Journal of Medical Laboratory Science, 2021; 31 (2): 52-72
http://jomls.org; info@jomls.org **Ebenebe *et al***
http://doi.org/10.5281/zenodo.5033303

Development and Evaluation of Vaginal Suppository Containing *Lactiplantibacillus pentosus* KCA1 for Treatment of Bacterial Vaginosis: Can Fourier Transform-Infrared (FT-IR) Spectroscopy be Used for Identification of *Lactiplantibacillus pentosus* KCA1?

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ABSTRACT
Background: Bacterial vaginosis is a major urogenital health problem for premenopausal women and the condition is misdiagnosed, mistreated and under-reported. Developing a user-friendly natural active product is more than ever needed. **Objectives**: The objectives of this study were to develop and evaluate vaginal suppositories containing *Lactiplantibacillus pentosus* KCA1 (Formally *Lactobacillus pentosus* KCA1) and to use FT-IR spectroscopy to identify the presence of *L. pentosus* KCA1. **Methods**: The vaginal suppository was developed using a glycerol-gelatin base. The prepared suppositories containing KCA1 were evaluated for viability and stability, and other physical properties. The *in vitro* release was done to determine the dissolution and the disintegration time of the suppositories. Fourier Transform-Infrared (FT-IR) spectroscopy was used to analyze *Lactobacillus pentosus* KCA1, formulated vaginal suppository containing *Lactobacillus pentosus* KCA1 and vaginal suppository glycerol-gelatin base without *Lactobacillus pentosus* KCA1, to reveal physical properties of KCA1 from the peak of the FT-IR spectra identified. **Results**: The result of the release study demonstrated that suppositories with glycerol-gelatin base releases *Lactobacillus pentosus* KCA1 faster and it was also found to be microbiological stable and viable after storage at 2-8^{0}C over the period of 3 months. The result of the FT-IR spectroscopy revealed that *Lactobacillus pentosus* KCA1 was present in the formulation. **Conclusion**: The FT-IR result showed that *L. pentosus* KCA1 is most likely to be identified between 1318.953-713.2706 cm^{-1} of the spectral regions. The FT-IR result in this study will support the development of the BACTI-FTIR database, which will continuously be updated to offer a practical tool for identifying microorganisms.

Key words: Vaginal suppository, probiotics, *Lactiplantibacillus pentosus* KCA1, Release study, FT-IR spectroscopy
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Author's contributions: This work was carried out and approved in collaboration between all the authors who take responsibility for its intellectual contents, accuracy and integrity. KCA, and COO designed the study; KCA and INE sourced for funding; INE and KCA wrote the protocol; INE and KCA contributed in literature search; INE did Lab experiments; INE did the statistical data analysis; INE and KCA did the discussions; INE drafted the manuscript; KCA, OEU and COO supervised the study.

Received: January/15, 2021; **Accepted**: May/012, 2021; **Published**: June/28, 2021.

Citation: **Ebenebe IN, Uduma OE, Esimone CO, Anukam KC**. Development and Evaluation of Vaginal Suppository Containing *Lactiplantibacillus pentosus* KCA1 for Treatment of Bacterial Vaginosis: Can Fourier Transform-Infrared (FT-IR) Spectroscopy be Used for Identification of *Lactiplantibacillus pentosus* KCA1? **J Med Lab Sci, 2021; 31 (2): 52-72**

INTRODUCTION

Bacterial vaginosis (BV) is under-reported, misdiagnosed and mistreated and the condition has emerged as a public health problem due to its association with sexually transmitted infections (STIs) (**1**). Our research group used 16S rRNA metagenomic method to determine the composition of vaginal and gut microbiota of Nigerian women diagnosed with BV, which revealed polymicrobial nature and decreased presence of Lactobacilli (**2**).

However, in healthy women, the vagina is colonized predominantly by lactobacilli as we demonstrated in our previous study whereby the vagina of healthy, non-BV Nigerian women were colonized by Lactobacillus-dominated bacterial communities (**3**). It is now a known fact that the healthy vaginal microbiota is dominated mainly by Lactobacillus species, which can used as a valuable biomarker for evaluating health and disease (**4**). The current treatment for BV is usually Metronidazole and Clindamycin. However, this treatment does not prevent BV recurrence, which is the main complaint of the patients as well as the side effects. The major reason for the recurrence following the treatment of BV is that the antimicrobials merely kill both disease causing and healthy microbes and unfortunately do nothing to restore to the vagina the levels of Lactobacillus and other healthy bacteria that are necessary for optimal vaginal health. The acidic environment, bacteriocins produced by lactobacilli in a healthy vagina makes the environment unfavorable for the proliferation of bacteria associated with BV and sexually transmitted pathogens (**5**).

The use of probiotics for the restoration of abnormal vaginal microbiota is gaining momentum (**6**, **7**) and most products are commercially available to consumers in many forms including probiotic supplements (capsules, gummies, liquids, powders, tablets, drops and suppository) and consumers interest in probiotics has risen in the last 30 years (**8**, **9**, **10**) and are now used as topical agents and vaginal suppositories (**11**). For example, *L. crispatus* strain CTV-05 has been developed as a vaginal suppository (LACTIN-V) for the prevention of recurrent urinary tract infections in women and found to colonize the vagina (**12**). Other authors found that vaginal suppositories containing Lactobacillus possess good properties that promote the replacement of the vaginal microbiota in cases of urinary tract infections (**13**)

In another study, the administration of vaginal suppositories containing *L. crispatus* GAI 98332 was found to be a safe and promising treatment for the prevention of recurrent urinary tract infections (**14**). One of the selective criteria for application of probiotics to the vagina is viability of the microorganisms and suppositories possess some advantages in terms of uniformity, and absence of irritation and not requiring large volume of dissolution for the release of the active substance (**14**). After preparation, identifying the active substance is very critical to ensure that viability is maintained throughout the shelf life.

A previous study (**15**) used Fourier Transform Infrared (FT-IR) spectroscopy to analyze 56 strains from four closely related species of *Lactobacillus*, (*L. sakei*, *L. plantarum*, *L. curvatus* and *L. paracasei*) but there are little or no published information on the use of FT-IR spectroscopy to identify Lactobacillus present in suppositories. Fourier Transform Infrared (FT-IR) spectroscopy has been used to identify bacteria in the last five decades. The FT-IR measures the total composition of bacterial cells in a nondestructive manner, producing an IR spectrum with bands from all cellular

components. The technique is rapid, easy to use and cost-effective (**16**)

The objectives of this study were folds; first to formulate and evaluate vaginal suppository containing *Lactiplantibacillus pentosus* KCA1 using glycerol-gelatin base that can be used for the treatment of bacterial vaginosis. Second, to use FT-IR spectroscopy to identify the presence of *Lactiplantibacillus pentosus* KCA1 in the suppository.

