Fungal Genomics & Biology
ISSN: 2165-8056
OPEN ACCESS Freely available online
Research Article

Antimicrobial Activities of *Moringa oleifera* and *Psidium guajava* against Bacterial and Fungal Strains

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ABSTRACT

Introduction: *Moringa oleifera* and *Psidium guajava* could be a potential candidate for the development of new strategies to treat bacterial infections. In the present study, the antimicrobial activities of their leaf extracts in the dry and rainy seasons against some bacteria and fungus species were studied.

Materials and methods: Three strains of bacteria and one strain of fungi were *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Candida albicans* and *Aspergillus Niger*. They were collected from the Microbiology Laboratory of Nile University of Nigeria. Extractions of ethanol, methanol, and aqueous leaf extracts were prepared. The media used was Mueller Hinton Agar.

Results: Both *Moringa oleifera* and *Psidium guajava* leaf extracts have both antibacterial and antifungal properties. Their antibacterial and antifungal activities were more in the rainy season than in the dry season.

Conclusion: The results of the present study show that *Moringa oleifera* and *Psidium guajava* leaf extracts have both antibacterial and antifungal properties. Their usage or consumption should be advised in especially in rainy season compared to in dry season because their antibacterial and antifungal activities were more in the rainy season than in the dry season.

Keywords: *Moringa oleifera*; *Psidium guajava*; Antibacterial activity; Antifungal activity

INTRODUCTION

Moringa oleifera has been grown and consumed in its original areas until recently (the 1990s) when a few researchers started to study its potential use in clarifying water treatments, while only later were its nutritional and medical properties "discovered" and the species was spread throughout almost all tropical countries [1]. In 2001, the first international conference on *Moringa oleifera* was held in Tanzania and since then the number of congresses and studies increased disseminating the information about the incredible properties of *Moringa oleifera* [1]. Leaves are rich in protein, mineral, beta-carotene and antioxidant compounds. In traditional medicine, its leaves are used to treat such as malaria, typhoid fever, parasitic diseases, arthritis, swellings, cuts, diseases of the skin, genito-urinary diseases, hypertension and diabetes. They are also used to elicit lactation and boost the immune system (to treat HIV/AIDS related symptoms) [2-5].

Moringa seed powder can be used for water purification, replacing dangerous and expensive chemicals such as aluminum sulfate [2]. Leaf and seed extracts show biopesticide activity, effective against larvae and adults of *Trigoderma granarium* and can reduce the incidence of fungi on groundnut seeds [6]. The powder from the leaves of *Moringa* showed potential antibacterial activity against the gram-positive bacteria; *Staphylococcus aureus* and gram-negative bacteria i. e. *Escherichia coli* and *Pseudomonas aeruginosa* [7].

In a recent study, the extract of Pisidium guajava leaves showed the highest activity against *B. cereus*, *B. subtilis* and *E. coli* while *M. Indica's* leaves extract was effective against *S. typhi* [8]. These plants (*Moringa oleifera* and *Psidium guajava*) could be a potential candidate for the development of new strategies to treat bacterial infections. In the present study, the antimicrobial activities of *Moringa oleifera* and *Psidium guajava* leaf extracts in the dry and rainy seasons against some bacteria and fungus species were studied.

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Received: 10-Jan-2022, Manuscript No. FGB-22-45167; **Editor assigned:** 12-Jan-2022, PreQC No. FGB-22-45167 (PQ); **Reviewed:** 26-Jan-2022, QC No. FGB-22-45167; **Revised:** 31-Jan-2022, Manuscript No. FGB-22-45167 (R); **Published:** 07-Feb-2022, DOI: 10.35841/2165-8056.22.12.177

Citation: Djadjiti N, Aziz MA, Esther OK, Dane S (2022) Antimicrobial Activities of *Moringa oleifera* and *Psidium guajava* against Bacterial and Fungal Strains. Fungal Genom Biol. 12: 177.

