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In vitro antimicrobial assessment of seeds of selected medicinal plants in Sri Lanka

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ABSTRACT

Introduction: People are suffering from many noncommunicable diseases as a result of the COVID pandemic and the stress that has followed after it. Diabetes mellitus is a complex non-communicable disease and its incidence in Sri Lanka is almost high. While a number of antidiabetic medications are available, herbal management of diabetes is encouraged due to its low side effects and efficacy. Examining the antibacterial properties of anti-diabetic plants may be highly valued because, diabetics are chronic immunocompromised individuals who are more prone to microbial infections. To focus this aim, the present *in-vitro* antimicrobial assessment carried out for the seeds of selected four medicinal plants, such as *Syzygium cumini* (L.) Skeels, *Sinapis alba* L., *Trigonella foenum-gra*ecum L. and *Nigella sativa* L. that are commonly used for diabetes management in Sri Lanka.

Materials and methods: Crude ethanol extract from the seeds has been studied for their antibacterial potential against three bacterial strains such as *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* by using agar well diffusion method in triplicates. The statistical analysis was performed using a one-way analysis of variance.

Results: The seed extract of *S. cumini* showed the highest value of zone of inhibitions (*E. faecalis*: 24.70±037, S. *aureus*: 16.15±1.20 and *E. coli*: 10.37±1.51 mm) and *S. alba* exhibited the lowest value of zone of inhibition (1.08±2.65, 1.08±2.65, 0 mm) for all selected pathogens respectively and which were comparable to the positive control *streptomycin* (*E. faecalis*: 25.45±1.18, *S. aureus*: 21.08±0.26 and *E. coli*: 19.37±1.35mm).

Conclusion: The result shows that *S. cumini* seed extract poses the highest antimicrobial activity in selected bacteria. Therefore, this seed is potential to be further developed as an herbal antibiotic for the management of infection in diabetes in future.

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Introduction

The number of people with diabetes is exponentially increasing world-wide and has become an important global public health problem (Global report on diabetes, 2016; Shehab et al., 2022). Sri Lanka, despite being a developing nation, has always maintained health indices on par with most developed countries. Currently, Sri Lanka is in the midst of its worst economic crisis in history (Pathmanathan & Abhayaratna 2022). In Sri Lanka, people are suffering from many noncommunicable diseases as a result of the COVID pandemic and the stress that has followed after it. That condition gets more critical as a result of the country's economic situation. Diabetes mellitus is one of the most common endocrine disorders in Sri Lanka. 10% of the Sri Lankan population and 18% of the urban population are affected by it (Pathmanathan & Abhayaratna 2022). Diabetes mellitus is characterized by hyperglycaemia and is especially classified into two types, Type-I (Insulin dependent diabetes) and type-II diabetes (non-insulin dependent diabetes) (IDF Diabetes Atlas, 2013; AOAC, 2016; American Diabetes Association, 2019; King, 2012). The incidence of diabetes (% of the population aged 20-79) in Sri Lanka was 11.3% in 2021 (The World Bank Group, 2022). This condition may be due to the common risk factors such as genetic, environmental, different life stylishness and physical inactivity (Wu et al., 2014; Arawwawala, 2006) and prevalence of depression (Akter & Latif 2021). There is another type is gestational diabetes which mainly arises during the pregnancy (Buchanan, Xiang & Page, 2012).

The oxidative stress is a recognized pathogenic mechanism in the development and progression of diabetes which reasons owing to augmented free radical production and weakened antioxidant defences (Unuofin & Lebelo, 2020). A study mentioned that the antiglycation properties of herbal extracts and their complexes powerfully interrelated with their antioxidant capacity with that antioxidant and anti-glycation activities are associated strongly with phenol and flavonoid contents (Babich et al., 2022).

Generally, antibacterial actions are facilitated by the immune-modulating and antioxidant capabilities of medicinal plants (Aryal et al., 2021). Antibacterial action is the most significant distinctive of medical textiles, to deliver satisfactory defence against microbes, biological fluids, and infection transmission (Alihosseini, 2016). Prevention of food spoilage and food intoxication pathogens is regularly attained by use of chemical preservers. Plant extracts have been used to control food poisoning diseases and preserve foodstuff (Mostafa et al., 2018). An extensive range of biological constituents as alkaloids, flavonoids, glycosides, terpenoids, phenols, and coumarins have been stated from different parts of the plant, which are responsible for numerous biological activities as well as antimicrobial, antioxidant, and anti-inflammatory properties (Sarkar, Salauddin & Chakraborty, 2020; Phuyal et al.,2020). While a number of antidiabetic medications are available, herbal management of diabetes is encouraged due to its low side effects and efficacy. Examining the antibacterial properties of anti-diabetic plants may be highly valued because, diabetics are chronic immunocompromised individuals who are more prone to microbial infections (Hegazy et al., 2021).