MATERIALS AND METHODS

Lyophilized *Lactiplantibacillus pentosus* KCA1 was a gift from Winclove Probiotics-The Netherlands and suppository molds that were used was donated by the Department of Pharmaceutics and Pharmaceutical Technology, Faculty Pharmaceutical sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. All other chemicals and reagents that were used are of analytical grade.

Culture collection

Lactiplantibacillus pentosus KCA1 strain, a previously genome-characterized (**17**) vaginal isolate from a healthy Igbo woman was employed for this study. The genome sequence and annotations can be found in the non-redundant database of the National Center for Biotechnology Information (NCBI) with gene Gene Bank accession number NZ_CM001538.1

Reconstitution of the culture

Lactiplantibacillus pentosus KCA1 strain in lyophilized (powered) form, was reconstituted in MRS broth and grown microaerophilically at 37^{o}C for 18 - 48 hours. Thereafter, it was harvested by centrifugation and washed with Phosphate Buffer Saline (PBS). The reconstituted culture of L pentosus KCA1 was stored in MRS broth with 20 % glycerol for subsequent use.

Formulation of Vaginal Suppository containing *Lactiplantibacillus pentosus* KCA1.

The method adopted for the formulation of vaginal suppository containing Lactobacillus spp., was obtained from the procedure described by Kaewnopparat and Kaewnopparat, (**18**).

The vaginal suppository was prepared by fusion method. Glycerogelatin, as a suppository base was melted over a water bath. Thereafter, 10^8 cfu of *Lactiplantibacillus pentosus* KCA1, was added to the melted base at a temperature of about 40-45^{0}C with gently stirring until a homogenous mass was formed. Then, the mixture was poured into a metal suppository mold at a temperature just above the congealing point of the suppository base and they were allowed to cool and solidify at room temperature. Each suppository contains 10^8 cfu of *Lactiplantibacillus pentosus* KCA1. And all the prepared suppository was kept in the refrigerator for further studies.

Pre-formulation studies of the vaginal Suppository containing *Lactiplantibacillus pentosus* KCA1.

Physical appearance

The physical appearance of the suppository was examined. These include the odour, surface condition, shape, texture and, colour and opacity.

Weight variation

These was carried out as described by (**19, 20**). Twenty (20) suppositories were randomly selected and weighed together, to determine the average weight of the 20 suppositories. Individually, each of the suppository was also weighed. Thereafter, the deviation of the individual suppository

from the average weight was calculated and expressed in percentage.

Dimensions of the suppositories

Three suppositories were randomly selected and, the length and the width of the formulated suppositories were measured (**21**).

Homogeneity Test

Three suppositories from each batch were cut longitudinally and drug distribution pattern (rough or dryness) in the formulated vaginal suppositories was observed and recorded (**21**, **22**).

Evaluation of the vaginal suppository containing *Lactiplantibacillus pentosus* KCA1.

Viability test and stability of *L. pentosus* KCA1

The Viability test and stability of the formulated vaginal suppository was done according to the method described by Kaewnoparat and Kaewnoparat, (**18**). The vaginal suppository containing *Lactiplantibacillus pentosus* KCA1, was kept in glass containers at ambient temperature (30 ± 2^0C) and for 3 months. Thereafter, at appropriate time intervals 0, 1 month, 2 months and 3 months, the survival of *Lactiplantibacillus pentosus* KCA1 was determined by plate count method using MRS agar medium.

Maximum release studies / Dissolution Test

The release of the *Lactiplantibacillus pentosus* KCA1, from the vaginal suppository was determined by the method described by Kaewnoparat and Kaewnoparat, (**18**). A rotating basket dissolution apparatus was used at 100 ± 1 rpm at a temperature of 37 ± 0.5^0C, with one thousand (1000) mililiter of citric acid/phosphate buffer solution at pH 4.4 (modelling the vaginal pH) which was used as the medium. Thereafter, each suppository was placed in the basket and lowered into the flask containing the dissolution medium. At appropriate time intervals, 4 miles of the sample was withdrawn and fresh buffer solution maintained at experimental temperature, was used to replace the same volume of withdrawn sample. The *Lactiplantibacillus pentosus* KCA1, was determined by plate method using MRS agar medium and incubated under anaerobic condition at 37^0C for 48 hours.

Disintegration Test

These was carried out as described by Kushwaha *et al*., (**19**, **20**). Six (6) suppositories were selected for the disintegration time test using digital micro-processor-based disintegration test apparatus. One suppository was introduced into each tube cylindrical glass container with a perforated end. The assembly was suspended in 1000 ml of citric acid/phosphate buffer solution at a pH 4.4 and maintained at 37 ±0.5^0C. Thereafter, the apparatus was operated by allowing it to move up and down in the buffer. The time of disintegration was noted when each of the suppositories has completely dissolved in the medium. The mean values will be calculated from six parallel measurement.

FTIR analysis

The FTIR spectrum of the vaginal suppository containing *Lactiplantibacillus pentosus* KCA1 was analyzed using the KBr Disc technique described by Frank (**23**). About 0.5 – 1 mg of the vaginal suppository was finely grinded and mixed with 0.5 g of KBr powder, using small mortar and pestle.

Thereafter, 1 ml of nujol (a solvent for preparation of sample by Buck 530 IR-spectrophotometer), was introduced into the sample (vaginal suppository) using a syringe, an in-house protocol used by springboard research laboratory, Awka Anambra State Nigeria. Briefly, the sample was introduced into the instrument sample mould and allowed to scan at a wavelength of 4000-600 cm^{-1} to obtain its spectra heights (Springboard Research Laboratory, Personal Communication, 2018).

RESULTS

Formulation of vaginal suppository containing *Lactiplantibacillus pentosus* KCA1

The vaginal suppositories were formulated according to the method described above. The formulated suppositories had bullet shape or cylindrical shape with a pointed end, as shown in Figure 1, because this shape was considered preferable by women (**24**). The formulation requirement was summarized in Table 1 below.

