Nigerian Journal of Pharmaceutical and Applied Science Research, 12(4): 19-27; December 2023 p-ISSN: 2971-737X; e-ISSN: 2971-7388. Available at www.nijophasr.net

Formulation of Lozenge of Methanol Root Extract of *Moringa oleifera* Lam (Moringaceae) and its Efficacy against some Clinically isolated Pathogens

[*1]Akeem A. Agboke, [2]Imoh I Johnny, [3]Musiliu O. Adedokun, [4]Owoidoho V. Paulinus [4]Nyong E. Etim and [5]Motunrayo P. Agboke

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
[2]Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria
[3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Federal University Oye-Ekiti, Ekiti, Nigeria
[4]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
[5]Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

ABSTRACT

Background: This research aimed to determine the antimicrobial activities of the methanol root extract of *Moringa oleifera* and its lozenge formulation against the clinically isolated cultures of *Staphylococcus aureus, Candida albicans*, and *Pseudomonas aeruginosa* using established procedures.

Method: The extract-formulated lozenges are 500 mg each, for five granule batches using the wet granulation method. The flow properties of the granules and tablet properties were evaluated using standard methods. The antimicrobial properties of the extract and the formulated lozenges batches A, B, C, D, and E were evaluated using a pour plate and Agar-well diffusion method respectively.

Results: The evaluation of the granules and tablets met the required standards and the organisms were susceptible to the extract at concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml. The excipients had no significant effect on the antimicrobial activity of the extract-formulated lozenges batches B, C, D, and E. Batch A which contained no extract, showed no activity as negative control.

Conclusion: The results confirmed the effectiveness of the methanol root extract of *M. oleifera* formulated as lozenges against the examined organisms. Hence, this plant can be used in the formulation of lozenges and retain its activities in the treatment of mouth and upper respiratory tract infections.

Keywords: *Moringa oleifera*, lozenge formulations, methanol extract, antimicrobial activities

1.0 INTRODUCTION

Oral dosage forms vary and have advantages over other dosage forms. They are economical and safe to the patient. They are the natural and easiest route of drug administration. No additional care is required for drug intake, so therefore patients can take it without help. Toxicity is delayed due to onset of action and allows for easier recovery as compared to other dosage forms. Oral dosage forms have disadvantages as well; they are not good choice of drug when the patient suffers chronic vomiting, when the patient is uncooperative such as children and infants, and also in the case of emergency and unconscious patients [1]. The oral cavity contains some of the most varied and vast flora in the entire human body and is the main entrance for two systems vital to human function and physiology; the gastrointestinal system and respiratory system [2]. Several diseases involve these two systems and manifest in the oral cavity. The oral cavity harbors more than 700 microorganisms as part of the residents and transient flora. In addition

* Corresponding author: Email: ayoagboke@gmail.com; Phone: +2348023707016

to bacterial organism, oral microorganism can include, protozoa, fungal and viral species. The bacteria include hundreds of types of microorganisms in the micro environment of the oral cavity which adhere to the teeth, the gingival cavity, the tongue and the buccal mucosal [3]. Each site has a unique way of allowing the microorganism to establish their residency. The normal flora in healthy individuals maintains similar patterns. Bacterial infection of the oral mucosa is becoming an increasing problem in many countries. Factors that impair host resistance may cause overgrowth of microorganisms. Fungal infections are classical oral opportunistic infection caused by either systemic or local impairment of the host [3].

Some oral pathogenic microorganisms
Candida albicans: This is an opportunistic fungal pathogen that causes candidiasis in human host. That which occurs in the mouth is known as oropharyngeal candidiasis. This occurs in the mouth or throat and it's identified by white plaque growth on oral mucous membrane. It is usually characterized by the formation of white patches on top of the tongue and throughout the mouth which is known as thrush. Thrush can be removed with a blade or cotton tipped swab, but the underlying tissue will be irritated and show a distinct redness. This infected area will cause soreness and difficulty during eating [4, 5].

