JOPAT Vol 20(2) 670– 689, July – Dec. 2021 Edition.

ISSN2636 – 5448 https://dx.doi.org/10.4314/jopat.v20i2.4

Physicochemical and *In Vitro* Antimicrobial Evaluation of Cream and Vaginal Suppository Formulations of the Extract of the Flower Buds of *Syzygium aromaticum* (Myrtaceae)

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ABSTRACT

Clove also known as *Syzygium aromaticum* (Myrtaceae) has been used for centuries due to its known antimicrobial properties. The aim of this study is to formulate and evaluate cream and vaginal suppository formulations containing the ethanol extract of clove. Crushed clove buds were macerated in ethanol (70 %) for 24 h at room temperature, the resulting extract (Cx) was used to prepare water-in-oil cream formulations at concentrations of 5, 10 and 40 %w/w, a macrogol-based suppository at 40 %w/w was also prepared. The creams were evaluated for organoleptic and physicochemical properties; color, odor, texture, pH, and spreadability. The suppository was evaluated for appearance, weight uniformity, pH, liquefaction time, melting point and adhesion/erosion time. Incompatibility was determined using the Fourier Transform Infrared (FTIR). *In vitro* antimicrobial evaluation of the formulations against selected microorganisms were also carried out using the agar dilution and agar diffusion method. Creams were brown to dark brown in color, had a characteristic odor and smooth texture and had good spreading ability, pH was between 6.08 and 9.17 and concentration dependent. The suppositories were brown in color, had an aromatic odor and were whole without pitting, exudates or sedimentation. The pH was 4.78 ± 0.09 with liquefaction time of 13.00 ± 3.61 min and melting point of 35.5 ± 0.5 ºC. Adhesion time of the suppositories on an excised cow vagina was about 2 h. FTIR returned spectra with no interaction. The cream and vaginal suppository formulations showed most significant antimicrobial activity against Gram-negative bacteria and *Candida albicans*. This study shows the propensity of clove extract to be formulated into standardized dosage forms for treatment of vaginal infections.

Keywords; *Syzygium aromaticum*, cream, vaginal suppository, antimicrobial evaluation

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INTRODUCTION

The use of herbal drugs for the management of ailments and treatment of diseases dates back to as long as life itself. The World Health Organization estimates that about 80 % of the world population use herbal therapies for their daily health issues and postulates that this number may likely increase in the coming years as research on the efficacy and safety of these herbal remedies are being actively investigated [1]. Herbal plants have been documented as an essential source for discovering new pharmaceutical molecules due to the presence of secondary metabolites which is responsible for some pharmacological activities of the plants. They have served as bio-starters for some conventional drugs that have been developed into acceptable forms and are currently in use today. For example, salicylic acid, a precursor of aspirin, was derived from white willow bark and the meadowsweet plant; (*Filipendula ulmaria*). Quinine and the popular Artemisinin used as antimalarials are derived from *Cinchona pubescens* bark and *Artemisia annua* plant, respectively. Vincristine, an anticancer drug was derived from periwinkle (*Catharanthus roseus*), while morphine and codeine are derived from the opium poppy, *Papaver somniferum* [2, 3, 4, 5, 6]. Many plants have demonstrated the ability to prevent bacterial growth, inhibit inflammation and also inhibit fungal infections *via* several mechanisms.

Infections of the urinary tract and Vaginitis are rapidly becoming a major health issue among local communities in Nigeria; they are caused by microbial attack on the urinary tract and vagina. Common pathogenic microorganisms implicated in these infections include *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and opportunist yeasts like *Candida albicans.* These infections are common causes of hospitalization (35 %) in developing countries especially among the women due to the anatomy of their urinary tract. Morbidity is high among women of childbearing age, sexually active females, diabetics, patients with sickle cell anemia and pregnancy [7].

In most cases, treatment is by the use of antibiotics but due to the persistence of the infections, overuse and misuse of the commonly prescribed chemical agents, the emergence of drug resistance has increased. Some investigations have shown evidence of fast worldwide spread of clinical isolates that are resistant to available chemical agents. A recent study [8] demonstrated that about 94 % of *Candida albicans* isolated from patients with vaginitis was resistant to fluconazole and econazole. The emergence of antibiotics resistance and contraindication of the use of some of these agents in certain conditions like pregnancy has stirred researchers to search for fresh leads in the development of new and effective molecules from cheap and readily available natural compounds.

Syzygium aromaticum (L.) also known as *Eugenia cariophylata* or *Eugenia aromaticum* and commonly called “clove” belongs to the

family Myrtaceae. It is an ancient plant which is native to India, Zanzibar, Iran, Indonesia, Brazil and Madagascar [9] but is imported into West Africa as an aromatic spice and food flavor.

Clove is primarily cultivated as a spice tree, it has a lifespan of about 100 years and grows all through the year owing to altered harvest seasons in various countries. The whole and ground clove buds are used for culinary purposes (spice, aromatherapy) but they also possess medicinal values such as gastro-protection, anticarcinogenic, antifungal, antibacterial, anti-inflammatory, antiulcerogenic, antiparasitic [10, 11]. Clove is rich in phenolic compounds, particularly eugenol which is responsible for its fungicidal, antioxidant and antibacterial properties [12]. However, different extracts of the clove plant contain varying bioactive compounds which accounts for varying pharmacological activities [9].