Table 1: Composition of the formulated vaginal suppository containing 10^8 CFU *Lactobacillus pentosus* KCA1

Ingredients	Composition (w/w, %)
Glycerol	14
Gelatin	70
Water	15
L.pentosus KCA1	1ml (10^8 cfu)

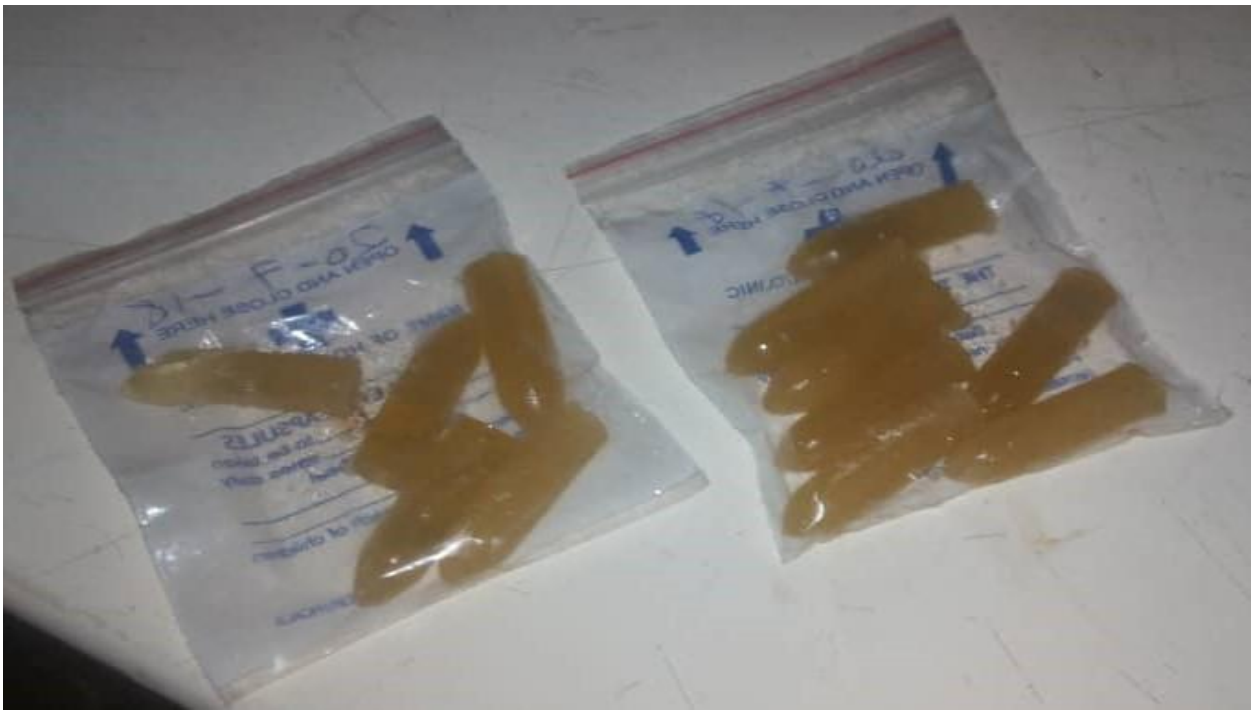


Figure 1: The formulated vaginal suppositories containing *L. pentosus* KCA1

Pre-formulation studies of the vaginal suppositories containing *L. pentosus* KCA1

Physical Appearance

The colour and opacity, shape, homogeneity, texture, surface condition was observed with naked eyes and the odour was perceived with nose. And the results were noted and shown in the Table 2 below.

Dimension of the suppositories

The length and width of the randomly selected vaginal suppositories were measured and the record shown in Table 2 below.

Homogeneity of the suppositories

The selected vaginal suppositories were cut longitudinally and their drug distribution pattern (rough or dry) was observed virtually and the result taken was also recorded in Table 2 below.

Weight Variation

Twenty suppositories were weighed individually and average weight was determined as recorded in the Table 2 above.

Viability and Stability Test

Viability and stability test carried out on the formulated vaginal suppositories containing *L. pentosus* KCA1 on day 0, 1 month, 2 months and 3 months at temperature 2-8^{0}C showed sufficient growth of *Lactobacillus*. From table 3, it was observed that when grown on a standard MRS agar plate, that there was a reduction in the microbial count in the 2 months, which continued even in 3 months. Colony characteristics and gram staining confirmed the presence of *Lactobacillus*. This indicates that the viability and stability test of the *Lactobacillus* was not affected during preparation of the formulation.

Maximum Release Studies / Dissolution Test

The study of the release profile of the formulated vaginal suppositories allowed the determination of the viability of *L. pentosus* KCA1 over the time. The release profile of the suppositories was shown in Table 4.

Disintegration Test

The disintegration test determines whether suppositories soften or disintegrate within the prescribed time when placed in an immersion fluid. According to the BP requirement, disintegration occurs in not more than 60 minutes. The result of the disintegration is shown in Table 5 below.

Table 2: Physical characteristics of the formulated vaginal suppository containing *Lactiplantibacillus pentosus* KCA1

Physical characteristics	Formulated vaginal suppository
Colour and opacity	Slightly yellowish and transparent
Shape	Cylindrical with a pointed end
Surface condition	Smooth and soft to touch
Texture	Jelly
Homogeneity	Homogenous
Odour	Odourless
Dimension (mm^2)*	22.3 x 5.3
Weight variation **	2.25 ± 0.03

*Results represented as Mean ± SD, n =3; **Results represented as Mean ± SD, n =20

Table 3: Viability and stability of the formulated vaginal suppository containing *Lactiplantibacillus pentosus* KCA1 and stored at temperature of 2-8^{0}C.

S/N	Number of period	Viable count (CFU/ML)	Physical changes
1	0 day	27.4 X 10^7	No significant changes were seen
2	1 month	41.6 X 10^7	No significant changes were seen
3	2 month	13.4 X 10^7	No significant changes were seen
4	3 month	6.1 X 10^7	No significant changes were seen

Table 4: Release studies of the formulated vaginal suppositories

Time (minutes)	*Lactobacillus pentosus* KCA1 released (CFU/ml)
5	25.7 X 10^8
15	47.7 X 10^8
30	64.7 X 10^8
45	57.0 X 10^8
60	140.3 X 10^8

All values represent n=3

Table 5: Disintegration studies of the formulated vaginal suppositories

No of suppositories containing *L. pentosus* KCA1	Disintegration time (minutes)
1	36.0 ± 1.0
2	26.6 ± 0.6
3	34.3 ± 0.6
4	24.3 ± 0.6
5	25.0 ± 1.0
6	26.3 ± 0.6

All values represent Mean± SD, n=3.

