

A Panoramic View of Phytochemical Analysis and Antibacterial Potential of *Moringa oleifera*

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Abstract

Moringa oleifera belongs to moringaceae family. Moringa derives from the Tamil word murungai. The Chinese name, pronounced la mu in Mandarin & lat mok in Cantonese. Evaluation of antibacterial activities from fresh and shade dry leaf extracts i.e. Aqueous Ethanolic, Chloroform & Methanolic were using filter paper disc & agar well diffusion method. The antibacterial activities of these leaf extracts were investigated against some pathogenic group of bacteria (Achromobacter, Bacillus, Coagulus Negative Staphylococcus (CONS), Ε. coli, Enterobacter, Enterococci. Klebsiella. Proteus, Pseudomonas. Staphylococcus aureus). The different crude extract showed remarkable & significant activity against the growth of bacteria. This investigation suggests that the extracts of Moringa oleifera can be use to discover antibacterial agent for control of pathogenic bacteria responsible for severe illness. It has a wide range of phytochemical compounds in the different leaf extract. The phytochemical screening indicated the presence of glycosides, alkaloids, saponins, tannins, reducing sugar, flavanoids, volatile oils, terpenoids etc, in the extracts. Keywords: Antibacterial Activity Aqueous, Chloroform, Ethanolic, and Methanolic extract, Phytochemical Activity, Moringa oleifera.

Introduction

Moringa oleifera is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. M. oleifera parts are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia⁽¹⁾. Moringa oleifera Lam is one of the best known, widely distributed and grown species of a monogeneric family moringaceae⁽²⁾. The plant is referred to as drumstick tree or the horse radish tree. Moringa seeds are also known for its coagulation properties for treatment of water and waste water $^{(3, 4)}$. The seeds are used as a sexual virility drug for treating erectile dysfunction in men and also in women for prolonging sexual activity. Previous studies have reported the antimicrobial properties of the various parts of Moringa roots, flowers, bark and stem including seeds (5, 6).

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Materials and Methods

- **Plant Collection:** Healthy disease free, mature fresh plant leaves were collected locally from Kota, District-Bilaspur (C.G.), India. Fresh leaves were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried without any contamination. The dried leaves were then powdered using a mortar pestle.
- Preparation of Leaf Extracts:
- Aqueous extract of fresh & dried leaves 100 g of fresh & dried leaves of *Moringa oleifera Lam*. were weighed out and crushed directly by grinder and dipped into 400 ml cold distilled water into an each conical flask stoppered with rubber corks and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask ⁽⁷⁾.
- Ethanol (95%) extract of fresh & dried leaves 100 grams of fresh & dried leaves of *Moringa oleifera Lam.* were weighed out and crushed directly by grinder and dipped into 400 ml ethanol (95%) into an each conical flask stoppered with rubber corks and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask.
- Chloroform extract of fresh & dried leaves For preparation of chloroform extract, 100gm of fresh & dried leaves of M. oleifera were crushed in mortar pestle and added in 200ml chloroform and left for overnight at room temperature. After 24 hours the extracts were separated using sterile muslin cloth and filter through sterile Whattman filter paper (no. 02).
- Methanol extract of fresh & dried leaves For preparation of methanol extract, 50gm of fresh & dried leaves of *M. oleifera* were crushed in mortar pestle and sequentially extracted by shaking for 2 hours on Wrist Action Shaker after overnight soaking in 150 ml of relevant solvent. After filtration, samples were rinsed with additional 3 x 60 ml portions of the solvent. Combined filtrates were dried at room temperature under electric fan ⁽⁸⁾
- Phytochemical Screening of Leaf Extracts:
- Alkaloids: 2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.
- Saponins: Saponins were detected using the froth test 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then

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allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

- Tannins: To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.
- Terpenoids: 4mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid.
- Flavonoids: 4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.
- Glycosides: 25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.
- Volatile oils: 2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.
- Reducing Sugars: To 0.5ml of plant extracts and 5-8 drops of Benedict solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.
- Collection and Maintenance of Bacterial Sample: The bacterial sample was collected from Chhattisgarh Institute of Medical Science (CIMS), Bilaspur. The sample was some pathogenic group of bacteria (Achromobacter, Bacillus, Coagulus Negative Staphylococcus (CONS), Ε. coli. Enterobacter, Enterococci, Klebsiella, Proteus. Pseudomonas. Staphylococcus aureus).