Literature shows many studies have investigated the traditional/folklore belief of the antifungal and antibacterial properties of clove (*Syzygium aromaticum*). A study demonstrated the effectiveness of the ethanol extract of clove against multidrug resistant (MDR) strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* [7]. Different extracts of clove (methanol, ethyl acetate, di- ethyl ether, and n-hexane) were found to possess activity against *Candida albicans*, *Candida glabrata* and *Candida tropicalis* at small concentrations however, the ethyl acetate extract exhibited the most significant activity [13]. The antifungal activity of ethanol extract of clove was compared with those of eugenol and amphotericin B and found to possess greater activity against *Candida neoformans* than the other agents [14]). Clove extract has also been reported to possess strong anti-fungal activity against *Candida aspergillus* and dermatophyte species [15, 16]. In a different study [17], the ethanol extract of clove was found to be more effective than the commercial clove essential oil in inhibiting *Proteus mirabilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherchia coli*, *Klebsiella pneumoniae* implicated in urinary tract infections. In yet another study, the ethanol extract of clove was found to be effective against 221 gram-negative beta-lactamase uropathogens isolated in the clinical study [18]. A different study [19] revealed that combination of antibiotics with clove extract produced a synergistic effect which was more effective than the antibiotics alone. The activity of eugenol and carvacrol (constituents of clove) against fungal dermatophytes such as *Onchomycosis* has also been demonstrated [20].

In spite of the numerous efficacies of herbal preparations, they are not readily accepted by patients in their unrefined forms (decoctions, infusions, macerates). Therefore, it is necessary to prepare them into suitable dosage forms like creams and suppositories that would be readily accepted and convenient to use. Creams are semisolid dosage forms prepared as emulsions of either oil in water (o/w) or water in oil w/o) types containing one or more active ingredients. They are applied topically unto the outer layer of the

skin or the mucous membranes to exact their localized effect. Creams unlike other dosage forms like ointments, have a soft feel, are easy to apply, have good permeability and aesthetic approval of most users [21]. Suppositories on the other hand are solid dosage forms with different sizes, shapes and weight that are meant to be inserted into the rectum, vagina, or urethra and allowed to melt with the body temperature [22]. They can be formulated using water-soluble or oil-soluble bases and are beneficial because they require little fluid to release the active ingredient [23].

Literature has shown the potential of *Syzygium aromaticum* in treatment and management of microbial infections of the vagina and urinary tract. Therefore, the aim of this study is to develop cream formulations and vaginal suppositories from the ethanol extract of *Syzygium aromaticum*, evaluate the physicochemical properties and assess the antimicrobial activity of the formulations against selected organisms (*Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pneumonia*, *Neisseria gonorrhea*, *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*) commonly implicated in vaginal infections.

MATERIALS AND METHODS

Materials

Flower buds of *Syzygium aromaticum* (clove) were purchased from a community market in Abuja, Nigeria. Other materials include; Cetyl alcohol (Sigma Aldrich, Germany), Liquid paraffin (Alpha, India), Glycerin (Guanghua, China), Stearic acid (BDH, England), Triethanolamine (Meru, India), Benzyl alcohol (A.B., India), Ethanol (Loba Chemie, India), polyethylene glycol (PEG) 1000 and 4000 (Merck, Germany), ethanol extract of *Syzygium aromaticum* prepared in the NIPRD laboratory. Muller Hinton agar (Titan Biotech LTD, India), Muller Hinton broth (Hi Media Laboratories Pvt. Ltd, India), Sabouraud dextrose agar (Hi Media Laboratories Pvt. Ltd, India), Sabouraud dextrose broth (Hi Media Laboratories Pvt. Ltd, India). All other chemicals used were of Analar grade.

Microorganisms

Typed bacterial and fungal cultures: *Staphylococcus aureus* NCTC 6571, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* 27853, *Klebsiella pneumonia* ATCC BAA 1705, *Streptococcus enterica subsp. enterica serovar typhymurium* ATCC 14025, *Streptococcus pyogenes* ATCC 12384, *Neisseria gonorrhea* ATCC 43069, *Proteus mirabilis* ATCC 12453, *Candida albicans* ATCC 10231; were obtained from the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Collection and preparation of plant material

Flower buds of *Syzygium aromaticum* were deposited at the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. It was subsequently identified and assigned a voucher number (NIPRD/H/7250). The dried buds were

washed, air-dried and pulverized using a mechanical grinder. The coarse powder obtained was packaged in an air-tight container and stored until further use.

Methods

Preparation of ethanol extract of clove

This was prepared according to an earlier method [24] with some modification. The dried pulverized clove buds (657.3g) were macerated in 70 % ethanol in the ratio 1:3 (plant material to solvent) at room temperature for 24 h. After maceration, the mixture was filtered using a muslin cloth, concentrated in a Rotary evaporator (Bibby, Germany) and further concentrated over a water bath (Karl Kolb, Germany) at 40 ^{o}C. The resulting product; ethanol clove extract (Cx), was kept in the refrigerator at 4 ^{o}C until further use.