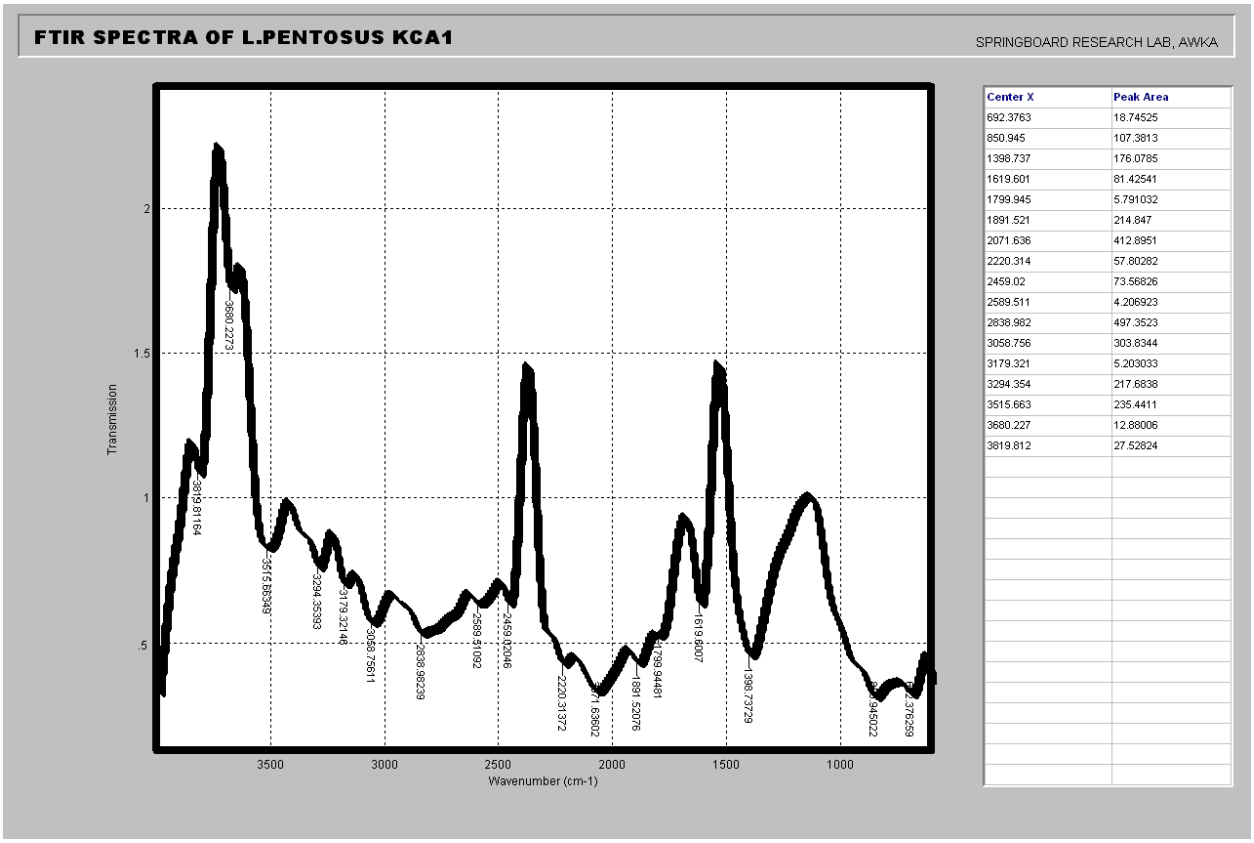
Fourier Transform Infrared (FTIR) Analysis

The FTIR spectra of *Lactiplantibacillus pentosus* KCA1, formulated vaginal suppository containing *L. pentosus* KCA1 and formulated vaginal suppository without *L. pentosus* KCA1 physical mixtures are shown in Figure 2 (A); (B); (C) and Table 6 (A); (B); (C) respectively.

From the result presented in Table 6(A) and Figure 2 (A) below, the peak values around 692.3763 cm^{-1} and 850.945 cm^{-1} were assigned to alcohol, OH out of plane bend and peroxide with C-O-O- stretching vibrations respectively. The absorbance around 1398.737 cm^{-1} and 1619.601 cm^{-1} were assigned to carboxylate (carboxylic acid salt) and organic nitrates with C-O stretch vibration and asymmetric/symmetric NO_2 stretching vibration respectively.

The strong band around 1799.945 cm^{-1} was assigned to acid (acyl) halide with C=O stretching vibration. Again, both the absorption band around 1891.521 cm^{-1} and 2071.636 cm^{-1} were assigned to a carbonyl group of Transition metal. The sharp absorption band around 2220.314 cm^{-1} was assigned to Nitrile with C≡N stretching vibration. The absorbance broad band around 2459.02 cm^{-1} was assigned to acids (carboxylic acid) with O-H bending stretch.

The weak band around 2589.511 cm^{-1} was assigned to Thiols, S-H stretching vibration (Coates, 2000). The absorbance band around 2838.982 cm^{-1} was assigned to Methoxy, methyl ether of C-H stretching vibration. The broad band around 3058.756 cm^{-1}, 3179.321 cm^{-1} and 3294.354 cm^{-1} were assigned to Ammonium ion of N-H stretching vibration. The absorbance band around 3515.663 cm^{-1} was aromatic primary N-H stretch. The broad band around 3680.227 cm^{-1} and 3819.812 cm^{-1} were assigned to tertiary alcohol with O-H stretching vibration.



Center X	Peak Area
692.3763	18.74525
850.945	107.3813
1398.737	176.0785
1619.601	81.42541
1799.945	5.791032
1891.521	214.847
2071.636	412.8951
2220.314	57.80282
2459.02	73.56826
2589.511	4.206923
2838.982	497.3523
3058.756	303.8344
3179.321	5.203033
3294.354	217.6838
3515.663	235.4411
3680.227	12.88006
3819.812	27.52824

Figure 2 (A): FTIR spectrum of *L. pentosus* KCA1

Table 6 (A): FTIR spectrum of *L. pentosus* KCA1

Centre X/ Origin	Wavelength range (cm^{-1})	Functional group	Functional class
692.3763	770-590	O-H	Alcohol, OH out of plane bend
850.945	890-820	C-O-O-C	Peroxides, C-O-O- stretch
1398.737	1420-1300	RCO_2H	Carboxylate (Carboxylic acid salt)
1619.601	1640-1620	$-NO_2$	Organic Nitrates
1799.945	1815-1770	C=O	Acid (acyl) halide
1891.521	2100-1800	RC=O	Transition metal carbonyls
2071.636	2100-1800	RC=O	Transition metal carbonyls
2220.314	2250-2230	R-C≡N	Nitrile, CN stretch
2459.02	3400-2400	O-H	Carboxylic acid OH bend
2589.511	2600-2550	-S-H	Thiols, S-H stretch
2838.982	2850-2815	$-CH_3$	Methoxy, methyl ether $O-CH_3$, C-H stretch
3058.756	3300-3030	$N-H^+$	Ammonium ion
3179.321	3300-3030	$N-H^+$	Ammonium ion
3294.354	3300-3030	$N-H^+$	Ammonium ion
3515.663	3520-3460	$-NH_2$	Aromatic 1^0 amine NH stretch
3680.227	3900-3690	R_3COOH	2^0 alcohol, OH stretch
3819.812	3900-3690	R_3COOH	3^0 alcohol, OH stretch