Staphylococcus spp: This is a gram-positive coccal bacterium. It is frequently found in the nose, respiratory tract and the skin. The species of *Staphylococcus* often found in the mouth include; *Staphylococcus epidermidis* and *Staphylococcus aureus.* These organisms are opportunistic pathogens and can cause infections in human given the optimal set of circumstances [6].

An illness in another part of the body may cause reduced immune function resulting in a secondary infection from *Staphylococcus. Staphylococcus spp* in the mouth can result due to unhygienic oral practices, diabetes and HIV, sharing dinning utensils with infected persons and kissing or having oral sex with infected person. *Staphylococcus* can invade the tonsil causing tonsillitis, the parotid gland causing parotitis and can also lead to bacterial epiglottitis; a condition which is indicated by painful swallowing, inflammation, redness of the mouth and ulceration at the infected site [6].

Pseudomonas aeruginosa: It is a gram-negative rod like bacteria that belong to the family Pseudomonadaceae. The organism has become an important cause of gram-negative infections, especially in patient with compromised host defense mechanism. It is the most common pathogen isolated from patients who have been hospitalized longer than one week. Pseudomonal infections are complicated and can be life threatening. The oral cavity can represent a reservoir for *Pseudomonas aeruginosa* especially in patients with periodonititis. This makes treatment more difficult and in the case of opportunistic infections, may worsen the condition of the debilitated patient such as the elderly and immune-suppressed [7].

Lozenges as a dosage form
Lozenges are the flavored medicated oral dosage form intended to be sucked and held in the mouth or pharynx, containing one or more medicaments usually in a sweetened base [8]. Lozenges are used for patients who cannot swallow oral dosage forms as well as for medications designed to be released slowly to yield a constant level of drugs in the oral cavity or to bathe the throat tissues in a solution of the drug. Lozenges are usually over the counter (OTC) and prescription products taken for local effect in the mouth, tongue and throat etc. It contains a variety of active ingredients like local anesthetics, antimicrobials, antibiotics, vitamins, decongestants, analgesics etc. Lozenges have a high advantage over conventional tablets as they have slower dissolution rate, pleasant taste, attractive appearance and slow release of the tablet [8]. Since lozenges are hygroscopic in nature, a complex and multiple packaging is adopted. The individual unit is wrapped in a polymeric moisture barrier material which are then placed in a tight or moisture resistant glass or container that is over wrapped by aluminum foil or cellophane to avoid absorption of moisture [8, 9]. Storage of lozenges should be away from heat and out of the reach of children. It should also be protected from extreme humidity. Depending on the storage of both the drug and the base, either room temperature or refrigerator temperature is usually indicated [9]. Since lozenges are taken orally under a slow-release situation, it is assessed to determine the antimicrobial properties of *Moringa oleifera* after formulation. Assessment is done after recognition of the bacteria that can cause mouth or throat infection and even upper respiratory tract infection [9].

Moringa oleifera roots
Traditional uses: *Moringa oleifera* roots have been reported to exhibit strong antibacterial activity due to the presence of some active principles which justifies its local use as chewing stick for oral health. The health benefits of moringa

roots have been used by Ayurveda practitioners for centuries to treat a wide variety of ailments. The roots are especially useful in controlling the disorders of the circulatory system including minor cardiovascular complaints. In small doses, moringa roots can be used to stimulate appetite and improve the function of the digestive tract making is useful for individuals with gastric upset and irritates bowel syndromes. In poultice form, the roots are used for cramps and arthritis pains. Moringa roots are diuretic and have some antiseptic qualities in topical use as well [10].

Modern medical uses: Moringa roots have been investigated by medical researchers to determine the effectiveness against a number of ailments. Scientific studies have shown its usefulness in kidney function, anti-inflammatory properties, analgesics, antibiotics and have also been found useful in supplementing pharmaceutical remedies allowing patients to experience longer, less interrupted sleep when taking pain medications [10, 11].