MATERIALS AND METHODS

Microorganism species

The antimicrobial activity of different plant extracts was tested against three strains of bacteria and one strain of fungi. They were *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Candida albicans and Aspergillus Niger*. These bacterial and fungal species were collected from the Microbiology Laboratory of Nile University of Nigeria, Abuja, Nigeria.

Preparation of plants extract

The preparations of plant extracts and the medias were carried according to the method of [7,8]. However, the extraction of Mango, *Moringa* and Guava leaf oil were carried out using a Soxhlet extractor and ethanol, methanol and water as solvents.

Thus, three hundred millilitres (300 ml) of each solvent were charged into the round bottom flask of Soxhlet apparatus. Subsequently, 100 g of crushed plant leaf was charged into the thimble and fitted into the Soxhlet extractor. The apparatus was assembled. The solvents in the set-up were heated based on their respective boiling points, and the vapor produced was then condensed by water flowing in and out of the condenser. This process of heating and cooling continued for 4 hours until enough plant leaf oil was obtained. At the end of the extraction, the thimble was removed while the remaining solvent is recharged into the round bottom flask. Finally, the set-up was then re-assembled and heated for several hours to recover the solvent from the oil or a rotatory evaporator was used to recover each solvent by charging the mixture into the flask of the rotatory evaporator assembled the set-up and separate solvent from the mixture.

Extraction of aqueous leaf extract

Fifty grams (50 g) of the powdered leaves, roots and barks were weighed and poured into 500 ml conical flask in which 400 ml. of distilled water was added. The mixture was kept for 12 hours with constant shaken at 30 minutes intervals. The extract was filtered using Whatman No.1 filter paper. Extracts (filtrate) were concentrated at 40°C under reduced pressure using evaporator, and then kept in a glass flask. The semi solid extract (residue) obtained was stored in a refrigerator for further use [7].

Extraction of ethanol leaf extract

Fifty grams (50 g) of the powdered leaves, roots and barks were weighed and poured into 500 ml conical flask in which 200 ml of ethanol was added. The mixture was kept for 12 hours with constant shaken at 30 minutes intervals. The extract was filtered using Whatman No.1 filter paper. Extracts (filtrate) were concentrated at 40°C under reduced pressure using rotary evaporator, and then kept in a glass flask. The semi solid extract (residue) obtained was stored in a refrigerator for further use [7].

Extraction of methanol leaf extract

Fifty grams (50 g) of the powdered leaves, roots and barks were weighed and poured into 500 ml conical flask in which 200 ml of methanol were added. The mixture was kept for 12 hours with constant shaken at 30 minutes intervals. The extract was filtered using Whatman No.1 filter paper. Extracts (filtrate) were concentrated at 40°C under reduced pressure using rotary evaporator, and then kept in a glass flask. The semi solid extract (residue) obtained was stored in a refrigerator for further use [7]. The extracts: crude aqueous extract, crude ethanol extract and crude methanol extract was used for antibacterial analyses.

Preparations of culture media

The media used was Mueller Hinton Agar (MHA), it was prepared according to the manufacturer's instruction, where 35 g of media was mixed with one liter of distilled water and enclosed in a container and autoclaved at 121°C for 15 minutes. The media were later dispensed into 90 mm sterile agar plates and left to set [7]. The agar disk diffusion method was followed to investigate the antimicrobial activity of plant extracts [8]. 0.1 ml of TSB broth culture of the test organisms were firmly seeded over the Mueller-Hinton agar plates. Then paper disks soaked in crude extracts of different concentration of plant extracts were placed on the surface of agar using sterile forceps [8]. The culture plates were kept at low temperature (4°C) for 2-4 hours and incubated at 37°C for 24 hours [8]. After the incubation period formation of zones around the disks, confirms the antimicrobial activity of the respective extracts [8]. Absence of any kind of growth after 24 hours showed that the plates were sterile [7,9].

