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Effect of Varying Drying Temperature on the Antibacterial Activity of *Moringaoleifera* Leaf (Lam)

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Abstract: *The use of herbal and medicinal plant for traditional therapeutic measure and health care services began since pre-historical time and currently exploited especially in modern medicine. This study investigated the effects of different drying temperature on the antibacterial activity of ethanolic extract of M. oleifera leaf. Fresh leaves of M. oleifera were dried separately at varying temperature by sun drying, oven drying and air drying methods, grinded into powdered form and extracted with ethanol. The ethanolic extracts were used for antibacterial susceptibility test using agar disc diffusion method. All the three clinical bacterial isolates such as E. coli, S. aureus and S. typhiwere susceptible to the ethanolic extract of M. oleifera leaf which signified its antibacterial potency. The air dried extract produced the highest zones of inhibition than the sun dried and oven dried ethanolic extract with 13 mm and 19 mm, 13 mm and 21 mm, 11 mm and 20 mm as the lowest and highest inhibition zones for E. coli, S. aureus and S. typhi respectively. This study inferred that drying temperature especially higher temperature has profound effects on the antibacterial activity of M. oleifera leaf as active component are depreciated.*

Keywords: *M. oleifera leaf, Drying temperature, Zone of inhibition, ethanolic extract, Antibacterial susceptibility, Clinical bacterial isolates.*

I. Introduction

Medicinal plants have been used in traditional health care systems since pre-historic times and are still the most important health care source for the vast majority of the population around the world [1].The use of herbal medicine has always been part of human culture, as some plants possess important therapeutic properties, which can be used to cure human and other animal diseases [2].

It has been reported that a substantial percentage (38%) of prescription contained one or more of the natural products of plant origin as the therapeutic agent [3]. Study of [4] had earlier pointed that more than 75% pure compounds derived from higher plants are used in herbal medicine but most of those applied in modern medicine are now produced synthetically. The reasons for the high patronage of herbal medicine are the high cost of very effective antibiotics and the problem of antibiotic resistance which is very common in developing countries [5].

In recent studies, extract of various parts of medicinal plants were found to have broad spectrum antimicrobial activities against pathogenic organisms ([6], [7], [8]). An instance is the *M. oleifera* plant; Nearly every part of this plant, including root, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil have been used for various ailments in the indigenous medicine [9].Recently, researches also indicated and emphasized on the active constituents for accepting its applicability in modern medicine.

Moringaoleifera 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. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β – carotene, amino acids and various phenolics [10].Reference [11] reported that the idea that certain plants had healing potentials, indeed, they contain what would be characterized as antimicrobial principles was well accepted long before mankind discovered the existence of microbes. The healing property of these medicinal plants is usually linked with the presence of secondary metabolites and these differ from one plant to another [12].

Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals which have very important medicinal values [13], with both antibacterial and antifungal activities [14].

In this regards, *M. oleifera*plant parts are being employed for the treatment of different ailments in the indigenous system of medicine.[15]reported on the antimicrobial properties of *M. oleifera* against gram positive and gram negative microorganisms. Aside the antibacterial activity of *Moringaoleifera* plant, it also posses anti-fungal activity [16]. In addition, *M. oleifera* seeds possess water purifying powers by flocculating gram positive and gram negative bacterial cells [17].

Countless studies proving the antimicrobial activities of *Moringaoleifera*leafabound today, but relatively fewer studies emphatically verifies the effect of different drying temperature (during processing) on the antimicrobial activities of *Moringaoleifera* leaf; thus this study aimed at determining the effect of varying drying temperature

on the antibacterial activity of *Moringaoleifera*leaf(Lam) extract tested on some clinical bacterial isolates hence the study essentially consider full exploitationof the antimicrobial activities of *Moringaoleifera*especially in modern medicine.

II. Materials And Methods

2.1 Collection of Plant Materials

The leaves of *Moringaoleifera* known as *Moringaoleifera*lam were neatly collected from Kumo, Akko Local Government Area of Gombe State Nigeria and taken to the laboratory forprocessing and extraction.