Results and Discussion

Phytochemical Analysis:

The phytochemical analysis of aqueous extracts using fresh and shade dry nature was showed in Table 1. Figure 1 showed the positive test of different phytochemical components. The fresh aqueous extract of *Moringa olifera* showed the presence of flavanoids, and saponins. Tannins, saponins, reducing sugar, and glycosides were observed in shade dry aqueous extract of *Moringa olifera*.

Antibacterial Studies:

The antibacterial activity of fresh and dry extract of *Moringa oleifera* leaves (i.e. aqueous, methanol, ethanol, chloroform) was investigated using filter paper disc method and agar well diffusion method against the selected human pathogenic bacteria. Table 2 & 3 shows diameter of zone of inhibition (in mm) of bacterial

Extract Used for	Fresh Aq.	Shade Dry Aq.
Extraction	Extract	Extract
Alkaloids	-	-
Saponins	+	+
Tannins	-	+
Terpenoids	-	-
Flavonoids	+	-
Glycosides	-	+
Volatile oils	-	-
Reducing Sugars	-	+

Table 1: Phytochemical Characteristics of LeafExtract of Moringa oleifera

growth and their values are presented as mean because the experiments were set in triplicate mode.

In this study the antibacterial activity of fresh and shade dried extract of aqueous, methanol, ethanol and chloroform against *Achromobacter* was studied, the respective diameter of zone of inhibition 30 ± 0.08 , 25 ± 0.19 , 15 ± 0.96 , 21 ± 0.78 and 22 ± 0.34 , 26 ± 0.16 , 21 ± 0.72 , 19 ± 0.53 and; *Bacillus* was 25 ± 0.41 , 16 ± 0.43 , 12 ± 0.84 , 15 ± 0.94 and 14 ± 0.01 , 14 ± 0.79 , 20 ± 0.01 , 12 ± 0.75 . The diameter of zone of inhibition against *Achromobacter* and *Bacillus* showed in figure 2 (a).

Thus, the antibacterial activity of fresh and dry extract of aqueous, methanol, ethanol and chloroform against Coagulus *Negative Staphylococcus (CONS)* was studied and the respective diameter of zone of inhibition 20 ± 0.05 , 23 ± 0.58 , 20 ± 0.68 , 24 ± 0.69 and 24 ± 0.67 , 17 ± 0.79 , 22 ± 0.76 , 21 ± 0.14 and; *Escherichia coli* was 19 ± 0.30 , 21 ± 0.34 , 10 ± 0.27 , 11 ± 0.18 and 16 ± 0.24 , 19 ± 0.02 , 09 ± 0.14 , 10 ± 0.65 . The diameter of zone of inhibition against *Coagulus Negative Staphylococcus (CONS)* and *Escherichia coli* showed in figure 2 (b)

Here the extensive antibacterial effect of fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Enterobacter* was studied and the respective diameter of zone of inhibition 24 ± 0.02 , 21 ± 0.32 , 15 ± 0.51 , 18 ± 0.73 and Nil, 13 ± 0.59 , 15 ± 0.48 , 16 ± 0.86 and; *Enterococci* was 32 ± 0.04 , 25 ± 0.06 , 24 ± 0.70 , 21 ± 0.28 and 29 ± 0.65 , 24 ± 0.01 , 22 ± 0.31 , 35 ± 0.46 . The diameter of zone of inhibition against *Enterobacter* and *Enterococci* showed in figure 2 (c).

The antibacterial activity of fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Klebsiella* was studied, the respective diameter of zone of inhibition 21 ± 0.81 , 19 ± 0.24 , 15 ± 0.58 , 14 ± 0.90 and Nil, 13 ± 0.39 , 13 ± 0.89 , Nil and; *Proteus* was 26 ± 0.56 , 15 ± 0.30 , 09 ± 0.90 , Nil and 17 ± 0.28 , 11 ± 0.67 , 18 ± 0.41 , 07 ± 0.52 . The diameter of zone of inhibition against *Klebsiella* and *Proteus* showed in figure 2 (d).