Other uses: The harvested roots of *Moringa oleifera* serve a wide variety of purposes which include; wound healing, nourishment, making of medicines, perfumes, fertilizers, natural pesticides and other import products [11].

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Dilution bottles, aluminum foil, face mask, hand gloves, cotton wool, disinfectant, masking tape, filter paper, electronic weighing balance, incubator, water bath, refrigerator, beakers, test tubes. Separating funnel, measuring cylinders, extraction tank, retort stand, spatula, test tube rack, corkborer, spreader, petri dishes, mortar and pestle, funnels, triple beam balance, Stoke's tablet hardness tester, Roche tablet friabilator, disintegration apparatus, single punch tableting press, sieve of mesh size 1 & 2mm.

2.1.2 Reagents

Methanol, distilled water (prepared in the Department of Pharmaceutics and Pharmaceutical Technology of the University of Uyo), chloroform, hydrochloric acid, dragendoff's reagent, Fehling's solution A & B, Sodium bicarbonate, ferric chloride solution, Bromine Water, potassium hydroxide (KOH), ammonia, sulphuric acid, benzene, magnesium metal, glacial acetic acid, acetic anhydride, sodium hydroxide, nutrient broth, nutrient Agar, talc powder and lactose (BDH chemicals Ltd, England), Polyvinylpyrrolidone (PVP) (FSA Lab Supplies, England), Magnesium stearate (Sigma-Alderich, USA), Microcrystalline cellulose, Extract of *M. oleifera* (extracted in the Department of Pharmacognosy and Natural Medicine Laboratory, University of Uyo).

2.1.3 Maintenance and standardisation of microorganisms

The following test organisms were obtained from the stock culture in the Department of Microbiology, Faculty of Science, University of Uyo, Nigeria: Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans. The bacteria were sub cultured on sterile Mueller Hinton agar (MHA) at 37 o C for 24 h and the fungi, on sterile sabouraud dextrose agar (SDA) at 28ºC for 48 h before use. Standard suspension of the cultures was prepared and the turbidity of each of the broth cultures adjusted to 0.5 McFarland Nephelometer Standards with an estimated cell density of $1x10^8$ C [12, 13].

2.2 Methods

2.2.1 Collection and identification of plant

The *Moringa oleifera* roots were collected from Itak Akap, Ikono Local Government Area of Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Imoh I. Johnny of the Department of Pharmacognosy and Natural Medicine, University of Uyo. A voucher specimen was prepared, assigned a herbarium specimen number [UUPH A 50(i)] and deposited in the herbarium of the same department, for reference purposes.

2.2.2 Preparation of extract

The roots collected were washed with clean water to remove dirt, air-dried and cut into small pieces. They were air-dried for four weeks and reduced to coarse powdered form using the mortar and pestle. A quantity of 3.5 kg was macerated using absolute methanol for 72 hours with intermittent shaking. The macerated preparation was filtered using funnel fitted with cotton wool. The filtrate was concentrated using rotary evaporator at 45^0C and was dried using the water-bath at 45^0C. The dried extract was weighed and percentage yield was calculated. The dried extract was stored in the refrigerator in an amber bottle at 4^0C prior to use.