2.1.1 Dryingand Processing of*Moringaoleifera* leaves

The leaves of *Moringaoleifera*collected were washed with clean water then dried using different drying methods (applying varying drying temperature) such as; ovendrying, sun drying and airdrying.
In Sun drying method;the leaves of *Moringaoleifera* were dried under the sun for three days at the average day temperature of 33^0C.For the airdrying method employed;*M.oleifera* leaves were dried at average room temperature of 24^0C for about 12 to 14days.While in oven drying method;*M.oliefera* leaves were dried in the oven only for 24hours at the temperature of 50^0C.Afterward, the dried *M.oleifera* leaves were crushed into powdered form using mortar and pestle then stored for extraction.

2.1.2 Extraction of*M. oleifera* Leaves extract

100 gram of powdered *M. oleifera*leaf was soaked in 1000 mL ethanol for five days at room temperature with shaking at various intervals; the resulting mixture was then filtered using muslin cloth thenfilter paper. The filtrate was evaporated to dryness at 40^0C to 50^0C on burner resulting toethanolicextracts of *Moringaoleifera* leaves.

2.2 Bacterial Isolates and Collection

Three clinical bacterial isolates namely: *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* were used in this study. Stock culture in an agar slant of these bacterial isolates were obtained from Specialist Hospital Gombe, Gombe State and subjected to proper identification and confirmatory test.

2.2.1 Identification & Confirmation of Test Organisms

Bacterial isolates such as*E.coli, Staphylococcus aureus* and *Salmonella typhi* were identified by employing Gram staining procedure and microscopy, and further confirmed using confirmatory biochemical tests including Catalase test, Coagulase test, Motility test, Indole test, Urease test, Oxidase test, Citrate test, KIA test, LDC test and H_2S production test as described by [18].

2.2.2 Sub-culturing andPurification of Test Organisms

Stock culture of the clinical bacterial isolates of *E.coli, S.aureus*and *Salmonella typhi* were sub-cultured on nutrient agar and incubated at 37^0C for 24hours. From the sub-cultured plate, a single of colony of each bacterial isolate was picked with a sterile inoculating loop and pure culture of these test organisms were obtained by streak plating method, then incubated at 37^0C for 24hours**.**Pure culture each of these test organisms were used for main analysis.

2.2.3 Standardization of Inoculum

A loopful of the colony of *E.coli, S. aureus*and *Salmonella typhi* from the pure culture plates were picked using sterile wire loop and emulsified in 2 to 3 mL nutrient broth then incubated overnight at 37^0C for24hours**.**For *S. aureus,*ten folds serial dilution was achieved to (10^{-10}) dilution while hundred dilutions(100^{-100}) for enterobacteriacea (*Salmonella* and *E. coli*); then turbidity was compared with 0.5 McFarland's standard [18].

2.3 Preparation of Disc and Concentrations of *M. Oleifera* Leaves Extracts

2.3.1 Concentration in mg/mL

Different concentrationswere prepared from the crude ethanolic extract of *M. oleifera* leaf by weighing the actual concentration/quantity (in milligram) and dissolved completely in one milliliter (1 mL) of distilled water. Various concentrations of ethonalic extract of *M. oleifera* prepared include 30 mg/mL, 50 mg/mL, 70 mg/mL, 100 mg/mL, and 150 mg/mL.

2.3.2 Disc Preparation

Discs used for sensitivity were punched from Whatman No. 1 filter paper and sterilized in Bijou bottle by autoclaving at 121 ^{O}C for 15 minutes, then various concentrations (30 mg/mL, 50 mg/mL, 70 mg/mL, 100 mg/mL and 150 mg/mL) of ethonalic extract of *M. oleifera* leaf prepared were impregnated in the sterilized

discs by wholly submerging the disc in differently prepared concentrations of *M. oleifera*extract contained in bijou bottle while disc soaked in distilled water was used as negative control disc.