The studies shows that antibacterial properties of fresh and dry extract of aqueous, methanol, ethanol and

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chloroform against *Pseudomonas* was obtained, the respective diameter of zone of inhibition 21 ± 0.06 , 23 ± 0.19 , 18 ± 0.25 , 08 ± 0.50 and 16 ± 0.73 , 19 ± 0.89 , 09 ± 0.45 , 10 ± 0.71 and; *Staphylococcus aureus* was 22 ± 0.08 , 23 ± 0.36 , 20 ± 0.20 , 18 ± 0.61 and 13 ± 0.21 , 24 ± 0.62 , 14 ± 0.49 , 17 ± 0.08 . The diameter of zone of inhibition against *Pseudomonas*, and *Staphylococcus aureus* showed in figure 2 (e).

In this study the antibacterial activity of fresh and shade dried extract of aqueous, methanol, ethanol and chloroform against *Achromobacter* was studied, the respective diameter of zone of inhibition 15 \pm 0.03, Nil, 10 \pm 0.26, 12 \pm 0.06 and 13 \pm 0.07, 08 \pm 0.23, 07 \pm 0.12, 10 \pm 0.86. The diameter of zone of inhibition against *Achromobacter* and *Bacillus* showed in figure 3(a).

The results obtained from in vitro antibacterial activity showed that fresh and shade dried extract of aqueous, methanol, ethanol and chloroform against *Bacillus* was studied, the respective diameter of zone of inhibition 16 ± 0.89 , 18 ± 0.56 , 23 ± 0.07 , 26 ± 0.4 and 21 ± 0.38 , 23 ± 0.40 , 25 ± 0.13 , 16 ± 0.76 . The diameter of zone of inhibition against *Bacillus* showed in figure 3(b).

The results reveal that the antibacterial activity of fresh and dry extract of aqueous, methanol, ethanol and chloroform against Coagulus *Negative Staphylococcus (CONS)* was studied and the respective diameter of zone of inhibition 22 \pm 0.19, 11 \pm 0.16, 14 \pm 0.47, 08 \pm 0.40 and 17 \pm 0.50, 09 \pm 0.63, 11 \pm 0.34, Nil. The diameter of zone of inhibition against *Coagulus Negative Staphylococcus (CONS)* showed in figure 3(c).

The growth of *Escherichia coli* was suppressed by antibacterial compounds of fresh and dry extract of aqueous, methanol, ethanol and chloroform of *Moringa oleifera* leaves and the respective diameter of zone of inhibition was 16 ± 0.49 , Nil, Nil, 09 ± 0.62 and 10 ± 0.60 , Nil, 08 ± 0.96 , Nil. The diameter of zone of inhibition against *Escherichia coli* showed in figure 3(d).

The result of this study showed that the antibacterial effect of fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Enterobacter* was studied and the respective diameter of zone of inhibition 23 ± 0.01 , 24 ± 0.47 , 16 ± 0.52 , Nil and 14 ± 0.32 , Nil, Nil, Nil. The diameter of zone of inhibition against *Enterobacter* showed in figure 3(e).

Thus, the antibacterial activity of fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Enterococci* was studied and the respective diameter of zone of inhibition07 \pm 0.84, 10 \pm 0.36, 09 \pm 0.20, Nil and Nil, 08 \pm 0.92, 11 \pm 0.46, Nil. The diameter of zone of inhibition against *Enterobacter* and *Enterococci* showed in figure 3(f).

This result, however, is at disparity with another report indicating that fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Klebsiella* was studied, the respective diameter of zone of inhibition 08 ± 0.75 , 13 ± 0.82 , Nil, 09 ± 0.79 and Nil, 12 ± 0.10 , Nil, Nil. The diameter of zone of inhibition against *Klebsiella* showed in figure 3(g).

The antibacterial activity of fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Proteus* was studied, the respective diameter of zone of inhibition 21 ± 0.58 , 19 ± 0.09 , 15 ± 0.39 , Nil and 16 ± 0.74 , Nil, Nil, Nil. The diameter of zone of inhibition against *Proteus* showed in figure 3(h).

Here the extensive antibacterial effect of fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Pseudomonas* was studied and the respective diameter of zone of inhibition 18 ± 0.06 , 12 ± 0.58 , 09 ± 0.01 , 11 ± 0.52 and 09 ± 0.34 , 11 ± 0.40 , 09 ± 0.78 , 08 ± 0.23 . The diameter of zone of inhibition against *Pseudomonas* showed in figure 3(i).

The studies shows that antibacterial properties of fresh and dry extract of aqueous, methanol, ethanol and chloroform against *Staphylococcus aureus* was obtained, the respective diameter of zone of inhibition 23 ± 0.72 , 13 ± 0.41 , 25 ± 0.89 , 14 ± 0.09 and 16 ± 0.12 , 12 ± 0.81 , 19 ± 0.64 , 13 ± 0.37 . The diameter of zone of inhibition against *Staphylococcus aureus* showed in figure 3(j).

