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MICROBIAL LOAD ANALYSIS OF *MORINGA OLEIFERA* LAM. LEAVES IN THE GUINEA SAVANNAH VEGETATION ZONE OF NIGERIA

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ABSTRACT
A study was carried out on the various means of drying on the microbial contents of M. oleifera leaves harvested from different zones of the Savannah vegetation of Nigeria. The microbial loads of leaf samples of M. oleifera subjected to five different drying methods (Sun-drying, Room drying, and Oven drying at varied temperatures of 50°C, 60°C, and 70°C.) and fresh leaves were analyzed using the Pour Plate techniques. Microbial load in each treatment were determined in triplicates and analysed with SPSS Version 16. The isolates were classified on the basis of Cultural Morphology, Gram reaction and Biochemical tests and matched against standard microbial cultures. Result shows that Nutrient media allows the growth of many bacteria. MacConkey and Sabouraud dextrose media recorded no growth in all the treatments. The presence of Pterygospermin in M. oleifera hinders the growth of some microorganisms such as coli forms and fungi/yeast on the tested materials. The microbial load in the dried leave extracts increased by 81.70 – 85.05% compared with the fresh leaves. In addition the Room drying was noticed to have enhanced the potency of antibiotics in M. oleifera leaves, as this method does not affect the active antimicrobial ingredient in Moringa Plant. The bacterial colony isolates encountered in the studied samples was identified as Staphylococcus aureus. Findings from this study portrayed room drying as the best method that can be adopted for conservation of the microbial content of moringa leaves in the Savannah vegetation of Nigeria.
***Keywords**:* Microbial load, *Moringa oleifera*, Guinea Savannah, Drying methods, Antimicrobial ingredient

INTRODUCTION

Moringa oleifera (Lam) is the most widely cultivated species of the family Moringaceae which is distributed in Sub-Himalayan region (Fahey, 2005) but it at present being cultivated in the tropics, Nigeria inclusive. It is a fast growing deciduous shrub that can grow 12 meters height and 30 cm in diameter. The leaves are alternate, trip innate compound, 20 – 70 cm long (Roa *et al*., 1983). The leaves are consumed by human and his livestock and at time form part of recipe for production of ethno-medicine for treatment of some ailments in man and his livestock.

M. oleifera leaves are rich in starch, minerals, iron, vitamins (A, B, β-carotene, α-tocopherol, C, and E), calcium, proteins, fats, amino acids, phosphorus and anti-nutritional factors (Price, 1985, Dahot and Memo, 1987, Dolcas Biotech, 2008). Also, Moringa plant is known for its nutritional values, medicinal benefits and industrial uses. Almost every part of the plant contain significant amount of nutrient components needed for livestock and human consumption.

Microbial load of food measures the degree of food contamination by micro-organisms and related contaminants. Drying techniques have a reducing effect on the microbial load of food when compared with the fresh food. Studies have shown drastic reduction in the microbial loads of various horticultural crops such as *Pepper guineense*, *Amaranthus hybridus*, *Telferia occidentalis*, *Corchorus oleitorus* and *Brassica oleraceae* (Omafuvbe and Kolawole, 2004; Awogbemi and Ogunleye, 2009; Bhila *et al.*, 2010; Khazaei *et al.*, 2011).

In Africa, the most common practice of preserving vegetables, fruits and seeds are sun and air drying (Lyimo *et al.*, 1991). The two techniques reduce the moisture contents of the crops and made them available during off season and thus proffer solution to food insecurity in the Continent. On the other hand, there are arrays of processing and preservative methods that can reduce moisture content and also prevent the growth of mould (fungi) and bacteria which can affect the nutritive value of vegetables.

Drying is a preservative technique for food and some other agricultural produce including green leafy vegetables. Drying techniques work by removing water by evaporation from food and related products thereby inhibiting the growth of micro-organisms which brings about food and vegetable spoilage. Various methods are employed and essentially include: Room, Sun, Solar, Oven, Lyophilization, Commercial food dehydrator (Dolcas Biotech, 2008).

The different processing and preservative methods of foods, seeds and vegetables may significantly affect the availability and concentration of the microbial load values of food samples (Price, 1985). The import of the various drying method necessitated this study that was aimed at ascertaining the influence of the various drying methods on the microbial contents of dried *M. oleifera* leaves harvested from different zones of the Savannah vegetation of Nigeria.