For this purpose, this *in-vitro* antimicrobial assessment was performed for the ethanol extracts of four seeds of medicinal plants namely *Syzygium cumini* (L.) Skeels, *Sinapis alba* L., *Trigonella foenum-gra*ecum L. and *Nigella sativa* L. against three bacterial strains as *E. faecalis, S. aureus* and *E. coli.*

Materials and methods

Chemicals and reagents

Ethanol, streptomycin, distilled water and nutrient agar were purchased from Sigma-Aldrich. All reagents and chemicals were of analytical grade.

Collection of medicinal plants and preparation of the seed extract

The plant materials with seeds of *S. cumini*, *S. alba*, *T. foenum - gra*ecum and *N. sativa* (Table 1) were collected and botanically authenticated by a Curator of the National Herbarium Center, Department of National Botanic Garden, Peradeniya, Sri Lanka.

The fresh seeds were washed in tap water for several times to remove the soil and dust particle. Then they were air dried in thoroughly at room temperature until dried and blended to form a fine powder and stored in airtight containers at room temperature until needed for analysis.

Fifty grams of powered materials of each seed were separately weighed and placed in 500 ml of culture bottles. As much as 150 ml of absolute ethanol was added to it and mixed well. Lid of each bottle were covered with para film. The solution was kept for 5 days with occasional shaking by using shaker at 150 rpm for 15 minutes in every morning and evening. They were filtered through Whatman filter paper No.1. The part of filtered content was concentrated by using rotatory evaporator (BUCHI, Chi Minh City, Vietnam) at 52 ^{0}C (Rajkumar, Jayasinghe & Sanmugarajah, 2021). Crude extracts were kept at 20 ^{0}C for further analysis.

Test microorganisms

Three bacterial strains were provided by the Faculty of Science, University of Jaffna were used for the antimicrobial tests, according to Table 2. All the test strains were preserved on nutrient agar slants at 4 ^{0}C and sub-cultured on to nutrient broth for 24 hours prior to testing. These bacteria served as test pathogens for this assay.

Assay of Antimicrobial activity using Agar well diffusion method

About 22.68 g of Nutrient Agar (NA) powder was dissolved in 810 ml of distilled water. Then 15 ml parts of the NA medium were poured into boiling tubes. Medium which was contained in the boiling tubes were autoclaved at 121 ^{0}C for 20-30 minutes. Then 15 mL of sterilized nutrient agar was mixed with 100 µl of bacterial suspensions inside the laminar air flow chamber. The mixture was stirred well and it was poured into sterile petri dishes separately (Dwivedi et al., 2017). After the solidification the wells were punched over the agar plates using sterile cork-borer (5mm in diameter) and 15 µL of plant extracts were added to the wells separately. The plates were incubated for 24 hours at 37 ^{0}C. Distilled water and Streptomycin (100 µg/µl) were used as the negative and the positive control respectively. After incubation the diameter of the formed inhibitory zones formed around each well were measured (mm) in four different fixed directions and recorded. Each experiment was conducted in triplicate.

Data analysis

Data were statistically analysed by one way Analysis of Variance and Tukey's multiple comparisons at probability value ($P<0.05$) using a SAS statistical package (version 9.1.3) and mean separation was performed by Least Significance Difference. Results are expressed as Mean ± SE and statistical significance was evaluated by ANOVA.

Results

This *in vitro* antimicrobial assay was done for four seeds of selected antidiabetic medicinal plants as *S. cumini*, *S. alba*, *T. foenum-gra*ecum and *N. sativa* against three selected common bacterial strains. Based on Figure 1, ethanolic seed extract of *S. cumini* exhibited the significant antimicrobial activity while the ethanolic seed extracts of *S.alba* represented minimum antibacterial activity against the all three bacteria as *E. faecalis*, *E. coli* and *S. auereus.*

The results showed that significant amount of inhibition zone was obtained against all the tested bacterial strains which was comparable to the positive control streptomycin.

Based on Figure 1, the ethanolic seeds extract of *S. cumini* exhibited the significant antimicrobial activity while the ethanolic seeds extract of *S. alba* showed minimum activity against all three bacterial strains at 37°C. There are also significant differences of antimicrobial activity among selected human pathogens. It showed that highest antimicrobial activity for *E. faecalis*, also it represented antimicrobial activity for both of gram negative and positive pathogens. While *S.alba* showed same antimicrobial activity against *E. faecalis* and *S. aureus*, inhibition zone is absent for *E.coli*.