Discussion

According to the researcher ⁽⁹⁾ studied that phytochemical analysis of *Moringa oleifera* leaves by using different solvent such as aqueous, chloroform, ethanol show the presence of tannins (cathecollic). Moringa olifera showed the presence of flavanoids, tannins, glycosides and terpenoids were found in presence of ethanol and aqueous extract. The chloroform extract of *Moringa olifera* showed the presence of alkaloids, tannins and saponins.

In filter paper disc method the aqueous and methanolic extracts of leaves posses significant antimicrobial activity against human pathogenic bacteria. However, both the aqueous and methanolic extracts of the leaf showed appreciable antibacterial activity on the human pathogens. The scientist, ⁽¹⁰⁾ evaluated antibacterial potential from the cold and hot aqueous extract of fresh and dried leaves of Moringa oleifera on some human pathogens and reported significant data. Similarly researcher (9) studied antibacterial activity from the ethanolic and chloroform extract of Moringa oleifera leaves. Both were carried out filter paper disc diffusion method for determination of antibacterial activity. The stronger antibacterial potential was showed against Achromobacter in fresh aqueous extract (30±0.08) while shade dried methanolic extract (26±0.16) also reported the effective results. The aqueous extract of fresh leaves (25 ± 0.41) showed great inhibitory property on *Bacillus* and shade dried nature of ethanolic extract

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(20 \pm 0.01) also showed great antibacterial activity. The chloroform extract of fresh leaves (24 \pm 0.69) of Moringa oleifera was more active against the *Coagulus Negative Streptococcus* (*CONS*) while shade dried aqueous extract (24 \pm 0.67) retain greater potential. In this study the Moringa oleifera fresh (21 \pm 0.34) and shade dried methanol extract (19 \pm 0.02) had more bactericidal properties against *Escherichia coli*. The strong ability of the fresh aqueous extract (24 \pm 0.02) to kills *Enterobacter* was more than other extract and shade dried nature of chloroform extract (16 \pm 0.86) had great potential.

whereas shade dried aqueous extract did not show any antibacterial effect. The present study, fresh aqueous extract (32±0.04) showed that great antibacterial activity against Enterococci and shade dried nature of chloroform extract (35±0.46) had great potential to kill *Enterococci.* The aqueous fresh extract (21 ± 0.81) had a higher zone of inhibition against Klebsiella, when compared with other than three extracts and shade dried methanol and ethanol extracts $(13\pm0.39, 13\pm0.89)$ showed approximately same result but aqueous and chloroform extract didn't show any significant result. In case of Proteus, the fresh aqueous extract (26±0.56) showed maximum zone of inhibition whereas chloroform extract had no zone of inhibition and shade dried extract of ethanol (18±0.41) showed maximum zone of inhibition. The antibacterial activity against Pseudomonas and Staphylococcus aureus of fresh (23±0.19; 19±0.89) and shade dried extract of methanol $(23\pm0.36; 24\pm0.62)$ was more than others. Moreover, the filter paper disc method the most three prominent results was obtained from shade dried extract of chloroform against Enterococci (35±0.46), than fresh aqueous extract against Enterococci (32±0.04) and the same extract showed against Achromobacter (30±0.08).

In agar well diffusion method, the antibacterial properties of the leaf extract of Moringa oleifera as shown in the present study corroborate the earlier claims by ⁽¹¹⁾, who reported that antibacterial properties of chloroform and ethanolic extract of Moringa oleifera leaves and according to their studies proved that Moringa ethanol extract was more active than chloroform extract. Similarly (12) studied antibacterial activity from the Moringa chloroform extract and Moringa aqueous extract, and the chloroform extract showed significant results than aqueous extract. The stronger antibacterial potential was showed against Achromobacter in fresh (15±0.03) and shade dried (13±0.07) aqueous extract. The chloroform extract of fresh leaves (26±0.45) showed great inhibitory property on Bacillus and shade dried nature of ethanolic extract (25±0.13) also showed great