These values represent: 1^0 (primary), 2^0 (secondary), 3^0 (tertiary)

From Table 6(B) and Figure 2(B) below, the spectra height around 713.2706 cm^{-1} and 836. 6365 cm^{-1} were assigned to alcohol, OH out of plane bending vibration and peroxide with C-O-O- stretching vibrations respectively. The absorbance band around 1318.953 cm^{-1} and 1443.583 cm^{-1} were assigned to aromatic primary amine, with CN stretch and Methyl due to C-H asymmetric/symmetric bending vibration. The peak values around 1608.422 cm^{-1} and 1836.644 cm^{-1} were to carboxylate/carboxylic acid salt and open-chain anhydride of carbonyl group.

The peak values around 2022.03 cm^{-1} and 2176.483 cm^{-1} were assigned to CO and SCN stretching vibration of Transition metal carbonyl and Thiocyanate ion respectively. The broad absorption peak values around 2458.714 cm^{-1}, 2581.909 cm^{-1}, 2781.714 cm^{-1} and 2993.25 cm^{-1} were assigned to carboxylic acid of OH stretching vibration. The absorbance band around 3202.06 cm^{-1} and 3322.169 cm^{-1} were assigned to ammonium ion and aliphatic primary amine with NH stretching vibration. The peak value around 3442.638 cm^{-1} was assigned to Heterocyclic amine with NH stretching vibration. The broad band around 3663.801 cm^{-1} and 3817.049 cm^{-1} were assigned to the OH (hydroxy) stretching vibration of secondary and tertiary alcohol.

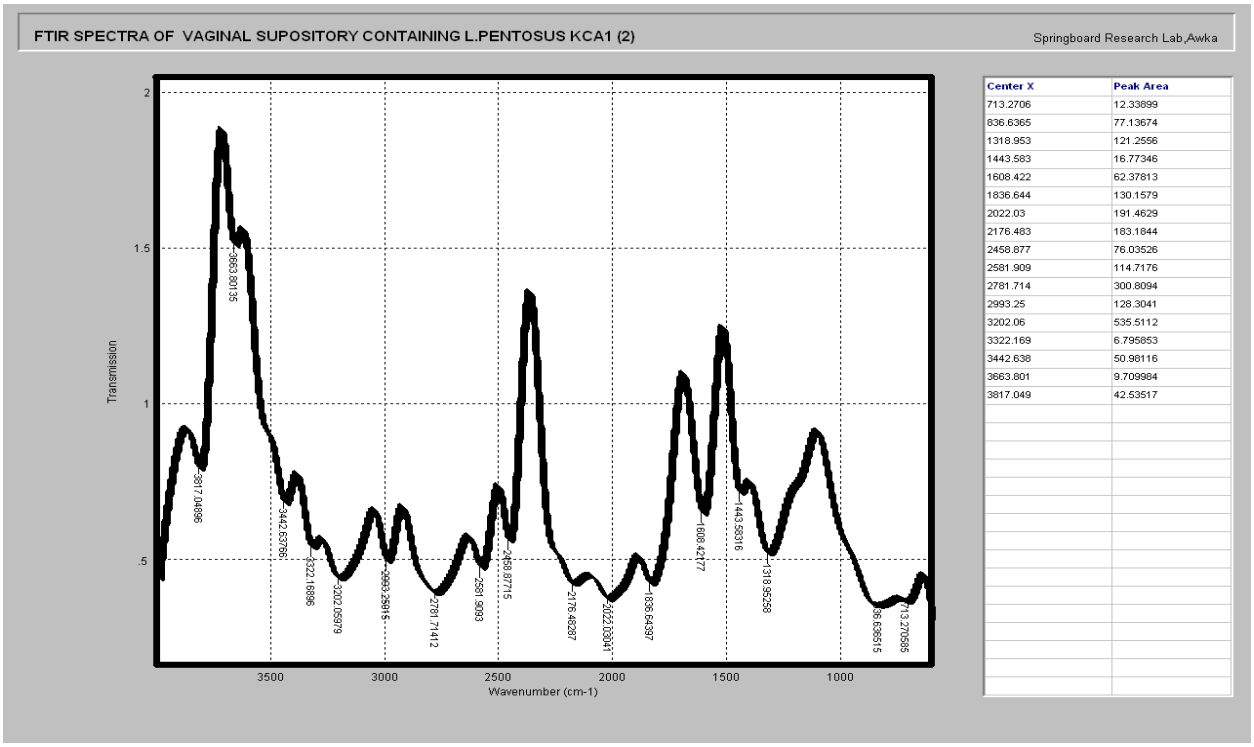


Figure 2(B): FTIR spectrum of vaginal suppository containing *Lactiplantibacillus pentosus* KCA1

Table 6(B): FTIR spectrum of vaginal suppository containing *L. pentosus* KCA1

Centre X/ Origin	Wavelength range (cm^{-1})	Functional group	Functional class
713.2706	770-590	O-H	Alcohol, OH out of plane bend
836.6365	890-820	C-O-O-C	Peroxides, C-O-O-stretch
1318.953	1340-1250	C-N	Aromatic 1^0 amine, CN stretch
1443.583	1470-1430	$-CH_3$	Methyl, C-H asym./symmetry bend
1608.422	1610-1550	RCO_2H	Carboxylate (carboxylic acid salt)
1836.644	1850-1800	$R_2C=O$	Open-chain acid anhydride of carbonyl group
2022.03	2100-1800	RCOH	Transition metal carbonyls
2176.483	2200-2000	$-SCN^+$	Thiocyanate ion
2454.877	3400-2400	O-H	Carboxylic acid
2581.909	3400-2400	O-H	Carboxylic acid
2781.714	3400-2400	O-H	Carboxylic acid
2993.25	3400-2400	O-H	Carboxylic acid
3202.06	3300-3030	$N-H^+$	Ammonium ion
3322.169	3345-3325	N-H	Aliphatic 1^0 amine, NH stretch
3442.638	3490-3430	N-H	Heterocyclic amine, NH stretch
3663.801	3900-3690	R_2COOH	2^0 alcohol, OH stretch
3817.049	3900-3690	R_3COOH	3^0 alcohol, OH stretch

These values represent: 1^0 (primary), 2^0 (secondary), 3^0 (tertiary)

From Table 6 (C) and Figure 2 (C) below, the absorbance around 757.1253 cm^{-1} was assigned to OH, out of plane bending vibration of Alcohol, while the peak values around 1351.603 cm^{-1} and 1630.713 cm^{-1} were assigned to $–NO_2$ asymmetric/symmetric stretching vibration of aromatic nitro compounds and organic nitrates respectively.