Based on Table 3, moderate antibacterial activity was showed by *T. foenum-graecum* and *N. sativa* against *E. coli* & *E. faecalis and E. coli* respectively. There is no any inhibition zone against *S. aureus* by seeds extract of *T. foenum-graecum* but it showed relative values of inhibition zones against both of *E. faecalis* and *E. coli*. There is no any inhibition zone against *E. faecalis* by *N. sativa*, and inhibition zones are represented against *S. aureus* and *E. coli* human pathogens respectively.

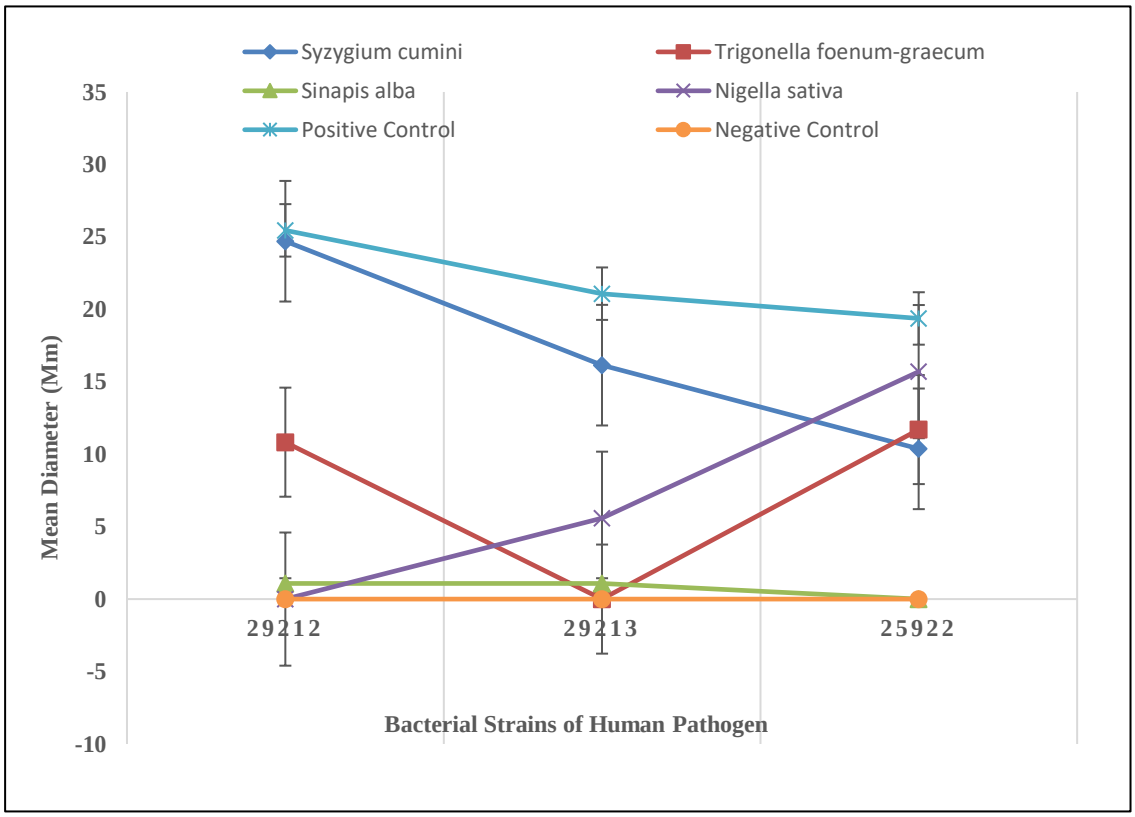
Positive and negative control are represented by *Streptomycin* and distilled water respectively. When consider about positive and negative controls, Streptomycin exhibited the highest inhibitory effect against *E. faecalis* and the lowest inhibitory effect against *E. coli*. In negative controls there were no any inhibitory zones.

Table 1: Medicinal plant seeds tested for their antibacterial activity in the study

Botanical name	Family name	Common name		
		Sinhala	**Tamil**	**English**
Syzygium cumini	Myrtaceae	*Mahadan*	*Naval*	Black Plum
Sinapis alba	Brassicaceae	*Aba*	*Kaduku*	Mustard
Trigonella foenum-graecum	Fabaceae	*Asumodhagam*	*Vendayam*	Fenugreek
Nigella sativa	Ranunculaceae	*Kaluduru*	*Karumjeerakam*	Black cumin

Table 2: Pure bacterial cultures

Name	Type	ATCC No.
Enterococcus faecalis	Gram positive	29212
Staphylococcus auereus	Gram positive	29213
Escherichia coli	Gram negative	25922