2.2.3 Susceptibility and Minimum Inhibitory Concentration (MIC) Determination

The susceptibility test was carried out using the pour plate method. The plate was aseptically filled with 15ml of the culture media, Sterile molten Muller Hinton agar (MHA) for the bacteria and Sabourand dextrose molten agar (SDA) was also transferred into plates for fungi. The different concentrations of the diluted extract 3ml each was swirled together with the media and allowed to solidify. The plate was inoculated with the organisms by streaking and incubated at 37^0C while the plate for fungi kept at room temperature for 7 to 14 days for fungi and bacteria for 24 hours to determine susceptibility of the organism to the extract [12]. The MIC was performed using Agar-well diffusion method. Sterile petri dishes were aseptically inoculated by dropping 0.1ml of suspension of the test organism in a petri dish of 15ml of the culture media (molten MHA) for the bacteria and SDA for the fungi. The mixture was swirled gentle to mix and allowed to solidify for each organism [12]. Using the sterile cork-borer, holes were bored on the seeded agar plates, discarding the removed agar rings into the disinfectant solution; the wells were aseptically filled with the different dilutions of the extract (12.5, 25, 50, 100, 200mg/ml of each extract) using a Pasteur pipette, care was taken to avoid spillage. The plates were allowed to stay for 30mins on the bench before incubation to allow for the diffusion of the extracts. The plates were incubated at 37^0Cfor 24 hours for bacteria but 25^0C or room temperature for 7 to 14 days for fungi. The plates were observed and diameter of the inhibition zone was measured [12]. These steps were repeated for formulated lozenges of 100 mg/ml of the extracts with the varying concentrations of the excipient and commercially available lozenge (containing 50, 25, 12.5, 6.25, 3.125, 1.563 µg/ml as positive control respectively).

2.2.4 Granule formulation

Each batch of granule (in grams) was prepared to give 30 tablets per batch. The method of preparation was by wet granulation. [13]. The following excipients were employed, *Moringa oleifera* extract as active ingredient, microcrystalline cellulose (MCC); Avicel as disintegrant, polyvinylpyrrolidone (PVP) as binder, magnesium stearate as lubricant, talc as glidant and lactose as bulking agent. The granules were produced by simple bulk mixing. Appropriate quantities of lactose, polyvinylpyrrolidone, and microcrystalline cellulose were weighed out and incorporated with the *Moringa oleifera* extract and triturated in a mortar. This was then made damp by dispersion of a small amount of water to form a damp coherent mass. The wet mass was passed through a 2.00mm stainless steel sieve with the aid of a spatula. The wet granules were dried in the hot air oven at a temperature of 50^0C for about an hour and further passed through a 1.00mm stainless steel sieve to obtain fine granules. The formula for each batch, percentage composition and weight of ingredient for each batch is as shown in the tables 1 and 2 below [13,14].

2.2.5 Determination of Granule Flowability and Compressibility Parameters

2.2.5.1 Granule flow rate and angle of repose

For the determination of flowability parameter of the granules, the angle of repose method and flow rate method were used [15]; using a funnel, retort, cotton, wool, plain sheet of paper, pencil to trace the outline made by the powder heap with the horizontal (paper surface), a stop watch (for flow rate) 15g samples were used in each case. The angle of repose was determined by clamping a dry funnel in a retort stand such that its tip is above 2cm above a plain white sheet of paper placed on a flat surface. The granules were passed through the funnel until the apex of the cone formed. The mean height of the granules heap was obtained likewise the radius of base of cone [16].

Flow rate was determined by noting the time of flow of granules from the funnel to the horizontal plane using a stop watch. Flow rate was determined by dividing the mass of a granule with its time of passage through the funnel. this was carried out for each batch of granules [15, 16].

2.2.5.2 Bulk and Tapped Densities

The weights of each batch of granules were determined using an electronic weighing balance. For each batch, the weighed granules were placed in a 100ml clean measuring cylinder and the volume V0 without tapping was determined. After which 100 mechanical taps of the cylinder was done as the tapped volume V1. The bulk and tapped volume were calculated as the ratio of granules weight in grams to volume for V0 andV1 respectively [16].

2.2.5.2.1 Hausner's Ratio:

This was calculated as the ratio of the bulk to the tapped volume of each batch of granules.

2.2.5.2.2 Carr's Index:

This was determined by using the formula below,

Carr's index = bulk volume-tapped volume bulk volume ×100%

2.2.5.3 Tablet compression

Magnesium stearate and Talc were weighed out according to their appropriate quantities and mixed with each batch of granule and transferred to a beaker.