2.4 Antibacterial Susceptibility Testing: Agar Disc diffusion method

Agar disc diffusion methodas described by [19]was used to test the antibacterial activity of ethanolic extract of *M.oleifera*leaf on the three clinical isolates (*E. coli, Staphylococcus aureus* and *Salmonella typhi*). From the standardized inoculum, the test organisms were inoculated using sterile swab stick by spread plating on Mueller Hinton agar plates. The prepared sensitivity discs impregnated with different concentrations (30 mg/mL, 50 mg/mL, 70 mg/mL, 100 mg/mL and 150 mg/mL)of the ethanolic extract of *Moringaoleifera*leafwere picked using a sterile forceps and then dropped on the inoculated plates; also equivalent quantity of distilled water impregnated in the disc termed blank disc (0 mg/mL) was used as negative control. The plates were incubated at 37^0C for 24 hours. Clear inhibition zones around the discs denoting antimicrobial activity of *Moringaoleifera* leaf extract were observed and measured. The results were compared to see the effect of drying temperature on antibacterial activity of *M.oleifera* leaves extracts.The diameter zones of inhibition (DZI) were measured in millimeter using a meter rule.

III. Results And Discussion

The leaves of M.oleifera dried separately at different temperature (oven-died, sun-dried and air-dried) and the ethanolic extract of M. oleifera leaves were tested on some clinicalbacteria isolates producing zones of inhibition which signifies susceptibility of the test organisms to the extract.

Table 1 Morphology and Biochemical properties of clinical bacterial isolates used**:**

Organisms	Shape	Grxn	Cat	Coag.	Mot.	Ind.	Cit.	Ur.	Ox.	Mred.	KIA	LCD	H_2S
E. coli	*srs*	-	+	-	+	+	-	-	-	+	*ND*	+	-
S. aureus	*cc*	+	+	+	-	-	-	-	-	+	*ND*	*ND*	-
S. typhi	*srs*	-	+	-	+	-	+	-	-	+	+	+	+

(+): Positive reaction, (-): No reaction, srs: short rods in singles, cc: cocci in clusters, ND: Not done, Grxn: Gram reaction, Cat: Catalase test, Coag: Coagulase test, Mot: Motility test, Ind: Indole test, Cit: Citrate test, Ur: Urease test, Ox: Oxidase test, Mred: Methyl red test, KIA: Kligler Iron Agar test, LCD: Lysine Decarboxylase, H_2S: Hydrogen sulphide test.

Table 1 relates the Gram reaction, microscopic examination/shapes and biochemical properties of the three bacterial isolates used in this study. These tests results confirmed isolates used for antibacterial sensitivity test.

The ethanolic extract of*Moringaoleifera* leaf has a varying degree of antibacterial activity on the tested bacterial isolates for*E. coli*producing lowest inhibition zones of 11 mm at 30 mg/mL concentration for sun dried and oven dried extract of *Moringaoleifera* leaf; andhighest inhibition zones of 19 mm at 150 mg/mLconcentration was produced by both sun dried and air dried extracts.

In table 2, the antibacterial activity ofethanolic extract of*Moringaoleifera* on*S. aureus* also varies for different drying temperature as 10.5mm was the lowest zone of inhibition at the lowest concentration of 30 mg/mLfor sun dried extract while the air dried extract produced highest zone of inhibition of 21 mm at 150 mg/mL concentration of same ethanolic plantextracts. Also the antibacterial activity of ethanolic extract of *Moringaoleifera* leaf on*Salmonella typhi*produced 10 mm at 30 mg/mL concentration as the lowest inhibition zone for both oven dried and sun dried extract while the air dried extract produced the highest zone of inhibition of 20 mm at 150 mg/mL of the extract of *Moringaoleifera* leaf.

Table 2 Antibacterial susceptibility of *E. coli, S. aureus* and *S. typhi*todifferent concentrations of ethanolic extract of *Moringaoleifera*leaf.

