a wrong way. There is lot of demand for finding /searching new natural antimicrobial agents 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 efficiency of the anti-bacterial effect of Polyphenols enriched extract of Drum stick plant leaves (*Moringa oleifera*) against staphylococcus aureus. The materials involved in this study include *Drum stick plant leaves*, micro-organism *staphylococcus aureus* in the bacterial type culture collection, agar, and blood-agar plates. At 10% concentration, of Polyphenols enriched extract of *Drum stick leaves* had zero anti-bacterial activity, while between 20 to 25% concentrations revealed high activity against the bacteria. Thus, increased in the anti-bacterial activity was promising as the concentration augmented from 20 to 25%. The results acquired from this study points that polyphenol enriched extract of Drum stick leaves (*Moringa oleifera*) had antibacterial property against *Staphylococcus aureus* when obtained to a necessary concentration.

Keywords: Drum stick plant leaves; *Moringa oleifera; Stephylococcus aureus*; Anti-bacterial effect; Polyphenols enriched extracts

1. Introduction

Edible, spices and herbal /medicinal plants are becoming promising alternative for microbial diseases and conditions. These sources are also valued to add flavor foods, giving the food a dual role like flavor and bioactive compounds. Furthermore, they are inexpensive, easily available and tend to have negligible side effects than available synthetic drugs. The presence of above mentioned active components are also acting as host defense mechanisms in variety of infectious conditions [1-5]. From ancient times, sources likey garlic (*Allium sativum* L.) and onion (Allium cepa L.), have represented important components of typical recipes and traditional healing systems. Not the least of which, their use as food biopreservatives is well documented, due to the relevant antibacterial activity of their extracts and essential oils

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in addition to garlic and onion [6-7]. Researchers reported that, extracts of edible plants from China, Japan, Thailand and Yemen are shown antibacterial activity when they were screened against *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella infanti* [8]. It was reported that, nanoemulsions of *Thymus daenensis* was analyzed for its antibacterial activity. The results revealed that it had shown a noticeable antimicrobial activity against selected microorganism *S. aureus* [9]. A study was done by focusing on terpene and terpenoid compounds presented in selected edible and aromatic plants, the results revealed the health beneficial effects of the above compounds like antimicrobial, antiviral, cytotoxic, anticancer, anti-inflammatory and many other pharmacological activities [10]. A study was conducted to find the anti-microbial activity of crude extracts (hexane, acetone, ethanol, and aqueous) of 46 edible plants from Odisha, India using a broth microdilution assay against 8 common food-borne pathogens. The results showed that some edible Indian plants with antibacterial properties have potential as herbal preservatives in the food industry, and perhaps also as alternative therapeutic agents, particularly against food-borne bacterial diseases [11]. 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 [12-14].

In this study, we focused on antibacterial activity against human pathogenic bacteria S. aureus bypolyphenol enriched extract of Drum stick leaves.

2. Material and methods

The present *in-vitro* study was piloted to study the antibacterial efficiency of different concentrations of polyphenols enriched extract of Drum stick plant leaves (*Moringa oleifera*) 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. Leaves of Drum stick plant are washed thoroughly with water and rinsed in 0.5% KMnO4 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. Polyphenol enriched extraction was done by mixing 25g of Drum stick leaves powder 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 [15].

2.1. Proximate analysis

The extract was subjected to phytochemical analysis to check the presence of bioactive compounds by using standard protocols [16-19].

The protein estimation was carried according to Bradford's method [20] using BSA as standard and absorbance was read at 535nm. Total phenolics was determined according to the method of Folin Ciocalteu reaction [21] using gallic acid as a standard and absorbance was read at 750 nm. Ascorbic acid estimation was carried out according to Sadasivam S., Manickam[20] and the absorbance was read against a reagent blank at 540nm. Total sugar estimation was done according to Dubois method [22] and the absorbance was read at 520 nm. Flavonoids estimation was done according to Cheon et al [23] 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 *Moringa oleifera* 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 *Moringa oleifera* 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% *Moringa oleifera* 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 [23].

3. Results and discussion

The Drum stick 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 Effects of different concentrations of Polyphenol enriched Drum stick plant leaves extracts on *S. aureus*.

Concentration of Drum stick plant leaves extract (%)	Zone of inhibition (in mm)
10	0
15	12
20	19
25	19

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

There was nearly zero zone of inhibition detected with 10% Drum stick leaves extract. Zone of inhibition of 12.0 mm was witnessed with 15% extract and zone of inhibition of 19.0 mm was witnessed with 20 and 25% extract.

A polyphenol producing endophyticfungus was isolated from *Zingiber officinale* rhizome. The ethyl acetate extract of *Aspergillus austroafricanus* (EAE) was studied against five human pathogenic bacteria by disc diffusion method. It showed significant antioxidant, antimicrobial activity and DNA damage protection capacity [25-31]. It was reported that, combination of cow urine and pepper extract enhance the Antibacterial Activity of *Azadirachta Indica* leaves [32]. Another similar type of animal study was conducted by the combination of seven different herbs along with a broad spectrum antibiotic Ciprofloxacin [33]. Drum stick is a common aromatic plant with lot of medicinal properties. It has been long known as an herbal remedy, easing queasy stomachs, calming stress and anxiety, and also rich source of Vitamin A [34-36]. In our study, the polyphenol enriched extract of *Moringa oleifera* was dose as explained in materials and methods. It analyzed for its antibacterial activity against human pathogenic bacteria by well plate method, where streptomycin was used as positive control. The results showed a promising inhibition of bacterial growth which was compared with standard. In MIC studies, it was observed that the 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 19 to 20 µg (1:10 w/v). Thus the polyphenol enriched extract of Drum stick leaves is inhibiting the growth of *S.aures* strain and further studies to be done in this direction to find which active polyphenol is responsible for the above.

4. Conclusion

The anti-bacterial activity of the Drum stick 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 Drum stick leaves have anti-microbial property against *S. Aureus*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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