MATERIALS AND METHODS

Collection of Materials

Leaves of *M. oleifera* were randomly collected from three locations in Guinea Savanna Vegetative Zones of Nigeria. These include: Abuja 9.07° N, 7.48° E (Southern Guinea Savanna); Kaduna 10.33° N, 7.75° E (Northern Guinea Savanna), and Zamfara 12.16° N, 6.25° E (Sudan Guinea Savanna) late in the evening to avoid wilting and loss of valuable properties.

Pre-treatment of Plant Samples

Branches of *M. oleifera* were defoliated to the petioles; leaves with deformity and signs of senescence were discarded. The healthy fresh leaves were washed thoroughly for 5 times under a running tap in order to remove extraneous substances on the leaves. Thereafter, the leaves petioles were tied together in small bunches and hung in an airy space to drain for 15 minutes and air dried for 30 minutes after which the leaves were weighed on Ohaus Adventurer analytical weighing balance (Scout ProSpu401Model). The samples were divided into six equal batches i.e. the one fresh sample and the other five batches were subjected to different types of drying procedures. Namely: Sun-drying, Room drying, and Oven drying at varied temperatures of 50ºC, 60ºC, and 70ºC. All the samples were processed and used for the microbial analyses (Olutiola *et al.*, 1991).

Microbial Loads Determination

The microbial loads of samples of leaves of *M. oleifera* subjected to five different drying methods and fresh leaves were analyzed using the Pour Plate techniques described by Olutiola *et al.* (1991). Microbial load in each treatment were determined in triplicates, and their means recorded.

Preparation of Materials

A. Glassware: Test tubes, pipettes (1 and 10ml), 250ml and 100mls beakers and conical flasks respectively, laboratory mortar and pestle, were washed and sterilized in the hot air drying oven (202-00A Model) for 45 minutes.

B. Rubber Tips: Automatic micropipette rubber tips were surface sterilized with 70% alcohol.

C. Media: Three media namely: Nutrient agar (General purpose), MacConkey agar (Differential and selective), and Sabouraud Dextrose agar (selective), were used for the determination of the microbial loads. One liter of each media was prepared thus:

Nutrient Agar: Twenty eight grams (28 g) of Nutrient agar powder (TM Media, Titan Biotech. LTD) was weighed in an Ohaus Adventurer analytical balance (Scout ProSpu401Model), and dissolved in 1000 cm^3distilled water in 2 liter conical flask. Complete dissolution of the media was achieved by boiling for 2 – 3 minutes on a Bunsen burner.

MacConkey Agar: Fifty two grams (52 g) of MacConkey agar powder was weighed in an Ohaus Adventurer analytical balance, and dissolved in 1000cm^3 distilled water in a 2 liter conical flask. Complete dissolution of the media was achieved by boiling for 2 – 3 minutes on a Bunsen burner.

Sabouraud Dextrose Agar: Fifty two grams (52 g) of Sabouraud dextrose agar powder was weighed in an Ohaus Adventurer analytical balance (Scout ProSpu401Model), and dissolved in a 1000cm^3 distilled water in a 2 liter conical flask. Complete dissolution of the media was achieved by boiling for 2 – 3 minutes on a Bunsen burner.

All the dissolved media were autoclaved (YX-280A Model) at 121ºC for 15 minutes under 1.6 $kgcm^2$ pressures, in an autoclave instrument.

D. Filtrates of Leaves: One hundred grams (100 g) of fresh leaves were grounded to paste using a sterile mortar and pestle. The pastes were each separately transferred into different 250 ml sterile beakers and 100 ml sterile distilled water added. These were thoroughly mixed using a sterile glass rod, and each filtered into different 200 ml conical flasks, through 30 cm Whattman filter paper in glass funnel. Similarly, one hundred grams (100g) of dried powered leaves sample was mixed in one hundred milliliter (100 ml) sterile distilled water in a 250 ml sterile beaker, and the filtrate was extracted in the same way as the fresh leaves (Olutiola *et al.*, 1991).