Preparation of clove cream formulations

The ingredients and their composition used for the formulation of clove cream are listed in Table 1. The aqueous phase containing water-soluble ingredients; glycerin, triethanolamine, benzyl alcohol and water were weighed and mixed together over the water bath at 75 °C. The appropriate quantity of the extract was added to the aqueous phase by stirring. The oily phase containing liquid paraffin, stearic acid and cetyl alcohol were weighed and melted over a water bath at 70 °C and incorporated into the aqueous phase with continuous stirring until completely mixed and cooled. The resulting cream formulations were packaged into airtight containers and stored at room temperature until further use.

Table 1: Formulation composition of clove cream formulations

Ingredients	CxC_0	CxC_5	CxC_{10}	CxC_{40}
Cx (g)	-	1.5	3	12
Stearic acid (g)	3	3	3	3
Cetyl alcohol (g)	1.5	1.5	1.5	1.5
Liquid paraffin (mL)	2.4	2.4	2.4	2.4
Glycerin (mL)	1.5	1.5	1.5	1.5
Triethanolamine (mL)	0.6	0.6	0.6	0.6
Benzyl alcohol (mL)	0.6	0.6	0.6	0.6
Water (mL)	20.4	18.9	17.4	8.4

Key: Cx = crude extract of clove; CxC_0 = cream formulations containing Cx at 0 %w/w; CxC_5 = cream formulations containing Cx at 5 %w/w; CxC_{10} = cream formulations containing Cx at 10 %w/w; CxC_{40} cream formulations containing Cx at 40 %w/w

Preparation of clove suppositories

Two types of suppository bases were intended for this study; cocoa butter a fatty hydrophobic base and macrogol (PEG) representing a water-soluble base. However, incorporation of the extract into the cocoa butter did not produce whole suppositories, therefore only macrogol was used for the suppository preparation.

In order to calculate the displacement value of the extract and determine the final quantity of base to use, 2 g of the suppository molds were calibrated using 40 %w/w of the extract with appropriate

quantities of the base. After the displacement value was obtained, a batch size of 50 suppositories was prepared. Twenty (20) grams of PEG 4000 was weighed into a beaker and melted in a water bath (Karl Kolb technical Supplies, Germany) at 55 °C. This was allowed to cool to 35 °C, and 80 g of PEG 1000 was added to the content of the beaker. Afterwards, 40 g of the clove extract was added to the base and mixed thoroughly, after which it was poured into the suppository mold and allowed to solidify at room temperature (27 ºC). Subsequently, the molds were unscrewed and the suppositories were removed, wrapped in aluminum foil and coded as CxCS. They were stored in the refrigerator at 4 ºC for further analysis.

Evaluation of cream formulations

Physical evaluation

The organoleptic characteristics such as appearance, color and odor of the cream formulations were assessed. Other physical parameters like texture, ease of application and removal, phase separation after 30 days at room temperature were also evaluated.

Chemical evaluation

Determination of pH

The pH of the undiluted cream formulations was determined by using digital pH meter (Mettler Toledo, Switzerland). Triplicate determinations were made, the average and standard deviation were calculated.

Determination of cream spreadability

An established method [25] was adopted for this procedure with slight modifications. Two (2) glass slides of standard dimensions (7 cm × 2.3 cm) were selected, the cream formulation (0.2 g) was placed on one of the slides and covered with the other slide. A 100 g weight was placed on the covered slides for 5 min (in such a way that the formulation was sandwiched between them). After the weight was removed, the extent of spread of the cream on the slide was measured in four different directions and the average calculated in cm. Furthermore, the time taken for the upper slide to be separated from the lower slide was noted and spreadability (S) was calculated using the equation below;

$$S = \frac{MxL}{T} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, M= weights placed to upper slide, L= length of glass slide, T= time taken to separate slide

Physicochemical evaluation of suppositories

Appearance

Six (6) suppositories were selected at random and observed for intactness, colour, odour, shape, presence/absence of pitting, exudation and sedimentation.

Weight uniformity

This was performed by selecting ten (10) suppositories at random from the batch. Each suppository was weighed using an analytical balance (Mettler Toledo, Switzerland). The average weight of the suppositories was computed and the standard deviation calculated.

Determination of pH

One (1) suppository was melted at 37 ± 1 ºC in a beaker and the pH was determined using the pH

meter (Jenway, UK). Three determinations were made, the average and standard deviation were calculated from the results obtained.

Liquefaction Time

This was determined according to an earlier method [26]; one (1) suppository was selected at random from the batch. It was placed into a beaker containing 60 mL of phosphate buffer (7.4) maintained at 37 ± 1 ºC. The time taken for the suppository to completely melt or dissolve was noted as the liquefaction time. This was repeated for two (2) other suppositories, the average and standard deviations were computed from the results obtained.