antibacterial activity. The aqueous extract of fresh leaves (22±0.19) of Moringa oleifera was more active against the Coagulus Negative Streptococcus (CONS) while shade dried aqueous extract (17±0.50) retain greater potential but shade dried chloroform extract didn't show any kind of zone of inhibition. In this study the Moringa oleifera fresh (16±0.49) and shade dried (10±0.60) aqueous extract had more bactericidal properties against Escherichia coli whereas fresh and shade dried methanol extract; fresh ethanol and shade dried chloroform extract didn't show any antibacterial effect. The strong ability of the fresh methanol extract (24±0.47) to kills Enterobacter was more than other extract and shade dried nature of aqueous extract (14±0.32) had only potential to kill bacteria but other three extract didn't show any significant result. The present study, fresh methanol extract (11±0.46) showed that great antibacterial activity against Enterococci and shade dried nature of ethanol extract (10±0.36) had great potential to kill Enterococci while shade dried aqueous extract; fresh and shade dried nature of chloroform extract didn't show any kind of zone of inhibition. The methanol fresh (12 ± 0.10) and shade dried extract (13±0.82) had a higher zone of inhibition against Klebsiella, but other than three extracts of shade dried nature and fresh ethanol extracts had no significant antibacterial effect. In case of Proteus, the fresh (21±0.58) and shade dried (16±0.74) aqueous extract showed maximum zone of inhibition whereas other three shade dried extract and fresh chloroform extract had no zone of inhibition. The antibacterial activity against Pseudomonas of fresh aqueous (18 ± 0.06) and shade dried methanol extract (11 ± 0.40) of methanol was more than others. The methanol extract of fresh (25±0.89) and shade dried (19±0.64) leaves of Moringa oleifera against Staphylococcus aureus was more active than other extracts. The overall three prominent results in agar well diffusion method was observed in fresh extract of chloroform against Bacillus (26±0.45), than shade dried extract of ethanol against Staphylococcus aureus (25±0.89) and the same extract showed against Bacillus (25±0.13).

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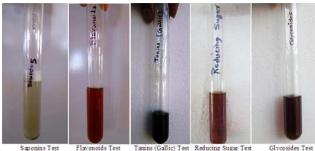


Figure 1: Phytochemical Analysis

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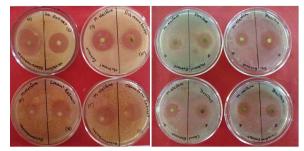


Figure 2(a): Achromobacter and Bacillus

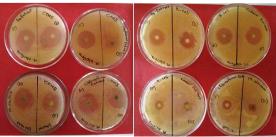


Figure 2(b): CONS and E. coli



Figure 2(c): Enterobacter and Entero

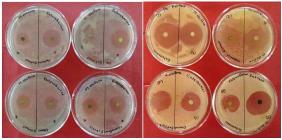


Figure 2(d): *Klebsiella* and Proteus

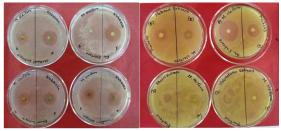
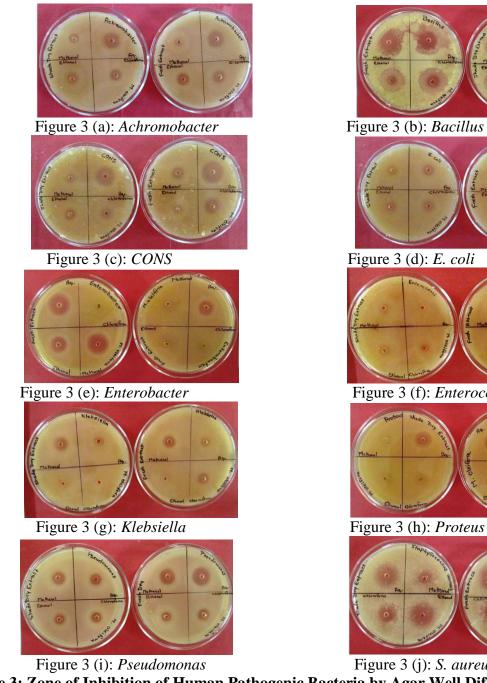


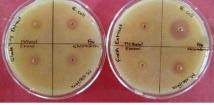
Figure 2(e): Pseudomonas and S. aureus



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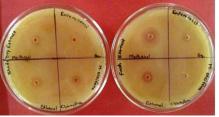


Figure 3 (f): Enterococci



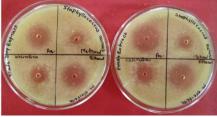


Figure 3 (j): S. aureus Figure 3: Zone of Inhibition of Human Pathogenic Bacteria by Agar Well Diffusion Method