The absorption peak around 1902.673 cm^{-1} and 2039.602 cm^{-1} were assigned to C=O of transition metal carbonyls. The broad absorptions around 2683.621 cm^{-1} and 2865.364 cm^{-1} were assigned to O-H stretching vibration of carboxylic acid. The absorption band around 3195.901 cm^{-1} and 3467.551 cm^{-1} were due to NH stretching vibration of ammonium ion and a H-bended OH-stretching vibration of hydroxyl group.

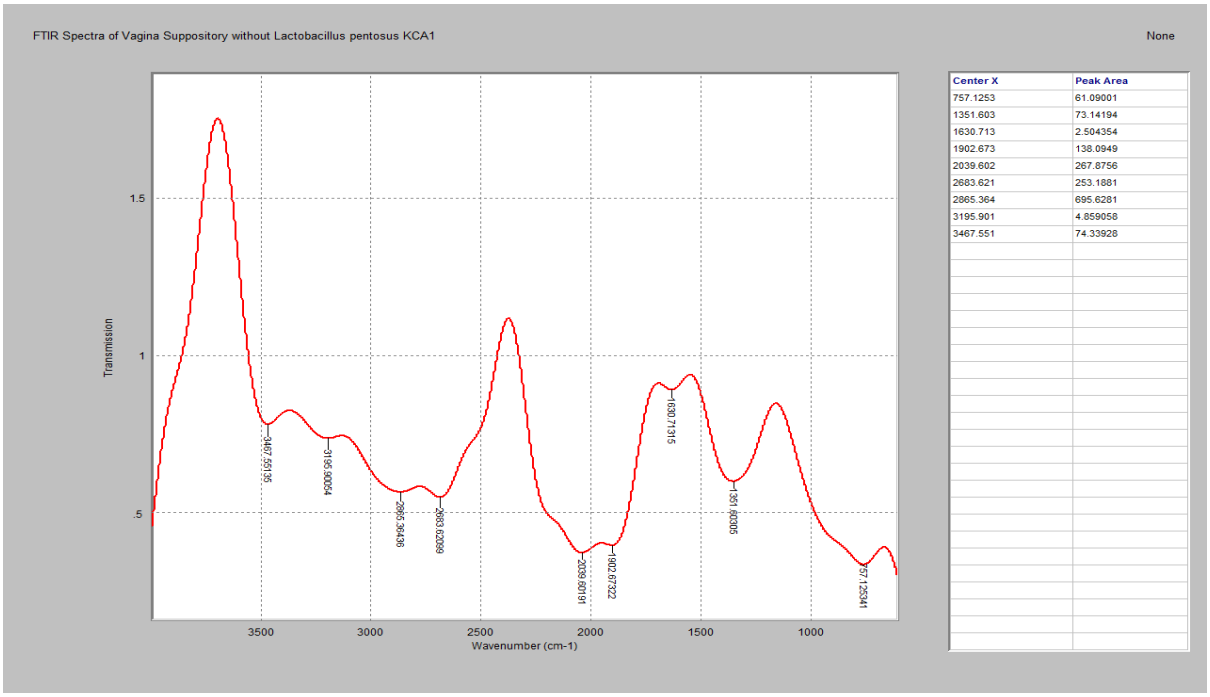


Figure 2 (C): FTIR spectrum of vaginal suppository without *L. pentosus* KCA1

Table 6 (C): FTIR spectrum of vaginal suppository without *L. pentosus* KCA1

Centre X/ Origin	Wavelength range (cm^{-1})	Functional group	Functional class
757.1253	770-590	O-H	Alcohol OH, out-of-plane bend
1351.603	1355-1320	ArO_2N-	Aromatic nitro compounds
1630.713	1640-1620	$-NO_2$ / O_2N	Organic nitrates
1902.673	2100-1800	RC=O	Transition metal carbonyls
2039.602	2100-1800	RC=O	Transition metals carbonyls
2683.621	3400-2400	RCO_2H	Carboxylic acids & derivative (C-H overlap; O-H very broad)
2865.364	3400-2400	O-H	Carboxylic acid
3195.901	3300-3030	$-NH_4+$	Ammonium ion
3467.551	3570-3200	OH^-	Hydroxy group, H-bended OH-stretch

DISCUSSION

The formulated vaginal suppositories were characterized with respect to the colour and opacity, shape, odour, dimensions, homogeneity, and weight variation. The results of the physical characteristics studies are reported in Table 2 above.

The formulated vaginal suppositories were found to be homogenous when cut longitudinally to expose the interior part. These was found to be in accordance with the work done by Reddy *et al*., (**25**), on the prepared fluconazole vaginal suppositories using glycerol-gelatine base.

All the formulated vaginal suppositories were cylindrical with a pointed end. The dimension of the formulated vaginal suppositories containing *L. pentosus* KCA1 was 22.3 X 5.3 mm^2 which may be due to its softness and jelly nature. This result was slightly less than the suppositories dimension obtained from the study done by Reddy *et al*., (**26**), which was 22.0 X 6.0 mm^2.

The results obtained of the weight variation as shown in Table 2, were in conformity with official limits which state that; No suppository should deviate from the average weight by more than 5 % except two, which may deviate by more than 7.5 %.

The survival of *Lactiplantibacillus pentosus* KCA1 in the formulated vaginal suppositories stored at temperature of 2-8C for 3 months revealed a reduction in the microbial count at day 0 after formulation. This may be attributed to the heating process during the preparation. At 1 month, there was an increase in microbial count of *L. pentosus* KCA1 and at 2 months the microbial count of *L. pentosus* KCA1 showed a reduction in the microbial count. At 3 months, there was a marginal reduction in the microbial count of *L. pentosus* KCA1. All the changes observed during the 3 month's period may due to temperature effect. Sufficient growth of *L. pentosus* KCA1 on day 0, month 1, 2 and 3 at 2-8^{0}C temperature, was observed when grown on a standard MRS medium plate as shown in table 3. This indicate that the viability and stability of *L. pentosus* KCA1 was not affected during the preparation of the formulation and when stored at 2-8^{0}C over the period of study of 3 months, this was in support with similar study carried out by Kaewnopparat and Kaewnopparat (**18**).