500mg of each batch of the granules was manually fed into the die cavity of the single punch tableting machine and then compressed [15-17].

2.2.5.4 Tablet Assessment

2.2.5.4.1 Tablet Hardness test: The hardness tests on the tablets were carried out using a Monsanto Tablet Hardness tester to measure the force required to break a tablet when the force generated by a coiled spring is applied diametrically. The force was measured in kilogram force (kgF). 10 tablets per batch were used for this determination. The mean hardness was calculated as (H1 + H2…+ H10)/10. The standard deviation was calculated.

2.2.5.4.2 Tablet friability test: The friability tests were conducted using Roche Friabilator, using 10 tablets for each batch with 100 revolutions (i.e., 25 revolutions per minute for 4 minutes). The tablets were tumbled for four minutes at 25 revolutions per minute. The tablets were then removed, dusted and weighed again. Friability, F was calculated as: F = {Wo-W1/Wo} x 100%.

2.2.5.4.3 Tablet Weight uniformity This test was done using an electronic weighing balance to weigh 20 tablets picked randomly from each batch individually. Then, the average weight, percentage deviation and standard deviations were calculated for each table batch.

*2.3.5.4.4 Tablet Disintegration test***:** Disintegration time test was performed using a B.P. Disintegration Apparatus. Six tablets were tested at the same time of which the dissolution medium was 500ml of distilled water [17, 18].

2.3.5.4.5 Tablet Thickness: The thickness was measured using micrometer screw gauge and expressed in mm. 10 tablets each from the different batches were obtained and their individual diameter and average diameter for each batch determined [18].

2.4 Statistical Analysis.

The experiments were carried out in triplicates and the results obtained were expressed in Mean and Standard error of mean (Mean ± SEM).

3.0 RESULTS

Table 1: Formula for each batch of formulated lozenge A, B, C, D and E for 500 mg tablet.
Quantities of the components per tablets (mg) are reflected

Batch	Extract (mg)	microcrystalline cellulose (MCC) mg	Polyvinyl pyrrolidone (PVP) mg	Magnesium stearate (mg)1%	Talc (mg) 1%	Lactose (mg)
A	0	50(10%)	25(5%)	5	5	415
B	100	40 (8%)	15 (3%)	5	5	335
C	100	60 (12%)	25 (5%)	.5	5	305
D	100	50 (10%)	25 (5%)	5	5	315
E	100	40 (8%)	40 (8%)	5	5	310

Total tablet weight = 500 mg, A is a negative control tablet

Table 2 Quantity of components in each batch of 30 Tablets

Batch	Extract (g)	Microcrystalline cellulose (MCC)g	Polyvinyl pyrrolidone (PVP)g	Magnesium stearate(g)	Talc (g)	Lactose (g)
A	0	1.50	0.75	0.15	0.15	12.45
B	3.0	1.20	0.45	0.15	0.15	6.30
C	3.0	1.80	0.75	0.15	0.15	9.15
D	3.0	1.50	0.75	0.15	0.15	9.45
E	3.0	1.20	1.20	0.15	0.15	9.30
Total (g)	15g	7.2g	3.90 g	0.75 g	0.75 g	46.65 g

Table 3: Susceptibility of organism to the extract

Concentration of extract (mg/ml)	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Candida albicans***
200	-	-	-
100	-	-	-
50	-	-	-
25	-	-	-
12.5	+	-	+
6.25	+	+	+

KEY: + = Growth - = No Growth

Table 4: Zone of inhibition of extract (IZD) (mm)

Concentration of extract (mg/ml)	*S. aureus* inhibition zone diameter (IZD) (mm)	*P. aeruginosa* inhibition zone diameter (IZD) (mm)	*C. albicans* inhibition zone diameter (IZD) (mm)
200	21	22	30.50
100	16	19	28
50	15	12	6.15
25	7	8	5
12.5	-	4	-
6.25	-	-	-

