Comparative analysis of the antibacterial properties of moringa flower (Moringaoleifera) chloroform extract and moringa leaf against food-borne bacteria

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ABSTRACT

Aim: To evaluate and compare the antibacterial activity of moringa flower and moringa leaf *(Moringa oleifera)*against food spoiling bacteria. **Materials and methods:** Chloroform extract of moringa flower (N=6) and moringa leaf (N=6) were prepared and the antibacterial activity was carried out using Agar-well diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. G power was kept as 80% and alpha 0.05. **Results:** The zone of inhibition of the moringa flower chloroform extract was 2 mm against *S.aureus* and 3 mm against *P.aeruginosa*, whereas the zone of inhibition of the moringa leaf chloroform extract was 6 mm and 10 mm against *S.aureus* and *P.aeruginosa*, respectively.The mean value of % of susceptibility for moringa flower chloroform extract against *S.aureus* is 34.36, while it is 33.34 against *P.aeruginosa*. Moringa leaf chloroform extract had a mean value of 43.70 against*S.aureus* and 84.14 against *P.aeruginosa*. **Conclusion:** The antibacterial activity of Moringa flower and leaf chloroform extract was performed and moringa leaf extract exhibited better antibacterial activity than moringa flower extract.

Keywords: Novel Antimicrobial Activity, *Moringa oleifera*, Antibacterial, Susceptibility, Zone of Inhibition, Chloroform Extract

INTRODUCTION

Food spoilage is caused by food contamination, which can occur at any point along the food manufacturing, delivery, and consumption chain.This can lead to foodborne diseases or food poisoning. Bacteria such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and others are responsible for the majority of food poisoning cases (Mostafa et al. 2018). Diarrhoea and vomiting are the most prevalent symptoms of food poisoning.These contamination of food can be prevented by the addition of chemical preservatives or natural preservatives . The persistent use of chemical preservatives can have disastrous effects on human health due to its toxicity. This difficulty enables customers and manufacturers to rely on natural alternatives to prevent food deterioration and foodborne illness, such as utilizing plant extract (Gull et al. 2016) due the assumption that naturally generated compounds from plants can help to control food pathogens (Hsieh, Mau, and Huang 2001). Plants have served as natural

sources of compounds with pharmacological and nutritional qualities that have assisted people in the prevention and treatment of various ailments (Brilhante et al. 2017). Moringa oleifera is a plant that belongs to the family Moringaceae. Almost all parts of the plant are used as food or have some beneficial properties . *M. oleifera* Lam. is a nutrient-dense edible plant that is high in proteins, amino acids, minerals, and vitamins. Polysaccharides, flavonoids, alkaloids, glucosinolates, and isothiocyanates are some of the bioactive phytochemicals found in it (Wang et al. 2021). The leaf contains bioactive chemicals that have physiological and biochemical activities that are useful to humans and also has anti-cancer. Antibacterial,antifungal properties Due to its industrial, medicinal and nutritional values, *Moringa oleifera* can be an excellent source to explore for antibacterial activity, and its discovery could lead to more widespread use in food preservation, medicine research, and other fields.Previously our team has a rich experience in working on various research projects across multiple disciplines(Madhesh et al. 2021; Bishir et al. 2020);(Vimalraj et al. 2020; Sivasamy, Venugopal, and Mosquera 2020)(Madhesh et al. 2021; Bishir et al. 2020)

In the last five years, 16 research articles have been published in Science Direct and 905 research articles have been published in Google Scholar. The chloroform extract of *Moringa oleifera* leaf exhibited antibacterial activity against S.aureus The flower pods extracts of *Moringa oleifera* using chloroform inhibited the growth of *E.coli* , *B.subtilis* showing antibacterial activity (Gull et al. 2016). *Ageratum conyzoides* leaf chloroform extract showed bacterial inhibition against *S.aureus*(Sarvesh Kumar*1,2, Vijay Jyoti Kumar2, Ranjit Singh 2020). Chloroform extract of Moringa leaf powder possessed antibacterial activity against *E.coli* and *S. aureus*(Ahaotu et al. 2018). The antibacterial activity of the extract is determined by the kind of solvent used and the method of extraction (Adetitun et al. 2013) because the solvent removes both polar and nonpolar molecules.