Antimicrobial screening test procedure

This was done following a modified method Agar diffusion technique by displaying the screen of antibacterial and antifungal sports of various solvent extracts as displayed through [3]. One ml of sparkling bacterial or fungi tradition changed into pipetted with inside the middle of sterile Petri dish. Molten cooled Muller Hinton agar (MHA) for microorganism lines or Potato dextrose agar (PDA) for fungi changed into then poured into the Petri dish containing the inoculum and blended properly.

Upon solidification, wells had been made the use of a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, a hundred ml of every extract (20% w/v) changed into delivered to respective wells. The awareness of extracts (20% w/v) has been decided on primarily based totally on our pre-experiments, and former literature. The plates had been positioned withinside the fridge for 30 min to permit the extracts diffusion properly into the agar. Then, the plates had been incubated at 37°C for 18 hours.

Antimicrobial interest changed into detected through measuring the quarter of inhibition (which includes the wells diameter) regarded after the incubation period. DMSO at an awareness of 10% changed into hired as a bad control.

Determination of minimum inhibitory concentration (MIC)

All examined extracts exhibited antimicrobial pastime at an awareness of 20% (w/v). Therefore, this awareness became manipulated to decide their minimal inhibitory concentrations (MIC) the usage of agar properly diffusion method, and to assess their effectiveness in controlling meals pathogens and spoilage microorganisms [10]. Different concentrations 10, 5, 2.5, and 1.25% had been organized through two-fold serial dilution. 1 ml of every organized inoculum became pipetted into sterile Petri dishes observed through the addition of molten agar and blended properly. Then, 4 wells had been made on every plate, and a hundred ml of 10, 5, 2.5, and 1.25% of every extract became transferred to the respective wells. Plates had been stored withinside the fridge for 30 min after which incubated at 37°C

for 18 hours. The MIC was considered as the bottom awareness which inhibited the increase of the respective microorganisms. All assays had been completed in triplicate. DMSO became served as a manage for ethanolic extracts and distilled water became used as a manage for water extracts.

RESULTS AND DISCUSSION

In Table 1 shows the antimicrobial activities of *Moringa oleifera* and *Psidium guajava* leaf extracts in the dry season against some gram-positive and gram-negative bacteria. In general, as concentration of extract increases, the level of antibacterial activity (zone of inhibition) increases. Their antibacterial activities in this dry season were found to be lower compared to the rainy season.

In Table 2 shows the antimicrobial activities of *Moringa oleifera* and *Psidium guajava* leaf extracts in the rainy season against some gram-positive and gram-negative bacteria. In general, as concentration of extract increases, the level of antibacterial activity (zone of inhibition) increases. Their antibacterial activities in this rainy season were found to be higher compared to the dry season.

Table 1: Antimicrobial Activities of *Moringa oleifera* and *Psidium guajava* leave extracts on bacterial and fungal isolates during the dry season.