Table 5: Zone of inhibition of formulated lozenges (IZD) (mm)

Batches (500 mg each)	*S. aureus* inhibition zone diameter (IZD) (mm)	*P. aeruginosa* inhibition zone diameter (IZD) (mm)	*C. albicans* inhibition zone diameter (IZD) (mm)
A	-	-	-
B	10	6	8
C	10	6	10
D	8	6	10
E	10	6	8

Table 6: Zone of inhibition of commercial lozenge (Sample X) (mm)

Concentration of drug (µg/ml)	*S. aureus* inhibition zone diameter (IZD) (mm)	*P. aeruginosa* inhibition zone diameter (IZD) (mm)	*C. albicans* inhibition zone diameter (IZD) (mm)
25.00	11	10	8
12.50	8	7	6
6.25	6	5	4
3.12	5	4	-
1.56	-	-	-

Table 7: Characterization of granules

Batches	Bulk density(g/cm^3)	Tapped density(g/cm^3)	Angle of repose	Carr'sindex (%)	Hausner's ratio	Flow rate
A	0.500	0.660	21.80	24.24	1.32	5.39
B	0.512	0.645	26.56	20.62	1.25	5.39
C	0.504	0.606	30.39	16.83	1.20	5.32
D	0.511	0.647	25.34	21.02	1.26	5.50
E	0.511	0.632	25.27	19.14	1.23	5.51

Table 8: Physical evaluation of tablet

Batches	Mean Hardness (kgf)± D	Mean Friability (%) ± SD	Mean Diameter(mm) ± SD	Mean Thickness(mm) ± SD	Disintegration(min) ± SD	Mean Weight variation(g) ±SD
A	6.80± 0.5	0.99± 0.2	12.529± 0.02	2.54± 0.03	35.15± 1	0.503± 0.01
B	6.40± 0.6	0.79± 0.2	12.523± 0.02	2.55± 0.03	23.05± 1	0.503± 0.01
C	6.25± 0.8	1.19± 0.2	12.535± 0.01	2.54± 0.03	40.10± 1	0.504± 0.01
D	6.95± 0.4	1.19± 0.2	12.541± 0.01	2.54± 0.03	42.12± 0.9	0.503± 0.02
E	6.20± 0.9	1.38± 0.1	12.531± 0.02	2.71± 0.02	30.18± 1	0.504± 0.01

4.0 DISCUSSION

4.1 Susceptibility and minimum inhibitory concentration test result

Susceptibility test is usually a test to evaluate the preliminary antimicrobial activities of an antimicrobial agent as shown in table 3; the organisms, *S. aureus, P. aeruginosa and C. albicans* were susceptible to the extract at the concentrations; 200, 100 and 50mg/ml, 25 mg/ml, 12.5 mg/ml as there were inhibition of the organisms. While at lesser concentration 6.25mg/ml, there was no inhibition [19]. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism [19]. The minimum inhibitory concentration was seen at 12.5 mg/ml for *P. aeruginosa*, 25 mg/ml for *S. aureus* and 25 mg/ml for *C. albicans* as shown in table 4. Batches B, C, D and E of the extract formulated lozenges showed zone of inhibitions which were smaller in diameter compared to that obtained from the raw extract, this was due to the reduction in the surface area of the extract, exposed to the microorganisms due to the presence of the excipients [1]. Batch A which contained no extract had no zone of inhibition. Zone of inhibition of the commercial lozenges (Sample X) of active ingredient, Dequalinium chloride was at the concentrations of 25, 12.5, 6.25, 3.12 µg/ml. While there was no zone of inhibition for *C. albicans* at concentrations 3.12 µg/ml and the MIC for *S. aureus and P. aeruginosa* was 3.12 mg/ml, while for *C. albicans* was 6.25 mg/ml as shown in table 6.