Drying temperature	Zones of inhibition (mm) for different concentrations (mg/mL)														
	E. coli					*S. aureus*					*S. typhi*				
	30	50	70	100	150	30	50	70	100	150	30	50	70	100	150
Oven dried at 50 ^{0}C	11	12	13	16	17	11	12	14	15	17	10	11.5	13	15	18
Sun dried at 33 ^{0}C	11	12	14	16	19	10.5	12	13	14	16	10	11	12	14	16
Air dried at 24 ^{0}C	13	15	16	18	19	13	15	17	19	21	11	13	14	17	20

In this study,the antibacterial activity ofethanolic extract of*Moringaoleifera*leaftested ondifferent clinical bacteria isolates including *E.coli, S. aureus* and *S. typhi*produced differentzones of inhibition; this is

highly indicative that the plant *Moringaoleifera*has reasonable antibacterial activity against these test organisms. This corroborate with the work of [20] who confirmed the presence of the common phytochemical constituent's like saponins, tannin, phenol and alkaloids in *M. oleifera* plant.The Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals which have very important medicinal values [13].

Previous studies ([21], [20],[13]) have demonstrated the presence of active antimicrobial components in *M. oleifera* plant. It is worthy to state that these active components of *Moringaoleifera*leaf are importantly responsible for its antimicrobial potency; as [20] inferred that the presence of these constituents has been reported to account for the exertion of antimicrobial activity by *M. oleifera*plants.

In a recent study by [22] aqueous extracts of *Moringaoleifera*was found to be inhibitory against many pathogenic bacteria, including *Staphylococcus aureus, Bacillussubtilis, Escherichia coli,*and *Pseudomonas aeruginosa* in dose dependent manner. *Moringaoleifera*extracts was also found to be inhibitory against *Mycobacterium phleiand B. subtilis.* The leaf extractwas found to be effective in checking growth of fungi *Basidiobolushaptosporus*and *Basidiobolusranarums.*

Also according to [21], tannins and phenolic compounds have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection. Emphasizing on the medicinal effect of the active ingredients of *M. oleifera,* Tannins have also been reported to have antidiarrheal, homeostatic and antihemorrhagal activity [23].

From table 2, fig. 1, 2 and 3, it is mostly evident that the zones of inhibition produced by the ethanolicextract of *Moringaoleifera* is proportional to the dose concentration of the extract of *M. oleifera*leaf used in this study; this importantly implies that the zones of inhibitions which indicates the susceptibility of the test organismsto *M. oleifera*leaf extract increases as the concentration of theethanolic extract of*Moringaoleifera*leaf increases. This phenomenon is reasonable and conformed to the study of [24].

On a general note, this work (fig. 1, 2 and 3)reveals that the air dried ethanolic extract of *M. oleifera* has higherantibacterial activity on *E .coil, S. aureus* and *S. typhi* than the sun dried and oven dried samples, this may be best attributed to the low drying temperature (24^0C) of the air-drying methodemployed in the drying of *M. oleifera* leaves as compared with oven-drying temperature (50^0C) and sun drying temperature (33^0C) considered higher than the former. The work of [25] relates that the extract of *Moringaoleifera*is more effective under low temperature, or moderate temperature conditions. Also [26]revealed that at high temperature, the activity is lost, which pointed that the antibacterial compounds might be some protein which may result in membrane permeabilization resulting from binding of cationic proteins to the negatively charged membrane surface and subsequent pore-formation; with increase of temperature, these proteins might be degraded.

It is imperative to state that the essence of drying of medicinal plant leaves is to expel water, subsequently concentrating the active ingredients in the leaf. The findings of this work reveal that higher drying temperatures otherwise termed processing temperature of *Moringaoleifera*leaf have effect on its active ingredients and the antibacterial potency of *Moringaoleifera* leaf extract. This is base on the facts of [26],who stated that although processing temperature may have effects on the antibacterial activity of *M. oleifera* extracts also supported by the study of [25].

IV. Conclusion

Several studies including this have demonstrated antimicrobial activity *M. oleifra*leaf extract on some bacteria isolates revealing positive results and inferences. Based on the findings of this study, higher or excessively highdrying or processing temperature of *M. oleifra*leaf havedepreciative effect on antibacterial potency/activity of such medicinal plant.

V. Recommendations

It is recommended that temperature should be importantly considered when drying or processing most medicinal plant like*M.oleifera*leaf and more research should be done to ascertain the appropriate drying temperature and suitable processing method for any herbal plant intended for therapeutic use to avoid excessive loss of important and active phyto ingredients.

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