Determination of melting point

An earlier method [27] was adopted; one (1) suppository was placed in a beaker which was then placed (6 cm deep) in a water bath (Karl Kobb, Derieich West Germany) containing water regulated at 1 °C/2 min. The temperature at which the suppository just started melting as determined using a thermometer was recorded as the melting point. This was repeated to obtain three (3) determinations from which the average and standard deviation were calculated.

Determination of adhesion/erosion of vaginal suppository

The determination of adhesion was conducted according to the method of Ofoefule and Ike-Unor [28]. Freshly excised cow vagina was cut into 11.5 cm by 7.5 cm and pinned unto a plastic support and the tissue was flushed with phosphate buffer (pH 4) maintained at 37 ± 1 ºC. The suppositories were placed unto the pinned tissue and allowed to equilibrate for 5 min before placing under the buffer solution which was set at 10 drops per min. They were monitored under this condition until the suppositories dissolved and bioadhesion (%) was calculated using the equation below;

$$bioadhesion\ (\%) = no.of\ suppositories\ after\ test / no.of\ initial\ suppositori$$

The time taken for the suppositories to dissolve was also noted and recorded.

Fourier transform infra-red spectra studies (FTIR)

The extract alone, cream formulations and the suppository were triturated with potassium bromide powder and made into pellets (1 ton/cm^2). Infra-red (IR) spectra were obtained between scanning ranges of 4000 and 400 cm^{-1} from the Magna-IR, 560 spectrometer (Perkin Elmer, USA).

Microbiological assay of prepared cream and suppository formulations

Agar diffusion method

The cup plate agar diffusion method was adopted according to CLSI [29] with slight modifications. Mueller Hinton agar was inoculated with 0.5 McFarland suspensions (approx. 1-2 x 10^8 cfu/mL) of the test organisms. Wells bored on the agar with a 6 mm cork borer were filled with 100 µL of the diluted cream formulations (40 mg/mL) and incubated at 37ºC for 24 h (bacteria) and 25 ºC for 48 h (fungi). On the other hand, the suppository formulation was melted, diluted appropriately to obtain (40 mg/mL) and 100 µL

was placed into the bored wells as earlier described. A commercial cream comprising of the combination of clotrimazole, betamethasone and neomycin was used as positive control. The diameter of the zones of inhibition were accurately measured and the procedure was repeated to obtain duplicate readings.

Agar dilution method

One (1) millilitres of 80 mg/mL of each of the cream and suppository formulations was diluted appropriately with 19 mL of molten Mueller Hinton agar and allowed to solidify. Subsequently, 10 µL of standardized inoculum (approx. 1-2 x 10^8 cfu/mL) was spotted on the surface of the agar and incubated at 37 ^oC for 24 h (bacteria) and 25 ^oC for 48 h (fungi). The plates were assessed for growth and the procedure was repeated to obtain duplicate readings.

Determination of in-vitro release

The *in-vitro* release of the suppository was performed using the agar diffusion method (26). One (1) suppository was melted in an empty beaker and 0.25 mL transferred into a 25 mL volumetric flask. The volume in the flask was made up to 25 mL with sterile phosphate buffer (pH 7.4) and mixed thoroughly. Ferric chloride, 5 %w/v (1 mL) was spread unto the surface of Nutrient agar and the excess solution was drained off. With the aid of a 6 mm cork borer, four (4) holes were bored on the agar surface. Subsequently, 200 µL of the diluted suppository was added to the wells and the plates allowed to stand for 30 min in the biosafety cabinet. The plates were then incubated at 37 ^oC and the zones of colour change were measured at 1, 2, 3, 4, 5, 6 and 12 h.

RESULTS

Physicochemical evaluation of cream formulations

Organoleptic and physical properties of the formulated creams are presented in Table 2. Organoleptic evaluation of the creams revealed the creams containing clove extract (Cx) were light brown to dark brown in color. However, formulation CxC_0 was white in color due to the absence of the extract. All the cream formulation had uniform appearance and smooth texture implying that the extract was homogenously dispersed into the other ingredients of the formulation. The creams containing the extract (CxC_5, CxC_{10} and CxC_{40}) were observed to be moderately to easily applied and washed-off. No phase separation was observed in all the cream formulations.

Table 2: Organoleptic and physical properties of clove cream formulations

Parameters/Batch	CxC_0	CxC_5	CxC_{10}	CxC_{40}
Appearance	Uniform	uniform	uniform	Uniform
Color	White	light brown	brown	dark brown
Odor	odorless	characteristic	characteristic	Characteristic
Texture	Smooth	Smooth	smooth	Smooth
Ease of application	+++	+++	++	++
Wash ability	+++	+++	++	++
Phase separation	-	-	-	-

Key: +++ = easy to apply, easy to wash off; ++ = moderately easy to apply, moderately easy to wash off; - = no phase separation

Table 3 shows the pH values of the prepared cream formulations and they were between 6.08 and 9.17 and these values were found to decrease as the concentration of Cx in the formulation increased. Spreadability was observed to be between 9.24 and 19.26 (g.cm/sec); CxC_0 was found to have the least value while higher values observed for creams containing the extract decreased with increase in the extract concentration.