4.2 Compressibility and flowability properties of the granules

The results obtained from the determination of the granules flow properties and compressibility indicates that the Carr's index, Hausner's ratio flow rate and angle of repose comply with the official standard for powder good flowability. These flow properties help determine the quality of the tablets to be produced and ensures for good tablet properties.

4.3 Tablet properties

Weight uniformity: The uniformity of drugs formulated is essential to determine the dose uniformity of a drug. From the obtained result, there was little or no variation in the weight of formulated drugs thereby meeting the official standard of a good tablet property [17, 18]. Any observed variation maybe due to inconsistent powder content.

Tablet hardness: Tablet hardness usually ranges from 4-6 KgF according to (Indian Pharmacopoeia, 2007) [20]. The hardness of a tablet affects the dissolution and the disintegration rate of a drug and a tablet of adequate hardness can withstand pressure applied to it. The friability obtained from the result indicates that it meets the stipulated quality standard.

Tablet diameter and thickness: The thickness and diameter of a tablet influences the weight uniformity thereby affecting the dose uniformity. The diameter and thickness of the tablet is dependent on the die and punches selected for making the tablet. The tablet diameter and thickness obtained in the results conforms to the limit for tablets [21, 22].

Tablet friability: The standard limit for friability is less or equal to 1% [22, 23]. The results showed that the tablets in the different batches conform to the standard limit. In the case of a higher friability this would mean that the tablet would not easily withstand abrasion during mechanical processes.

Tablet disintegration: Disintegration test is carried out to determine how long it takes for a drug to disintegrate in a given medium. If the disintegration time is too long it means the tablet is too hard and a case of batch inconsistency, if it is not in uniform. The tablets meet the standard limit when all six tablets disintegrate according to the stipulated timing [24].

5.0 CONCLUSION

The study showed that the extract formulated lozenges had antimicrobial properties in different concentrations and effective at high concentration against the examined microorganism *Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans* which covers a broad spectrum of gram positive, gram negative and fungal agent found in the mouth or oral cavity. Therefore, *Moringa oleifera* can be used in the formulation of lozenges as an active pharmaceutical ingredient for its antimicrobial properties in the treatment of mouth infections or that associated with the upper respiratory tract.

Acknowledgement

We wish to acknowledge the support of the Centre for Research and Development (CR & D), University of Uyo and the Tertiary Education Trust Fund (TETFund) of Federal Republic of Nigeria, for funding this research, through Institution- Based Research Grant (TETFundIBRG) 2021/2022 session.

Conflict of Interest

There is no conflict of interest as declared by the Authors.

Contribution of the Authors

Akeem A. Agboke: Corresponding Author, handled the microbial aspects of the research.
Imoh I Johnny: The author identified the plant, handled the extraction of the plant extract.
Musiliu O. Adedokun, Owoidoho V. Paulinus and Nyong E. Etim were involved in the formulation of lozenge tablets and the assessment of the tablet's properties. Motunrayo P. Agboke was involved in some microbial aspect of the research.

6.0 REFERENCES

[1] Peters, D. (2005). Medicated lozenges. In Lieberman H.A, Lachman, L., Schwartz, J.B; Pharmaceutical dosages forms: Tablet 2nd edition, Marcel Dekker Inc, 419-577.

[2] Carinci, F., et al. (2013). Oral microflora and periodontal disease: New technology for diagnosis in dentistry. 4(2):170-173.

[3] Aas, A.J., Bruce, J.P., Laureen, N.S., Ingar, O., Floyd, E.D. (2005). Defining the normal bacterial flora of the oral cavity. Journal of Clinical Microbiology; 46(4):1407 – 1417.

[4] Pfaller, M.A., Diekema, D.J. (2007). Epidemiology of invasive candidiasis. A persistent public health problem: Clinical Microbiology Review, Virulence (2):119-128.

[5] Masalha, M., Borovok, I., Schreiber R., Aharonowitz, Y., Cohen, G. (2001). "Analysis of transcription of the Staphylococcus aureus, aerobic class1b and anaerobic class III Ribonucleotide reductase gene in response to oxygen". Journal of bacteriology, 183(24):7260-7272.