From the result of the release studies shown in Table 4, it was revealed that the microbial count of the *L. pentosus* KCA1 continued to increase until at 45 minutes, which showed a decrease in the microbial count of *L. pentosus* KCA1 and with a higher count at 60 minutes. The fast release of *L. pentosus* KCA1 from the glycerol-gelatin base may be due to the water solubility of the base, which allows *L. pentosus* KCA1 to be released by both diffusion and erosion mechanism (**27**).

The disintegration test determines whether suppositories fragment within a prescribed time when immersed in a fluid. According to the BP (**20**) requirement, disintegration occurs in not more than 60 minutes. From this study, it observed that all the six suppositories used disintegrate before 60 minutes with the highest disintegration time at 36.0 ±1.0 minutes. Therefore, all suppositories were found to satisfy the BP requirement for disintegration.

The characterization of compounds via infrared spectroscopy are not limited to organic compounds, but also to any inorganic compounds that forms double bonds of a covalent nature within a molecular ion fragment, cation or anion, which will produce a characteristic absorption spectrum, with

associated group frequencies (**28**). A typical FTIR spectrum of *Lactobacilli* and assignment of the various spectral regions are given in (Figure 2 (A) (B) (C)). The choice of spectral regions depends both on the purpose of the analysis and on the type of microorganisms under examination.

From Table 6 (A), presented above, the FTIR spectra with peak around 692.3763 cm^{-1} is absorption of lower importance, but often characteristic, is assigned to alcohol with out of plane bending vibration of O-H. Normally, O-H out - of- plane bending is broad, with only moderate absorption occurring around this region. And the peak around 850.945 cm^{-1} which assigned to peroxide is typically weak, and not very characteristic in the infra-red but tends to be more in Raman spectrum (**28**). The absorbance around 1398.737 cm^{-1}, which was assigned to carboxylic acid salt is highly characteristic. Absorption around 1619.601 cm^{-1} was assigned to organic nitrate, with asymmetric and symmetric NO_2 stretching vibration.

Interestingly, the nitro group ($-NO_2$) is isoelectric with the carboxylate ion group ($-CO_2^-$) and both provide very similar spectra for the main functional group (Coates, 2000). The strong band around 1799.945 cm^{-1} is indicative of the presence of carbonyl group (C=O) which was assigned to acid (acyl) halide with C=O stretching vibration, has a double bond which gives it the characteristic strong intensity band. The extremely intense absorption band around 1891.521 cm^{-1} and 2071.636 cm^{-1} respectively were provided by the multiple bonded CO (carbonyl) group, which was assigned to transition metal carbonyls. The actual position of the band(s) and the complexity of the bands is dependent on the structure of the compound. Metal carbonyls have unique bonding, where the bond order between carbon and oxygen is between two and three, and where electrons are provided by dπ back-bonding from the accompanying transition metal atom (Coates, 2000). The absorption band around 2220.314 cm^{-1} which was assigned to Nitrile with CN stretch has a medium intensity band with a very characteristic sharp and strong vibration than alkynes (Beauchamp, 2009). The broad band around 2459.02 cm^{-1}, which was assigned to acid (carboxylic acid) O-H bending vibration which usually overlaps with C-H stretch, with strong intensity. The carboxylic acids exhibit extremely strong hydrogen bonding, forming a stable dimeric structure which is highly characteristic with a large shift to lower frequencies is observed. The weak band around 2589.511 cm^{-1} was due to the S-H stretching vibrations of thiols compound which can be easily overlooked (**28**, **29**). The absorbance band around 2838.982 cm^{-1} was assigned to Methoxy, methyl ether of C-H stretching vibration, which is known as one of the special methyl ($-CH_3$) frequencies. The broad absorption band around 3058.756 cm^{-1}, 3179.321 cm^{-1} and 3294.345 cm^{-1}, which were assigned to ammonium ion due to the fact that the N-H stretching vibration was intense. The absorption band around 3515.663 cm^{-1} was assigned to aromatic primary amine due to N-H stretching absorption, which is less sensitive to hydrogen bonding than O-H absorptions. The primary amine displays two band peaks due to asymmetric (higher frequency) and symmetric N-H. The broad band around 3680.227 cm^{-1} and 3819.812 cm^{-1} were due to the hydroxyl compound of secondary (2^0) and tertiary (3^0) alcohol and this may be reflected in the position of the OH stretch absorption, but typically can be determined by other absorption, in particular C-O- stretching frequency (**28**).

From Table 6 (B) presented above, the spectrum height around 713.2706 cm^{-1} was due to OH bending or wagging vibration of alcohol, out-o-plane. This absorption is of lower importance, but often characteristic.

The OH bending vibration are broadened by hydrogen bonding as in the stretching absorption, but often to a lesser extent. The absorption peak around 836.365 cm^{-1} was assigned to peroxides, due to the C-O-O- stretching vibration, that are typically very weak and not very characteristic in the infrared but tend to be more characteristic in the Raman spectrum. The peak around 1318.955 cm^{-1} and 1443.583 cm^{-1} were due to the CN stretching vibration and C-H asymmetric /symmetric bending vibration of Aromatic primary amine and methyl compounds. The C-H stretch vibration of methyl is one of the most characteristic in terms of recognizing the compound as an organic compound containing at least one aliphatic fragment or center. The bending vibration helps to talk more about the basic structure (**28**). In contrast, strong methyl bands, showing significant splitting and comparatively weaker methyl/methylene band indicate chain branching and the possibility of isopropyl or tert-butyl substituents (depending on the amount of splitting and relative band intensities). The C-N stretching vibration of Aromatic primary amine are uncertain. The peak values around 1608.422 cm^{-1} and 1836.644 cm^{-1} were due to the C-O-H and C=O vibration of carboxylic acid salt (carboxylate) and open-chain anhydride of carbonyl group. Carboxylic acids have a very distinctive OH band that are highly characteristic. In open-chain acid anhydride of carbonyl group, it is important to point out, that the C=O absorption ranges for the various carbonyl-containing functional groups overlap significantly, so it is difficult to make a definitive identification of a functional group based solely on the position of the carbonyl peak. However, most of these functional groups show other diagnostic absorption that assist in identification. They have a strong intensity with two bands. The peak around 2022.03 cm^{-1} and 2176.483 cm^{-1} were due to the CO and SNC stretching vibration of Transition metal carbonyl and Thiocyanate ion respectively. The thiocyanate ion has an intense and broad absorption. All complex ionic compounds (containing more than one atom) and coordination compounds produce characteristic spectra. The multiple bonded CO group of transition metal carbonyl provides an extremely intense absorption band close to 2000 cm^{-1} (typically between 2100 and 1800 cm^{-1}), the actual position of the band(s) and the complexity of the bands being dependent on the structure of the compound. The broad peak values around 2458.877 cm^{-1}, 2581.909 cm^{-1}, 2781.714 cm^{-1} and 2993. 25 cm^{-1} were due to the OH stretching vibrations. The carboxylic acids are unique because the hydroxy group has direct interaction with the carbonyl group, by the formation of a stable dimeric hydrogen-bonded structure in the condensed phase (solid and liquid).