Table 3: pH and spreadability of the clove cream formulations

Batch	pH	Spreadability
CxC_0	9.17± 0.03	9.24 ± 1.09
CxC_5	8.46 ± 0.01	19.26 ± 7.61
CxC_{10}	7.68 ± 0.06	18.11 ± 1.47
CxC_{40}	6.08 ± 0.07	12.00 ±1.87

Physicochemical properties of clove suppository formulation

On physical examination, the suppositories were observed to be brown in color (Table 4) and they all had uniform color. The suppositories had an aromatic odor, with intact, smooth shiny surfaces devoid of cracks or fractions. There was no sign of pitting, exudates or sediments. The average weight of the prepared suppositories was 2.48 g with low standard deviation (0.02), implying they had similar weights. The pH of the formulated suppositories was 4.78 ± 0.09, liquefaction time was about 13 min while the melting point was 35.5 ± 0.5 ºC and the time taken for the formulated suppositories to adhere to and erode from excised cow vagina was about 2 h.

Table 4: Physicochemical properties of clove vaginal suppository formulation

Evaluation Parameters	Result
Intactness	Intact
Color	Light-Dark brown
Odor	Aromatic
Shape	Bullet
Pitting	None
Exudation	None
Sedimentation	None
Mean weight (g)	2.48 ± 0.02
pH	4.78 ± 0.09
Liquefaction Time (min)	13.00 ± 3.61

Melting point (ºC)	35.5 ± 0.5
Adhesion time (h)	2.00 ± 0.05

Fourier Transform Infrared (FTIR)

Spectra of the clove extract, prepared clove vaginal suppository and prepared clove cream formulation are displayed in Figure 1. FTIR spectrum of clove extract (Figure 1A) shows strong stretching vibrations of the OH groups of the alcoholic extract at 3514.9 cm^{-1} which was also detected in spectra of prepared cream (3265.9 cm^{-1}) and suppository (3447.8 cm^{-1}) formulations as displayed in Figures 1B and 1C. The moderate bands between 2937.1 and 2870.1 cm^{-1} represents asymmetrical patterns of group CH_2 and CH_3 of alcoholic compound. The short absorption band at 2844.0 cm^{-1} (Figure 1A) which represents the aromatic C-H stretch was also observed in Figures 1B and 1C as 2847.7 and 2870.1 cm^{-1}. The bands representing the C=C aromatic carbonyl group was observed in the 3 spectra at between 1640.0 and 1636.6 cm^{-1}. Frequency of pattern of group CH_2^- was present in Figure 1A at 1461.1 cm-1 and also in Figure 1B at 1461.1 cm^{-1} while it was observed at 1453.7 cm^{-1} in Figure 1C.

Microbiological assay of prepared creams

Antimicrobial activity was observed to be dependent on the concentration of the extract in the cream formulations while CxC_0 containing only the cream base showed no activity (Table 5). The most significant activity from the cream formulations was observed with CxC_{40} which was found to be comparable to the activity observed with the suppository formulation (CxCS). The observed activity was also found to be comparable with the control used against most of the microorganisms tested. The zones of inhibition were observed to be greater for Gram-negative organisms (*S. enterica*, *P. aeruginosa*, *E. coli*, *P. mirabilis*, *K. pneumoniae*, *N. gonorrhea*) than for the Gram positive bacteria (*S. aureus*, *S. pyogenes*).

Table 5: Inhibition zones of clove cream and suppository formulations

Organisms	**Diameter of Inhibition Zones (mm)**					
	CxC_0	CxC_5	CxC_{10}	CxC_{40}	CxCS	Control
E. coli ATCC *11775*	0.0	0.0	12.0	18.0	20.0	24.0
P. aeruginosa 27853	0.0	0.0	16.0	20.0	15.0	25.0
K. pneumonia ATCC BAA *1705*	0.0	0.0	14.0	17.0	15.0	20.0
P. mirabilis ATCC *12453*	0.0	0.0	16.0	18.0	20.0	25.0
N. gonorrhea ATCC *43069*	0.0	0.0	11.0	15.0	15.0	26.0
S. aureus ATTC *6571*	0.0	0.0	13.0	15.0	15.0	23.0
S. enterica ATCC *14025*	0.0	10.0	13.0	20.0	15.0	20.0
S. pyogenes ATCC *12384*	0.0	0.0	14.0	19.0	20.0	23.0
C. albicans ATCC *10231*	0.0	0.0	10.0	17.0	15.0	25.0

Table 6 below shows the results of the antimicrobial activities of the varying concentrations of the cream and suppository formulation also revealed a dose-dependent antimicrobial activity which was consistent for all the microorganism tested. Figure 2 on the other hand, shows very rapid diffusion of the formulation within 1 h after which a gradual release was observed over the 12 h period of the test. Plates I-VII show *in-vitro* diffusion of clove suppository over 12 h.