		Zone of inhibition (mm)						Zone of inhibition (mm)			
Extract	Conc. (µg/ml)	EC	ST	SA	AN	Extract	Conc. (µg/ml)	EC	ST	SA	AN
MLE	10%	2.40	2	2.74	1.75	GLE	10%	1.87	0	4.45	0.00
	5%	2	2.01	2.74	1.84		5%	2	0	4	0.00
	2.50%	2.01	1	2.70	2.10		2.50%	1.65	1.45	0	0.00
	1.25%	2.01	00.0	2.70	1.34		1.25%	2.5	1.97	2.78	0.00
	MIC (µg/ml)	>3 x10^3	>5 x10^3	6 x10^3	4 x10^3		MIC (µg/ml)	3 x10^3	>1 x10^3	>3 x10^3	0.00
	MBC (µg/ml)	3 x10^3	6 x10^3	4 x10^3	3 x10^3		MBC (µg/ml)	>3 x10^3	2 x10^3	2 x10^3	0.00
MLM	10%	2.46	2.10	2	1.90	GLM	10%	2.49	1.45	0.96	1.85
	5%	1.75	2.10	1.98	1.85		5%	2.85	1.5	0.00	0.00
	2.50%	2.09	2	1.90	2		2.50%	2	1.87	2.68	0.00
	1.25%	2	2.01	1.90	1.67		1.25%	2.7	2.15	3	0.00
	MIC (µg/ml)	2 x10^3	4 x10^3	4 x10^3	>4 x10^3		MIC (µg/ml)	4 x10^3	>2 x10^3	>3 x10^3	>1 x10^3
	MBC (µg/ml)	>1 x10^3	3 x10^3	4 x10^3	4 x10^3		MBC (µg/ml)	>3 x10^3	2 x10^3	3 x10^3	1 x10^3
MLA	10%	6	5	6.65	4	GLA	10%	4	3	4.25	3
	5%	5.86	3.85	6	4.45		5%	3.56	4	4	0.00
	2.50%	6	5.7	7	4.15		2.50%	3	2.54	1.85	1.35
	1.25%	6	5.15	5.75	4.40		1.25%	3	3	3.45	1.15
	MIC (µg/ml)	>6 x10^3	6 x10^3	4 x10^3	6 x10^3		MIC (µg/ml)	>4 x10^3	5 x10^3	5 x10^3	>2 x10^3
	MBC (µg/ml)	5 x10^3	5 x10^3	3 x10^3	6 x10^3		MBC (µg/ml)	4 x10^3	4 x10^3	5 x10^3	2 x10^3

Note: MLE: *Moringa* Leave Ethanolic Extract, MLM: *Moringa* Leave Methanolic Extract, MLA: *Moringa* Leave Aqueous Extract, GLE: Guava Leave Ethanolic Extract, GLM: Guava Leave Methanolic Extract, GLA: Guava Leave Aqueous Extract, MIC: Minimum Inhibition Concentration, MBC: Minimum Bactericidal Concentration, EC: *E. coli* , ST: *Salmonella typhi*, SA: *Staphylococcus aureus* , AN: *Aspergillus niger*.

Table 2: Antimicrobial Activities of *Moringa oleifera* and *Psidium guajava* leave extracts on bacterial and fungal isolates during the rainy season.

		Zone of inhibition (mm)						Zone of inhibition (mm)			
Extract	Conc. (mg/ml)	EC	ST	SA	AN	Extract	Conc. (mg/ml)	EC	ST	SA	AN
MLE	10%	10.15	10	12.50	11.75	GLE	10%	1.95	0	5	1.15
	5%	9	10.15	11.6	10		5%	2.45	0.98	4.45	0.00
	2.50%	11.5	1	13	9		2.50%	1.90	1.68	0.00	0.00
	1.25%	12.15	5	10	5.15		1.25%	2.89	2.25	3	0.00
	MIC (µg/ml)	4 x10^3	>4.6 x10^3	6 x10^3	5 x10^3		MIC (µg/ml)	>2 x10^3	2 x 10^3	4 x10^3	2 x10^3
	MBC (µg/ml)	4 x10^3	5 x10^3	5 x10^3	5 x10^3		MBC (µg/ml)	2 x10^3	2 x10^3	3 x10^3	3 x10^3
MLM	10%	8.5	6.15	5	4.95	GLM	10%	2.85	1.50	1	2
	5%	5.75	5.40	3.90	3.55		5%	3	1.59	1.45	1
	2.50%	7.55	5.25	3.70	8		2.50%	2.50	2	2.8	0.00
	1.25%	3.80	5.18	3.50	3.45		1.25%	2.98	2.60	3.65	0.00
	MIC (µg/ml)	5 x10^3	3 x10^3	3 x10^3	4 x10^3		MIC (µg/ml)	4 x10^3	2 x10^3	2 x10^3	>1 x10^3
	MBC (µg/ml)	5 x10^3	2 x10^3	3 x10^3	3 x10^3		MBC (µg/ml)	3 x10^3	2 x10^3	2 x10^3	2 x10^3
MLA	10%	24	18.47	10.45	9.19	GLA	10%	3.55	2.70	3.25	2
	5%	23.45	19	12	8.79		5%	3.15	3.4	3	0.00
	2.50%	20.65	18.26	11.15	10.55		2.50%	3.2	2.85	2.45	1.55
	1.25%	18.55	17.75	10	10		1.25%	2	3.2	2.45	1.25
	MIC (µg/ml)	>4 x10^3	4 x10^3	>5 x10^3	>5 x10^3		MIC (µg/ml)	>5 x10^3	4 x10^3	4 x10^3	>1 x10^3
	MBC (µg/ml)	4 x10^3	4 x10^3	4 x10^3	4 x10^3		MBC (µg/ml)	5 x10^3	4 x10^3	3 x10^3	2 x10^3