[6] Pollack, M. (2000). Pseudomonas aeruginosa. In Mandell, G.L., Bennett, J.E., Dolin, R.; Principles and practice of infectious disease: 5th edition, Churchill living stone, (23):10-27.

[7] Sastry, S.V., Nyshdham, J.R. (2000). Review of formulation used in oral cavity: Pharm. sciences and technology today, (3):138-145.

[8] Majekodunmi, S.O. (2015). Review on lozenges: American Journal of medicine and medical sciences, 5(2):99-104.

[9] Parotta, J.A. (1993). "Moringaoleifera lam, Reseda, horseradish tree. Moringaceae. Horseradish tree family". USDA Forest Service, International Institute of Tropical Forestry, 61:1-6.

[10] Fahey. (2005). Moringaoleifera: A review of the medical Evidence for its nutritional therapeutic prophylactic properties, Part1. Trees for Life Journal, 1:5.

[11] Madukwe, E.U., Ezeugwu, J.O., Eme, P.E. (2013). "Nutrient composition and sensory, Evaluation of dry Moringa oleifera Aqueous extract. International Journal of Basic and applied sciences, 13:3.

[12] Andrew J.M. (2001). Determination of minimum inhibitory concentration. Antimicrobial chemistry therapy. Journal of Antimicrobial Chemotherapy; 48:15-16.

[13] . Turnidge, T.D., Ferraro, M.J., Jorgensen, J.H. (2003). Susceptibility test methods: General considerations. In Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A., Yolken, R. H. Manual of clinical microbiology, 8thedtion; 1103.

[14] Bhattacharyya, L., Schuber S., Sheena C., William R. (2006). "Excipients: Background and introduction". In: Katdare A, Chubal M; Excipient development for pharmaceutical biotechnology and drug delivery systems, 1st edition, CRC Press. Boca Raton Pp 452-474. eBook ISBN9780429191046.

[15] Gohel, M. (2016). Excipient and their functionalities: Pharmaceutical Information. Journal of pharmaceutical Sciences, 13-15.

[16] British Pharmacopoeia (BP) 2018

[17] Tousey, M.D. (2002). The granulation process 101: Basic technologies for tablet making. Technology, Tableting and Granulation process, 11-13.

[18] Allen, V.L. (2001). Troches and lozenges, Secundum Artem; current and practical compounding information for the pharmacist; 4(2):23-25.

[19] Akeem A. Agboke, Imoh I. Johnny, Musiliu O. Adedokun, Owoidoho V. Paulinus and Nyong E. Etim (2023), Evaluation of anti-infective potential of methanol extract of Moringa oleifera root Lam (moringaceae) formulated as mouthwash. Journal of Pharmaceutical and Allied Sciences Vol. 20 No. 2 (2023) ISSN: 1596-8499 Websites: http://ajol.info/index.php/jophas http://www. facebook.com/; joph.website.co.in.

[20] Martindale, (2011). The complete Drug Reference, 37th Ed. Published by Pharmaceutical Press, London.

[21] Mendes, R.W., Bhargava, H. (2006). Lozenges. In SwarbickJ; Encyclopedia of pharmaceutical technology: 3rdedition, Informa Healthcare Inc, 2231-2235.

[22] Chiang, E. (2013). Measuring tablet hardness: A primer in crushing, fracturing and bending test quantify hardness. Journal of pharmaceutical technology, 37:6.

[23] Nelson, K.G., Wang, L.Y. (2006). Determination of time course of tablet disintegration. Journal of pharmaceutical sciences, 66(12): 1758-1761.

[24] Seitz, J.A., Friesland, G.M. (1965). Evaluation of physical properties of compressed tablets, tablet hardness and friability. Journal of Pharmaceutical Science, (54):1353-1357.