Table 6: Antimicrobial activity of clove cream and suppository formulations

	20 mg/mL					40 mg/mL				
Organisms/Batches	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
E. coli ATCC *11775*	+	+	+	-	-	+	-	-	-	-
P. aeruginosa 27853	+	+	+	-	-	+	-	-	-	-
K. pneumonia ATCC BAA *1705*	+	+	+	-	-	+	-	-	-	-
P. mirabilis ATCC *12453*	+	+	+	-	-	+	-	-	-	-
N. gonorrhea ATCC *43069*	+	+	+	-	-	+	-	-	-	-
S. aureus ATTC *6571*	+	+	+	-	-	+	-	-	-	-
S. enterica ATCC *14025*	+	+	+	-	-	+	-	-	-	-
S. pyogenes ATCC *12384*	+	+	+	-	-	+	-	-	-	-
C. albicans ATCC *10231*	+	+	+	-	-	+	-	-	-	-

Key: - = Inhibition (no growth), + = No inhibition (growth), 1 = CxC_0, 2 = CxC_5, 3 = CxC_{10}, 4 = CxC_{40}, 5 = CxCS (suppository formulation)

DISCUSSION

The color intensity of the creams increased as the concentration of the extract in the formulation increased. Color is a parameter that aids identification of dosage forms, it is also used to determine the stability of semisolids; a change in color over a period of time is an indication of decomposition or interaction. Our results show that the color of all the cream formulations was unchanged after 30 days' storage at room temperature. The odor of the creams was characteristic but not offensive and is attributed to the presence of the extract. Generally, the organoleptic properties of the creams were found to be appealing and this is important because it influences the patient's compliance to the medication. The uniform appearance and smooth texture of the cream formulations are an indication that the extract was homogenously dispersed into the other ingredients of the formulation. The results also show that the creams can be applied without the use of a lint and washed off without the use of soaps. The cream formulations were homogenous and stable over the period of storage depicting the possible use of the creams over a period of time.

The pH of the cream formulations was close to that recommended for the skin [25]. Topical preparations such as creams are required to have pH between 5.5 and 10 to prevent skin irritation

due to acidity and improve its acceptability by users. On the other hand, highly alkaline cream formulations with pH between 11 and 14 result in scaly skins [30] however, mildly alkaline topical creams have been found to be beneficial in the treatment of some skin inflammation like eczema [31]. Therefore, the clove cream formulations are found to be suitable for the skin.

Spreadability is the expansion of a cream formulation on a surface after a certain time, it assesses the ability of the topical formulation to be applied evenly on the skin with a small amount of pressure (32). This factor is critical as it affects the efficacy of the cream formulation because spreading ability influences the rate at which the active components of a formulation would be distributed and absorbed [33]. High spreadability values indicate that a shorter time is required to separate the two slides which further implies the formulation can be applied with greater ease with consequent faster release of the constituents of that formulation [25, 34]. The result shows that inclusion of clove extract in these cream formulations would not cause a drag or unnecessary resistance which invariably would influence distribution and absorption of the constituents of the formulation.

The color and aroma of the suppositories was due to the characteristic of the extract, the uniform appearance of the suppositories indicates that the extract and excipients were properly mixed without migration of ingredients. This is an important feature because uniform and smooth surfaces ensure ease of administration [35]. A distinctive physiology of the vagina is that it undergoes cyclical changes, with changes in hormones that alter its normal microflora, and pH. The pH of normal vaginal environment in adult women of reproductive age range between pH 4-5 [36] due to the presence/activities of *Lactobacilli* responsible for the secretion of lactic acid and hydrogen peroxide [37]. This acidic environment limits the overgrowth of opportunistic infections that causes irritations, discharge and odors [38]. The pH of the formulated suppositories was within the normal physiological pH of the vagina implying that this formulation would not cause irritation upon insertion.

The liquefaction time correlates with the time at which the suppository melts at body temperature and is expected to be within 30 min [22]. The liquefaction time reported here shows that the suppositories will melt rapidly when in contact with the body which is appropriate for the release of the active ingredient from the base and for rapid local effect at the site of action [39]. A higher liquefaction time however will cause expulsion of the suppository from the body before it elicits its effect. Other studies [26, 40] on herbal suppositories have also reported similar liquefaction time.

The clove suppositories were prepared using a combination of polyethylene glycol PEG (1000 and 4000). These are water soluble bases that are commonly used in the formulation of suppositories because they melt at body temperature [41]. Our results show that the

melting point of the suppositories is lower than that of the body but within the acceptable melting range of suppositories [42]. This implies that there will be rapid release of the active ingredient from the suppository as has been corroborated by the liquefaction time. Similar low melting points have also been observed with the use of water-soluble bases in the formulation of herbal extract-based suppositories [22, 43].

The time taken for the formulated suppositories to adhere to and erode from excised cow vagina can be regarded as the residence time or the removal time which is an indication of the average time for the suppository to stay on the excised cow vagina and the length of time it took to erode from that site. According to Acarturk [36], conventional vaginal dosage forms such as vaginal suppositories reside in the vagina for a fairly short duration after administration due to the self-cleansing actions of the vaginal tract, thus requiring multiple daily doses to achieve the desired therapeutic effect. The short residence time suggests that multiple doses will be required before a therapeutic effect can be achieved especially in consideration of the liquefaction time and the melting point observed in this study. Therefore, to achieve therapeutic efficacy of this formulation, a mucoadhesive vaginal suppository with extended/prolonged residence time may be developed.