Note: MLE: *Moringa* Leave Ethanolic Extract, MLM: *Moringa* Leave Methanolic Extract, MLA: *Moringa* Leave Aqueous Extract, GLE: Guava Leave Ethanolic Extract, GLM: Guava Leave Methanolic Extract, GLA: Guava Leave Aqueous Extract, MIC: Minimum Inhibition Concentration, MBC: Minimum Bactericidal Concentration, EC: *E. coli* , ST: *Salmonella typhi* , SA: *Staphylococcus aureus* , AN: *Aspergillus niger*.

Medicinal plants were regarded as valuable and most useful natural resources used for the invention of new novel drugs. Many compounds were better known as attributes to the efficacies of the used medicinal plants in treating many ailments caused by microbes [11]. *Moringa* plant can be grown easily and abundantly in Nigeria. The results of the present study are consistent with the previous studies about *moringa* plant's antibacterial features. In a few previous studies showed that *moringa* seed powder can be used for water purification, replacing dangerous and expensive chemicals such as aluminum sulfate [2], its leaf and seed extracts show biopesticide activity, effective against larvae and adults of *Trigoderma granarium* and can reduce the incidence of fungi on groundnut seeds [6]. Besides, the powder from the leaves of *Moringa* showed potential antibacterial activity against the gram-positive bacteria; *Staphylococcus aureus* and gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa* [7]. In a recent study, among infected children aged 5-15 from some selected hospitals in Abuja, Nigeria, *E. coli* (47.69%) was the most dominant species, followed by *Salmonella* (24.62%), *Klebsiella* (15.38%) while *Citrobacter* species was less prevalent (1.54%) mostly in their urine samples (70.18%) [12]. Therefore, it may be recommended to drink *moringa* tea instead of black tea before or after meals as a public health measure.

In a recent study, the extract of *Pisidium guajava* leaves showed the highest activity against *Bacillus cereus, Bacillus subtilis* and *E. coli* while *M. Indica's* leaves extract was effective against *S. typhi* [8]. Therefore, it can be stated that the results of the present study are consistent with previous studies. We also need to benefit from its antibacterial and antifungal activities.

In a recent study, four different coffee samples (Gorilla's Coffee, Nescafe 3 in 1, Café Najjar and Alcafe) were examined for fungi growth using potato dextrose agar and the samples were contaminated by two fungi *Aspergillus fumigatus* from Gorilla's Coffee and *Candida albicans* from Café Najjar with their occurrence frequencies of 4.16% [13].

CONCLUSION

Therefore, it can be stated that *Moringa oleifera* and *Psidium guajava* leaf extracts has both antibacterial and antifungal properties. Their antimicrobial activities were more in the rainy season than in the dry season. Therefore, their usage or consumption should be advised in especially in rainy season compared to in dry season. The results of the present study show that both *Moringa oleifera* and *Psidium guajava* leaf extracts have both antibacterial and antifungal properties. Their usage or consumption should be advised in especially in rainy season compared to in dry season because their antibacterial and antifungal activities were more in the rainy season than in the dry season.

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