Variation of mean diameter of seeds extracts of selected medicinal plants against selected human pathogens at 37℃. Mean followed by same letters are not significantly different by LSD at 5% level, P value <0.0001

Figure 1. Antimicrobial activity of selected seeds of anti-diabetic medicinal plants

Table 3: Bacterial growth inhibition zones of selected seeds of anti-diabetic medicinal plants

Plant species	Diameter of the inhibition zones (mm)		
	29212	**29213**	**25922**
Syzygium cumini	24.7±0.37	16.15±1.20	10.37±1.51
Sinapis alba	1.08±2.65	1.08±2.65	0
Trigonella foenum-graecum	10.83±0.34	0	11.7±1.97
Nigella sativa	0	5.58±4.37	15.7±1.56
Positive control	25.45±1.18	21.08±0.26	19.37±1.35
Negative control	0	0	0

Discussion

Based on this *in vitro* study the researchers found that the ethanolic seed extract of S. *cumini* had shown highest anti-bacterial activity against selected bacterial strains. *T. foenum-graecum* seed extract also shown higher antibacterial effect against some bacterial strains. But, *S.alba* had shown least antibacterial activity. Present study results could be comparable with the previous studies which were stated that the seed phenolic extract of *S.cumini* showed antibacterial activity against tested bacterial strains (Santos et al., 2020); the methanol fraction of ethanol extract from the seeds of *S. cumini* was found to have significant antibacterial activity (Yadav et al., 2011; Patoary et al., 2014; Das, Das & Dharani, 2019). However, the present results of *S.alba* is not in line with another study which was done by the Boscaro et al., 2018. That study found that the *S.alba* seed hydroalcoholic extract was effective against *E. coli* and *S. aureus* in disc diffusion test (Boscaro et al., 2018). The hexane extract of *S. alba* seeds showed the highest anti-microbial activity (Sujatha & Mariajancyrani, 2013). Sharma et al found that the methanol extract of *T. foenum-graecum* seeds shown maximum zone of inhibition against *E. coli* and *Staphylococcus* (Sharma, Singh & Rani 2017). Another study found that the oil which was extracted from fenugreek seeds has a good antimicrobial activity against some bacteria (Sara & Abdalbasit, 2022). Further another study found that the *N. sativa* seed oil had a strong antibacterial activity significantly (P<0.01) greater inhibition zone than that of gentamicin (Forouzanfar, Bazzaz & Hosseinzadeh, 2014). Bakathir and Abbas informed that the *N. sativa* ground seeds possessed antibacterial effect against the staphylococcus (Bakathir & Abbas 2011).

Based on all of the representation of antimicrobial activity, it showed that highest antimicrobial activity is showed by *S. cumini* rather than other extracts, also when consider about phytochemical screening there is highest representation in seeds extracts of *S. cumini*. Antimicrobial and antibiotic principle are highly showed by phytochemical compounds such as alkaloids, saponins, tannins, flavonoids and steroids which are known to be biologically active (Nethathe & Ndip, 2011; Patra, 2012; Mujeeb, Bajpai & Pathak, 2014; Ali et al. 2018; Pizzi, 2021; Nek Rahimi et al., 2022). Since Gram positive bacteria's cell walls are more permeable than Gram negative bacteria, whose outer membrane has a lipopolysacharide layer that prevents some antibiotics and antibacterial compounds from penetrating, most plant extracts are thought to be more effective against Gram positive bacteria (Wintola & Afolayan, 2015). Among the all-selected medicinal plants, *S. cumini* showed in high level of antimicrobial activity. Plant produces a range of chemical constituents to protect themselves from the attack of various pathogenic micro-organisms. Substances can either prevent the growth of microorganism or kill them. It can be considered as resources for developing new drugs for various infectious diseases. Antibiotic substances are recognized to vary in concentration in different tissues of same plant, between plants of same and different species and concentration of antibiotics in plant is determined by its environment (Sushil Kumar, Bagchi & Darokar, 1997). In seeds, reserve materials are starch, fixed oils, proteins, fixed oils, fatty acids, some proteins are known to possess good antimicrobial activity and antifungal protein (Cowan, 1999).

Conclusion

This research on the antimicrobial activity of the herbs helps to develop effective herbal remedies as anti-microbial activity to reduce the infection in diabetes. The result shows that *S. cumini* seeds extract poses the highest antimicrobial activity in gram positive and negative bacteria. Therefore, this seed is potential to be further developed as an herbal antibiotic for the management of infection in diabetes in future. More detail study such as are fractionation and characterization of active phytochemicals which are responsible for the antimicrobial activity, as well as *in-vivo* activities recommended to be conducted in the future study.

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Conflict of Interest

Authors have no conflict of interest.

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