When compared to other parts of *Moringa oleifera*, less study has been done on the antibacterial properties of Moringa flower against food pathogens (Bancessi et al. 2020). Hence as a result, the goal of this research is to assess and compare the antibacterial activity of moringa flower chloroform extract versus moringa leaf chloroform extract against food borne bacteria.

MATERIALS AND METHODS

The research was accomplished in the microbiology laboratory of Saveetha School of Engineering, Chennai. There are two groups in this study. Moringa flower (N=6) is the study group, followed by moringa leaf (N=6), which is the control group. The Total sample size is 12. The aforementioned sample size was generated with clincalc.com using the previously given data from (Mostafa et al. 2018), with a pretest power of 80%.

Moringa flower extract preparation

Fresh moringa flowers were purchased from a local market, washed, and air-dried for approximately 5 days. These dried flowers were then ground and sieved to produce a fine moringa flower powder. In a beaker, 10 grams of moringa

flower powder are combined with 50 ml of chloroform and covered with aluminium foil. The combination was then stored for 48 hours in a shaking incubator at 37.2°C. The resultant mixture was then filtered using a whatman filter paper after passing through a muslin cloth. The filtrate was then dried for 30 hours at 40°C in a hot air oven, and the dried extract was scraped and resuspended in known concentration chloroform. As a result, a chloroform extract of Moringa flower was created.

Moringa leaf extract preparation

Fresh moringa leaves were purchased from a local market, washed, and air-dried for approximately 5 days. These dried moringa leaves were then ground and sieved to produce a fine moringa leaf powder. In a beaker, 10 grams of moringa leaf powder are combined with 50 ml of chloroform and covered with aluminium foil. The mixture was then stored for 48 hours in a shaking incubator at 37.2°C. The resultant mixture was then filtered using a whatman filter paper after passing through a muslin cloth. The filtrate was then dried for 30 hours at 40°C in a hot air oven, and the dried extract was scraped and resuspended in known concentration chloroform. As a result, a chloroform extract of Moringa leaf was created.

Inoculum preparation

A loop of inoculum or 0.5 ml of the inoculum is taken from the mother culture and is suspended into the nutrient broth to make up the primary culture.therefore a primary culture of *Staphylococcus aureus*and *Pseudomonas aeruginosa* was prepared.

Media preparation

Standard Mueller Hinton agar was prepared using the standard instruction manual from the manufacturer. The agar was then poured onto the plates and allowed it to solidify. After solidification,using the back of the microtip, a well is made on the agar.

Antibacterial assay of *M.oleifera* leaf and flower extract

Using a sterile cotton swab, the inoculum were swabbed onto the agar in their respective labelled agar plates. 50 µL of the moringa flower and leaf extract were then poured into each well of their respectives plates. The extracts are then allowed to dissolve into the agar for 5 minutes.This method is the Agar-well diffusion method. The plates were then kept in an incubator at 37.2°C for 24 hours. Meanwhile, Nutrient broth is poured on a 96-well plate and inoculated with the organisms along with the extracts to observe the optical density. The data collected for this study includes the zone of inhibition (mm) and percentage of susceptibility(%)

Statistical analysis

The antibacterial activity of Moringa flower and leaf was determined statistically using IBM SPSS version 28.0.0.0. (190). There are no independent variables, and the optical density (O.D) value is the dependent variable. The mean, standard deviation, and ANOVA were all analysed(Mostafa et al. 2018).

RESULTS

M.oleifera is considered one of the new infection-fighting strategies in controlling bacterial infections. The antibacterial activity of chloroform extract

of *M. oleifera* is shown in terms of % of susceptibility in Table 1.