(Filter Paper Disc Method)							
S.	Name of Bacteria	Extract					
No.		Nature	Aqueous	Methanol	Ethanol	Chloroform	
			Extract	Extract	Extract	Extract	
1	Achromobacter	Fresh	30±0.08	25±0.19	15±0.96	21±0.78	
		Shade	22±0.34	26±0.16	21±0.72	19±0.53	
		Dry					
2	Bacillus	Fresh	25±0.41	16±0.43	12±0.84	15±0.94	
		Shade	14±0.01	14±0.79	20±0.01	12±0.75	
		Dry					
3	Coagulus	Fresh	20±0.05	23±0.58	20±0.68	24±0.69	
	Negative	Shade	24±0.67	17±0.79	22±0.76	21±0.14	
	Streptococcus	Dry					
	(CONS)						
4	Escherichia coli	Fresh	19±0.30	21±0.34	10±0.27	11±0.18	
		Shade	16±0.24	19±0.02	09±0.14	10±0.65	
		Dry					
5	Enterobacter	Fresh	24±0.02	21±0.32	15±0.51	18±0.73	
		Shade	-	13±0.59	15±0.48	16±0.86	
		Dry					
6	Enterococci	Fresh	32±0.04	25±0.06	24±0.70	21±0.28	
		Shade	29±0.65	24±0.01	22±0.31	35±0.46	
		Dry					
7	Klebsiella	Fresh	21±0.81	19±0.24	15±0.58	14±0.90	
		Shade	-	13±0.39	13±0.89	-	
		Dry					
8	Proteus	Fresh	26±0.56	15±0.30	09±0.90	-	
		Shade	17±0.28	11±0.67	18±0.41	07±0.52	
		Dry					
9	Pseudomonas	Fresh	21±0.06	23±0.19	18±0.25	08±0.50	
		Shade	16±0.73	19±0.89	09±0.45	10±0.71	
		Dry					
10	Staphylococcus	Fresh	22±0.08	23±0.36	18±0.61	20±0.20	
	aureus	Shade	13±0.21	24±0.62	14±0.49	17±0.08	
		Dry					

Table 2: Antibacterial Activity of Moringa oleifera Against Human Pathogenic Bacteria (Filter Paper Disc Method)

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Table 3: Antibacterial Activity of Moringa oleifera Against Human Pathogenic Bacteria	
(Agar Well Diffusion Method)	

S.	Name of Bacteria	Extract	Diameter of Zone of Inhibition (in mm)			
No.		Nature	Aqueous	Methanol	Ethanol	Chloroform
			Extract	Extract	Extract	Extract
1	Achromobacter	Fresh	15±0.03	-	10±0.26	12±0.06
		Shade	13±0.07	08±0.23	07±0.12	10±0.86
		Dry				
2	Bacillus	Fresh	16±0.89	18±0.56	23±0.07	26±0.45
		Shade	21±0.38	23±0.40	25±0.13	16±0.76
		Dry				
3	Coagulus	Fresh	22±0.19	11±0.16	08 ± 0.40	14±0.47
	Negative	Shade	17 ± 0.50	09±0.63	11±0.34	-
	Streptococcus	Dry				
	(CONS)					
4	E. coli	Fresh	16±0.49	-	-	09±0.62
		Shade	10 ± 0.60	-	08±0.96	-
		Dry				
5	Enterobacter	Fresh	23±0.01	24±0.47	16±0.52	-
		Shade	14 ± 0.32	-	-	-
		Dry				
6	Enterococci	Fresh	07±0.84	10±0.36	09±0.20	-
		Shade	-	08±0.92	11±0.46	-
		Dry				
7	Klebsiella	Fresh	08±0.75	13±0.82	-	09±0.79
		Shade	-	12±0.10	-	-
		Dry				
8	Proteus	Fresh	21±0.58	19±0.09	15±0.39	-
		Shade	16±0.74	-	-	-
		Dry				
9	Pseudomonas	Fresh	18±0.06	12±0.58	09±0.01	11±0.52
		Shade	09±0.34	11±0.40	08±0.23	09±0.78
		Dry				
10	Staphylococcus	Fresh	23±0.72	13±0.41	25±0.89	14±0.09
	aureus	Shade	16±0.12	12±0.81	19±0.64	13±0.37
		Dry				