Pour Plate Technique

1 milliliter (ml) of the leave filtrates were serially diluted up in 4 folds (1 ml filtrate + 9 ml sterile distilled water) each in separate sterile test tubes. 1ml of the small volumes of the most diluted (10^{-3} and 10^{-4}) leave samples were pipetted separately into different sterile Petri dishes. Twenty milliliters (20 ml) of sterile molten nutrients agar liquid that has been cooled to 45ºC was poured into each dish and the contents mixed together by swirling and allowed

to solidify. The cultured dishes were then incubated at 37ºC aerobically for 24 hours. Afterwards, plates containing between 30 and 300 colonies were counted with the colony counter. Total coli form count was determined on MacConkey agar as described by Barrow and Felthman (1993).

Characterization and Identification of Isolates

The isolates were classified on the basis of Cultural Morphology, Gram reaction and Biochemical tests (Olutiola *et al.*, 1991; Cheesbrough, 2006), and matched against standard microbial cultures.

Bacterial Cultural Morphology on Nutrient Agar

i. **Staphylococcus species** on nutrient agar media appeared Creamy large, smooth surface, circle and pasty.
ii. **Gram Reaction**: Staphylococcus species, reacts positively (purple colouration under the microscope) to gram stain, and appeared clustered cocci under the microscope.
iii. **Biochemical test**: Staphylococcus species reacts positively to coagulate/crumping factor test and catalase.

Statistical Analysis

Data were subjected to statistical analyses to evaluate the differences between microbial loads of studied samples. The data were expressed as mean and standard error of mean. Comparison of means was analyzed using one way analysis of variances (ANOVA) on a Statistical Package for Social Sciences (SPSS) version 16.0 for windows. The difference was considered significant at $P< 0.05$.

RESULTS AND DISCUSSION

The microbial loads in fresh (Raw), Sun, Room, and Oven dried powdered *M. oleifera* leaves extracts investigated are presented in Table 1. Bacterial growths were recorded in all the nutrient agar of the treatment. Nutrient media is a general purpose media that allows the growth of many bacteria. MacConkey and Sabouraud dextrose media recorded no growth in all the treatments (Table 1). This could be due to the selective and differential nature of the MacConkey and Sabouraud dextrose media, and explained that coli forms and fungi/yeast were not among the microbial contaminants or that the active ingredient content in the *M. oleifera* leaves extract inhibited the growth of micro-organisms in the studied samples. The active ingredient – Pterygospermin, was reported in Moringa leaves as heat Labile, thus experiences decreased potency with increased temperature (Sofowora, 1982). The mean of the microbial load in all the incubated samples have highest microbial load (115×10^3 cfu/ml) in the Sun dried powdered leaves extract from Zamfara, while the lowest microbial load (15×10^3 cfu/ml) was in the Fresh leaves extract from Kaduna (Table 1).

The microbial load in the dried leaves extracts increased by 81.70 – 85.05% compared to the microbial load in the fresh *M. oleifera* leaves of 17×10^3 cfu/ml, 15×10^3 cfu/ml and 16×10^3 cfu/ml in samples collected from Abuja, Kaduna and Zamfara respectively (Table 1). The microbial loads recorded in the Fresh *M. oleifera* samples studied showed non-viable count of 17, 15, and 16×10^3 cfu/ml colonies respectively (Table 1). There was distinct statistical significance ($p < 0.05$) among the obtained mean values of the microbial loads of the treated samples when compared with the microbial load in the fresh leaf samples.

The Cultural Morphology, Gram reaction and Biochemical characteristics of bacteria isolates in *M. oleifera* leave extracts at various locations/samples studied are as shown in Table 2. Room drying method enhances the potency of antibiotics in *M. oleifera* leaves, as this method does not affect the active antimicrobial ingredient content (*Pterygospermin)*, in Moringa Plant, when compared with the other treatments of this study (Table 2). Room drying method recorded a higher total mean bacterial count of 9.1×10^4 cfu/ml and a lower total mean bacterial count of 8.2×10^4 cfu/ml, compared to the other treatments (Table 2). This suggests that the higher the potency of the active microbial ingredient content in Moringa plants, the higher the degree of inhibition of microbial growth. Sofowora, (1982) and Price, (1985) reported that *pterygospermin* was antibacterial and fungicidal to gram positive and gram negative micro-organisms.