FTIR spectra of the clove extract showed some characteristic bands and peaks peculiar to the extract which were also observed in the cream and suppository formulations at varying intensities but no new peaks were observed. This implies that there was no interaction between the extract and the ingredients of the cream and vaginal suppository formulations.

Antimicrobial study showed significant activity against *Candida albicans;* worthy of note is the fact that the formulations also showed more significant activity against Gram-negative organisms which have been reported to be implicated in vaginal infections [44] than the Gram-positive organisms. This is of particular interest because most new drugs are developed for Gram-negative bacteria since they are more prone to resistance than the gram-positive bacteria [45]. This result shows the potential of development of clove formulations in treatment of infections caused by bacteria prone to resistance. Literature report of the antibacterial and antifungal activity of the clove extract is in tandem with results obtained in this study. This shows that incorporation of the extract into cream and suppository formulations did not interfere with the extent of activity of the extract.

In vitro diffusion as performed in this study is a precursor to the extent and duration of release of the suppository. The zone of color change which indicated diffusion, was found to increase with increase in time of incubation and agrees with a similar report [26]. Diffusion of the formulation into the agar matrix is critical to the resultant inhibition of the test organism and activity of the formulation. Thus, poor diffusion may result in reduced antimicrobial activity even with drug of known efficacy. This implies that there will be

rapid release of the extract from the formulation within 1 h after which there is gradual release of same.

CONCLUSION

The ethanol extract of the flower buds of *Syzygium aromaticum* has been successfully incorporated into cream and vaginal suppository formulations. Both formulations possessed optimum physicochemical properties. Antimicrobial activity of the formulations was found to be dose-dependent with the cream and vaginal suppository formulations containing 40 % of the extract showing the most significant potential to inhibit the growth of Gram-negative bacteria and *Candida albicans*. Therefore, the clove extract cream and vaginal suppository formulations can be further developed for commercial use in the treatment of vaginal infections.

ACKNOWLEDGEMENT

All authors are grateful to the National Institute for Pharmaceutical Research and Development, (NIPRD), Abuja, Nigeria for providing the facility to carry out most of this research.