The characteristic broad feature of carboxylic acid ranges from 3400-2400 cm^{-1}, that overlaps the C-H stretching region, and with a secondary absorption close to 3600 cm^{-1} (which occurred in Table 6 (B) at 2581.909 cm^{-1}), is observed for the hydrogen-bonded O-H of most carboxylic acids. The absorbance band around 3202.06 cm^{-1} and 3322.169 cm^{-1} were due to NH stretching vibrations of ammonium ion and aliphatic primary amine. The primary amine has weak hydrogen bonding, which makes the overall effect on the spectrum slightly less pronounced. This situation alters in the related ammonium and amino salts, where strong hydrogen is experienced and corresponding broadening of the associated NH absorptions is observed. Only the 1^0 and 2^0 amines exhibit the most characteristic group frequencies which are associated with the N-H bond. The peak value around was due to N-H stretching of heterocyclic amine.

The broad band around 3663.801 cm^{-1} and 3817.049 cm^{-1} were due to the OH stretching vibration of the secondary (2^0) and tertiary (3^0) alcohol. The OH absorptions are quite intense and smoothly curved (**28**).

From Table 6 (C) presented above, the spectrum height around 757.1253 cm^{-1} was sue to OH, out-of-plane bending vibration of alcohol, whereas the peak values around 1351.603 cm^{-1} and 1630.713 cm^{-1} were due to the $–NO_2$ asymmetric/symmetric stretching vibrations of aromatic nitro compound and organic is isoelectric with the carboxylate ion group ($-CO_2^-$), and both provide very similar spectra for the main functional group. The absorption peak values around 1902.673 cm^{-1} and 2039.603 cm^{-1} were due to the C=O of transition metal carbonyls.

The broad peak absorbance around 2683.621 and 2865.364 were due to the O-H vibrations of carboxylic acid that are highly characteristic with strong intensity. The absorption band around 3195.901 and 3467.551 were due to the narrow NH stretching vibration of Ammonium ion with weak to medium intensity and a broad H-bended OH-stretching vibration of hydroxyl group. The hydroxyl function is probably one of the most dominant and characteristic of all of the infrared group frequencies. In most chemical environments, the hydroxyl group does not exist in isolation, and a high degree of association is experienced as a result of extensive hydrogen bonding with other hydroxyl groups. These hydroxyl groups may within the same molecule (intra molecular hydrogen bonding) or they may most likely exist between neighboring molecules (intermolecular hydrogen bonding). The impact of hydrogen bonding is to produce significant band broadening and to lower the mean absorption frequency. The lowering of the frequency tends to be a function of the degree and strength of the hydrogen bonding. In special circumstances, where the hydroxyl group is isolated-either because of steric hindrance effects or because the sample is in the vapour state or in a dilute solution of a non-polar solvent- band is characteristically narrow, and is observed at the natural, high frequency. This absorption is important for the characterization of certain hindered phenol antioxidants, a commercially important class of compounds in the food, polymer and formulated oil industries. While, the hydroxy absorption is one of the most important bands in the infrared spectrum, other vibrations are also important for the characterization of the compound (**28**).

Recent finding has shown that *Lactiplantibacillus pentosus* KCA1 have not been studied with FTIR spectroscopy before now. According to the study by Ousta et al., (**15**), they revealed that the region between 1400-720 cm^{-1} was best suited for identification of *Lactobacillus* species based on their FTIR spectra. Naumann et al., (**16**), indicated in their work that the FTIR spectra of microorganisms are usually divided into five regions. These regions contain information from different cell components. They are:

1. 3000-2800 cm^{-1} (Fatty acids in the bacterial cell membrane)
2. 1800-1500 cm^{-1} (amides bands from protein peptides)
3. 1500-1200 cm^{-1} (mixed region: proteins and fatty acids)
4. 1200-900 cm^{-1} (polysaccharides within the cell wall)
5. 900-500 cm^{-1} (“true” fingerprint regions containing bands which cannot be assigned to specific functional group).

The spectral regions that fall within the wavelength bands of 1400-720 cm^{-1}, which lies within the mixed region, the polysaccharide region and the fingerprint regions, were found to be best suited for

differentiation and identification of *Lactobacilli* species based on their FTIR spectra. This is more or less in accordance with the range of the previous studies where a number of microorganisms have been differentiated and identified based on their FTIR spectra (**15**, **30**, **31**, **32**). In this study, it could be deduced from the result shown in table 6 (A) and (B) that spectral regions that falls within the wavelength bands of 1318.953-713.2706 cm^{-1} may the best for the identification of *Lactobacillus pentosus* KCA1.

In general, studies have shown that women prefer vaginal dosage forms to be colorless, odorless, adhesive enough to avoid leakage and messiness, safe and easy to use with no interference with sexual intercourse (**33**, **34**).

CONCLUSION

In conclusion, a pharmaceutical formulation of a probiotic preparation containing a strain of *Lactiplantibacillus pentosus* KCA1 was designed. It was observed from the pre-formulation studies, that the formulated suppositories met all the pharmaceutical requirement for suppository formulation standards. The stability and viability studies also showed that the formulation was not affected by the level of temperature used. The glycerol-gelatin base has proven to be a good vehicle for the preparation of *Lactobacillus* vaginal suppository in terms of the fast release and microbiological stability. The FTIR analysis revealed that *Lactiplantibacillus pentosus* KCA1 has spectral regions that fall within the wavelength band designated for identification of *Lactobacillus* species. Clinical studies to evaluate the effect of these suppositories in women with bacterial vaginosis is in progress.

Acknowledgements

Researchers were very much thankful to NAFDAC Zonal Laboratory, Agulu, Nigeria, Spring board research laboratory, Awka, Nigeria and Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University, Awka, Nigeria, for providing the working space and necessary facilities.

Conflict of interest: KCA isolated, sequenced the full genome of *Lactiplantibacillus pentosus* KCA1.

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