Leave extracts (1 ml) were inoculated in different media: Nutrient, MacConkey and Sabouraud dextrose agar (20 ml of each molten media), and incubated for 24 hours at 37ºC. Nutrient agar recorded bacteria colony in all the treatments (Table 2). MacConkey and Sabouraud agar showed no growth of bacterial colony (Table 2). All the bacteria colony growth in nutrient agar showed cultural morphology of creamy large, smooth surface, circle and pasty (Table 2).

Bacterial colony was gram stained in each of the treatments that recorded colony growth. Their reaction was dark purple and clustered cocci, under the microscope, indicating gram positive cocci, in clusters (Table 2). The bacterial colony tested positive to catalase and coagulase biochemical tests, confirming the bacteria isolates encountered

Table 1: Mean Count Microbial Loads (cfu/ml) of *Moringa oleifera* leaves at various locations

Samples	Treatment	Total Mean colony count on Nutrient Media	Coliform forming Unit (cfu/ml)	Total Mean colony count on Sabouraud Media	Total Mean colony count on MacConkey Media
	Fresh leaves extract.	1.7×10^{4a}	$17,000^{a}$	No growth	No growth
	Sun Dried Powdered leaves extract.	10.3×10^{4c}	$103,000^{c}$	No growth	No growth
	Room Dried Powdered leaves extract.	8.4×10^{4b}	$84,000^{b}$	No growth	No growth
	50°C	10.1×10^{4c}	$101,000^{c}$	No growth	No growth
	60°C	10.4×10^{4c}	$104,000^{c}$	No growth	No growth
	70°C	10.4×10^{4c}	$104,000^{c}$	No growth	No growth
	Fresh leaves extract.	1.5×10^{4a}	$15,000^{a}$	No growth	No growth
	Sun Dried Powdered leaves extract.	11.2×10^{4c}	$112,000^{c}$	No growth	No growth
	Room Dried Powdered leaves extract.	8.2×10^{4b}	$82,000^{b}$	No growth	No growth
	50°C	10.6×10^{4c}	$106,000^{c}$	No growth	No growth
	60°C	10.6×10^{4c}	$106,000^{c}$	No growth	No growth
	70°C	10.5×10^{4c}	$105,000^{c}$	No growth	No growth
	Fresh leaves extract.	1.6×10^{4a}	$16,000^{a}$	No growth	No growth
	Sun Dried Powdered leaves extract.	11.5×10^{4c}	$115,000^{c}$	No growth	No growth
	Room Dried Powdered leaves extract.	9.1×10^{4b}	$91,000^{b}$	No growth	No growth
	50°C	10.8×10^{4c}	$108,000^{b}$	No growth	No growth
	60°C	10.6×10^{4c}	$106,000^{c}$	No growth	No growth
	70°C	10.7×10^{4c}	$107,000^{c}$	No growth	No growth
	± SEM	6.33	7.684		

Means in the same column but with different superscripts differ significantly ($P < 0.05$).

Table 2: Cultural Morphology, Gram Reaction and Biochemical Characteristics of Bacteria Isolates in *Moringa oleifera* leave Extracts at various Locations

Samples	TREATMENT	Growth on NA	Growth on Mac	Growth on SDA	Cultural characteristics	Gram Reaction	Biochemical tests		Bacteria Isolates
							Catalase	Goagulase	
	Fresh leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Sun Dried Powdered leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Room Dried Powdered leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
		50°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus..*
		60°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
		70°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Fresh leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Sun Dried Powdered leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Room Dried Powdered leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
		50°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus..*
		60°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
		70°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Fresh leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Sun Dried Powdered leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
	Room Dried Powdered leaves extract.	Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
		50°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
		60°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*
		70°C Creamy large, smooth surface, circle and pasty	No growth	No growth	Creamy large, smooth surface, circle and pasty	Positive, Cocci in cluster	+	+	*Staphylococcus aureus.*

Key: NA = Nutrient Agar. Mac = MacConkey Agar. SDA = Sabaraud Dextrose Agar.
+ = Positive.

in the studied samples of *M. oleifera* were identified as *Staphylococcus aureus*.

CONCLUSION

This study has shown that fresh *M. oleifera* leaves contain ingredients (pterygospermin) that could be used to combat microorganisms. The ingredient is susceptible to heat as it was established that only bacterium isolate of *S. aureus* was encountered on *M. oleifera* leaves used in this study. This shows the import of integrating *M. oleifera* leave products among the conventionally used antimicrobial discs in the treatment of infections, considering its antimicrobial properties.

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