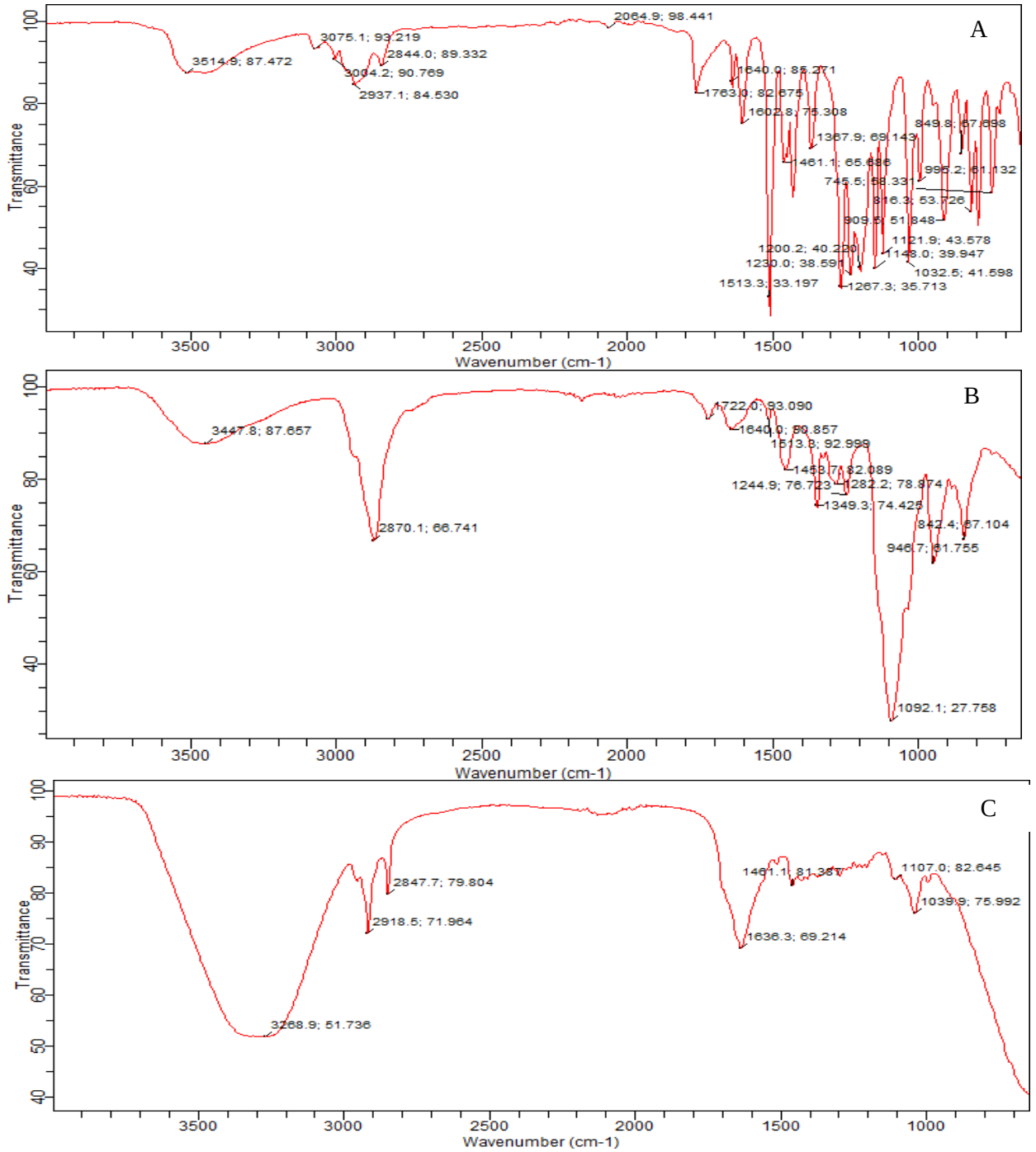


Figure 1: FTIR Spectra of clove extract (A), clove vaginal suppository (B) and clove cream (C)

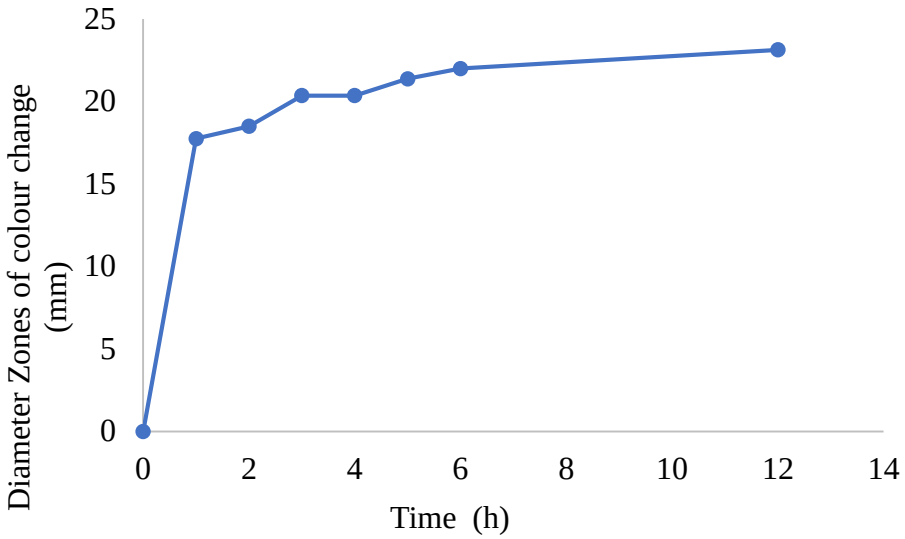
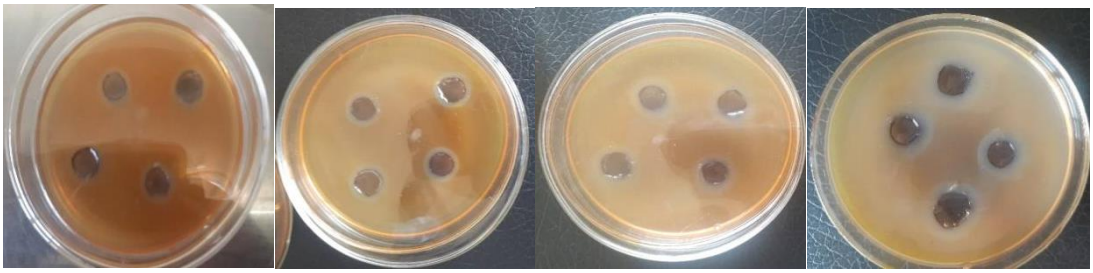


Figure 2: Diffusion profile for clove suppository formulation



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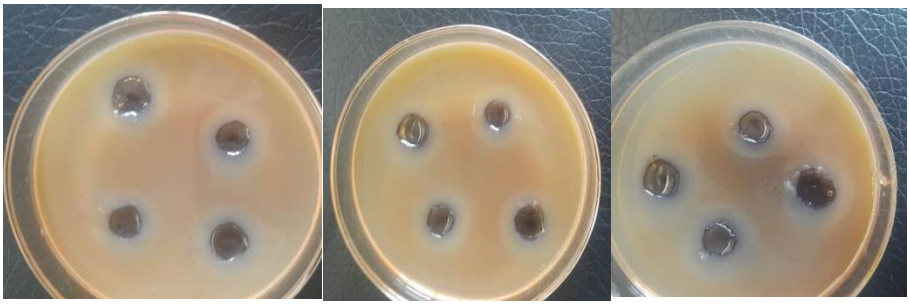

V VI VII

Plates I – VII: Zones of diffusion of suppository after 1, 2, 3, 4, 5, 6 and 12 h respectively

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