From Table 2, the mean value of % of susceptibility for moringa flower chloroform extract against *S.aureus* is 34.36, while it is 33.34 against *P.aeruginosa.* Moringa leaf chloroform extract had a mean value of 43.70 against*S.aureus* and 84.14 against *P.aeruginosa*.

Table 3 represents that Moringa leaf chloroform extract is statistically significant with p value (<0.001) and the Moringa flower chloroform extract is statistically insignificant (p=0.545). The chloroform extract of leaves and flowers expressed a relatively antibacterial effect against *S. aureus* and *P. aeruginosa* with their individual diameter zones of inhibition recorded ranging from 2 mm to 10 mm.

Figure 1 shows the comparison of mean percentage of susceptibility obtained for moringa flower chloroform extract and moringa leaf chloroform extract against *S.aureus* and*P.aeruginosa.* The chloroform extract of the leaves displayed a pronounceable better antibacterial effect against the tested *S. aureus* and *P. aeruginosa*.

DISCUSSION

From the results, it is evident that the chloroform extract of moringa leaf extract had better antibacterial activity than moringa flower chloroform extract. The moringa flower chloroform extract had a zone of inhibition of 2 mm against *S.aureus* and around 3 mm against *P.aeruginosa* whereas the moringa leaf chloroform extract exhibited a zone of inhibition 6 mm and 10 mm against *S.aureus* and *P.aeruginosa*respectively. The mean value obtained for moringa flower chloroform extract against *S.aureus* is 34.36 whereas for *P.aeruginosa* is 33.34 . The mean value for moringa leaf chloroform extract against *S.aureus* is 43.70, while it is 84.14 against*P.aeruginosa*. Hence the result of this study shows Moringa leaf chloroform extract had the broadest spectrum against the tested bacteria.

Moringa leaf chloroform extract has been shown to have antibacterial action in numerous investigations. Emad observed a zone of inhibition of 11.0 mm against S.aureus using chloroform extract of moringa leaf at 200 mg/ml concentration (Abdallah 2015). However this is not on par with the findings observed in this study. This could be due to various factors like environmental , or storage conditions etc. Ahaotu observed 13.92 mm of zone of inhibition against *S.aureus* using chloroform extract of moringa leaves at 100 mg/ml concentration (Ahaotu et al. 2018). However the result obtained is not in harmony with the results obtained in this study. *Moringa oleifera* chloroform leaf extract had a zone of inhibition of 9.5 mm against *P.aeruginosa* and 6.2 mm against *S.aureus* observed by Devendra (Devendra et al. 2011). This however the result is almost in accordance with the result observed in this study. Novel antimicrobial activity of chloroform extract of leaves of *Moringa oleifera* exhibits bacterial inhibition that ranges from 7 mm to 11 mm in different concentration against *S.aureus*(Kalpana, Moorthi, and Kumara 2013), also the results obtained is in

harmony with the results obtained in this study.

The antibacterial action of the Moringa plant can be further investigated by phytochemical investigations and other methods, which could lead to the discovery of chemicals that could be used in food preservation, drug development, and other applications.

CONCLUSION

The novel antimicrobial activity of moringa flower chloroform extract and moringa leaf chloroform extract was performed and it is observed that both the chloroform extract possess antibacterial activity. However, Moringa leaf chloroform extract was more effective than moringa flower and can be used for food preservation to prevent spoilage of food. Moringa leaf chloroform extract had a mean value of 43.70 against*S.aureus* and 84.14 against *P.aeruginosa*.

DECLARATIONS

Conflict of interest

In this manuscript, there are no conflicts of interest.

Authors Contribution

Author KS was involved in sample preparation, inoculum preparation, data analysis and manuscript writing. Author SS was involved in processing the idea, guidance, data verification and critical review of the manuscript.

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TABLES AND FIGURES

Table 1. Percentage of susceptibility (%) obtained for Moringa flower and Moringa leaf chloroform extract

	***S. aureus* (%)**	***P. aeruginosa* (%)**
MORINGA FLOWER	32.30	31.41
	34.56	33.64
	36.23	34.98
MORINGA LEAF	40.26	81.41
	43.88	84.76
	46.97	86.25

Table 2. ANOVA analysis of % of susceptibility obtained for moringa flower chloroform extract and moringa leaf chloroform extract (Mean and standard error of mean)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MORINGA FLOWER	*Staphylococcus aureus*	3	34.3633	1.97237	1.13875	29.4637	39.263	32.3	36.23
	Pseudomonas aeruginosa	3	33.3433	1.80339	1.04119	28.8635	37.8232	31.41	34.98
	Total	6	33.8533	1.7802	0.72676	31.9851	35.7215	31.41	36.23
MORINGA LEAF	*Staphylococcus aureus*	3	43.7033	3.35849	1.93902	35.3604	52.0463	40.26	46.97
	Pseudomonas aeruginosa	3	84.14	2.47885	1.43117	77.9822	90.2978	81.41	86.25
	Total	6	63.9217	22.3049	9.10592	40.5141	87.3292	40.26	86.25

Table 3. ANOVA analysis of chloroform extract of leaves and flowers showed a relatively antibacterial effect against *S. aureus* and *P. aeruginosa* with their individual diameter zones of inhibition recorded ranging from 2 mm to 10 mm

		Sum of Squares	df	Mean Square	F	Sig.
MORINGA FLOWER	Between Groups	1.561	1	1.561	0.437	0.545
	Within Groups	14.285	4	3.571		
	Total	15.846	5			
MORINGA LEAF	Between Groups	2452.69	1	2452.69	281.527	<.001
	Within Groups	34.848	4	8.712		
	Total	2487.53	5			

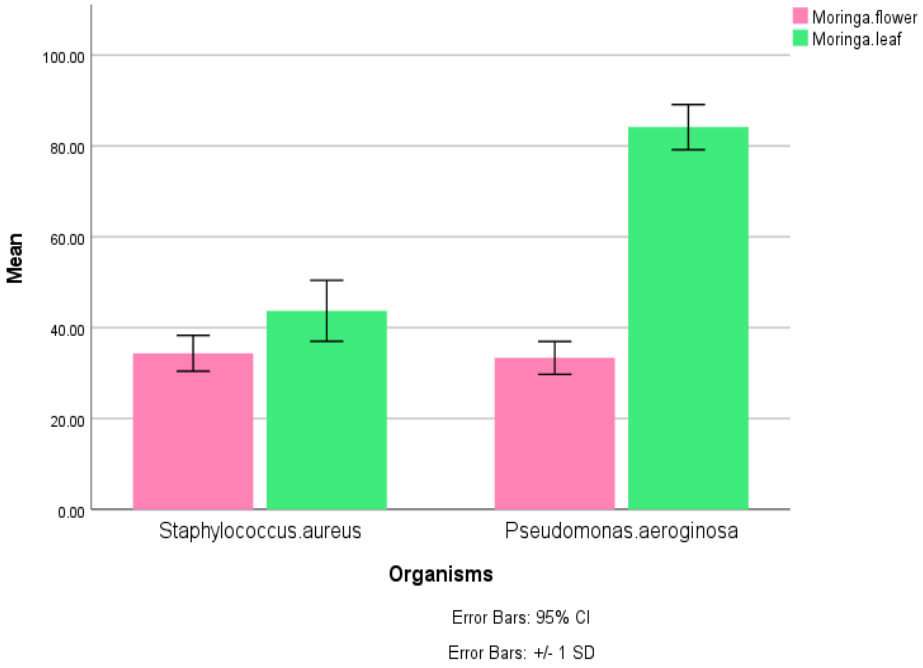

Fig. 1. Bar chart representing the comparison of mean % of susceptibility obtained for moringa flower chloroform extract and moringa leaf chloroform extract against *S.aureus* and*P.aeruginosa.* X axis: *Staphylococcus aureus* and *Pseudomonas aeruginosa*; Y axis: Mean % of susceptibility. SD+-1.