Original Research

FTIR Characterisation and *in vitro* Activities of *Bombacopsis buonopozense* and *Nauclea latifolia* Leaf Extracts against Bacteria Isolated from Cattle Rumen Waste and Vegetable Farm Irrigation Water

Akinde S.B., Ajayi E.I.O., Awojide O.I., Azeez O.K., Osunsanmi K.O., Olabiran O.A. and Fowosire A.A.

Abstract

Introduction: Symbiotic relationship between gut microbiota and animal models, as well as, between plant-parasitic microbes and economic vegetable crops act as a veritable tool to test and provide information on the characteristic changes in health and disease. It provides a framework for control of pathogenic microbes. In this work, therefore, we sought to study the susceptibility of cattle rumen waste and vegetable farm irrigation water bacterial flora to aqueous and ethanolic leaf extracts of *Bombacopsis buonopozense* and *Nauclea latifolia* Sm.

Methods: Following cold extraction, phytochemical screening of the leaf extracts was done. Paper disc diffusion method was employed to assay for antibacterial activities of the leaf extracts, while commercial Ofloxacin (5µg), Ciprofloxacin (5µg), Gentamicin (10µg), Chloramphenicol (10µg) and Cotrimoxazole (25µg) served as standard antibiotics. Predictions of the functional groups of bioactive compounds in the different fractions of the leaf extracts were made using FTIR spectroscopy.

Results: Approximately 94.87% of the bacteria were sensitive (17-38 mm Inhibition Zone Diameter [IZD]) to the standard commercial antibiotics; while they were not sensitive to the test concentrations of both the aqueous and ethanol leaf extracts of B. buonopozense (0-13.5 mm IZD) and N. latifolia (0-11.5 mm IZD).

Conclusion: The non- sensitivity of the plethora of the cattle rumen waste bacteria to aqueous leaf extracts of *B. buonopozense* and *N. latifolia* may be indicative of their possible usefulness in bioconservation and environmental protection. This means that the use of cattle rumen waste as manure for economic vegetables may not pose additional threat to the ecosystem as the vegetable farm irrigation water bacterial flora were not sensitive to *B. buonopozense* and *N. latifolia*.

Department of Biological Sciences, Osun State University, Osogbo, Nigeria.
*Akinde S.B.
Email: akindesb@uniosun.edu.ng

Awojide O.I.
Osunsanmi K.O.
Fowosire A.A.

Department of Chemical Sciences, Osun State University, Osogbo, Nigeria.
Ajayi E.I.O.
Azeez O.K.
Olabiran O.A.

Keywords: *Bombacopsis buonopozense*; *Nauclea latifolia*; FTIR spectroscopy; bioactive compounds; bacteria, antibacterial activity

UNIOSUN J. Sci. 1(1)
Online publication access at www.ujs.uniosun.edu.ng

The composition of the gut microbiota of cattle is to a large extent determined by the birth conditions and environment (Dominguez-Bello *et al.*, 2010). These serve as the initiating point of microbial colony into the newborn, typical of host-microbe mutualism (Chung *et al.* 2012; Neu and Rushing, 2011). As the calves breastfeed, graze, have contact with dung, and are being administered antiobiotics, there may be modifications in their intrinsic rumen microbiota (Tiwari *et al.*, 2006; Sonnenburg *et al.*, 2010).

Invariably, dietary ingredients confer modification on the mucosal antibacterial activities, which has been reported in gastrointestinal disorders affecting cattle (Lotz *et al.* 2006; Nuding *et al.*, 2007). For instance, microbe-infected vegetation or fodder contributes to changes in cattle rumen microorganisms leading to metagenomic shift in cattle rumen-adapted population and cattle rumen function adaptation. Alternative therapies, especially developed from organic plants are giving great promise, and scientific evidences are more abundant to attest to their efficacies in combating multidrug-resistant strains of microorganisms (Olasupo *et al.*, 2003).

In the same vein, efforts are geared towards bioconservation, by achieving pest control without causing imbalance in the ecosystem. There is evidence that as-yet-uncultured microorganisms represent the vast majority of organisms in most environments on earth (Handelsman, 2004). The discovery of novel compounds with antimicrobial activity against antibiotic-resistant bacteria will be a meaningful endeavour. Therefore, we sought to test the sensitivity of the cattle-rumen and vegetable irrigation water associated bacteria to the crude extracts of *B. buonopozense* and *N. latifolia*, towards a sustainable solution to environmental hazard posed by indiscriminate dissemination of potential bacterial pathogens.

Indiscriminate defecation by the free-grazing cattle, poor dung waste management by nomads and inappropriate manure handling techniques can lead to infection of economic vegetables by potential pathogenic microorganisms. In this study, we also endeavoured to characterise pure bioactive compounds present in the crude aqueous and ethanol extracts of *B. buonopozense* and *N. latifolia* leaves for possible field applications.

Materials and Methods

Plant Collection and Identification

Apparently healthy leaves of *Bombacopsis buonopozense* (*B. buonopozense*) and *Nauclea latifolia* (*N. latifolia*) were collected from Ado-Ekiti in Ekiti State and Ara in Osun State respectively. They were presumptively identified in the Department of Biological Sciences, Osun State University, Nigeria and authenticated in the herbarium of Department of Botany, University of Ilorin, Kwara State (Voucher Numbers: UIH/001/899 and UIH/004/506 respectively).

Crude Extract Preparation

Crude extracts of the two leaf samples were prepared by first dusting off the sand and debris attached to the leaves. The leaves were then rinsed and cut into pieces using a scalpel and air-dried for seven days until they were completely dry. To make crude extract, the dried leaves were ground using mortar and pestle into fine powder and weighed; it was then equal amount of leaf powder was soaked in 1 litre of cold water and ethanol for 48 hours in separating funnel. Only apparently healthy plant leaves were used for this study.

Preliminary Phytochemical Screening of the Leaf Extracts

Phytochemical screening of the leaves was carried out according to the method of Sofowora (1996).

Purification and Characterization of bioactive Compounds of Leaf Extracts

The crude extracts obtained were made into slurry with acme silica gel of 60-120 mesh in hexane. These were loaded on silica gel-packed column. A binary mixture of hexane: ethylacetate solvent system of increasing polarity was used to elute the compounds. A 10 ml volume of eluate was collected over time; the volumes was reduced in rotary evaporator and then analyzed on Thin Layer Chromatography (TLC) plates (Merck, Germany) to determine the number of bioactive compounds present as spots observed under UV light at 245 nm and 366 nm, respectively.

When spots were detected by TLC, the fractions (or eluates) collected from column chromatography were matched with the eluates corresponding to those with similar spots and pulled together. These portions were further dried in rotary evaporator to reduce the volume. A 1-mg of each sticky mass was then reconstituted in 1 ml of dichloromethane and then subjected to FTIR spectra analyses.

Crude Extract Reconstitution for Antibacterial Tests

A 3-g of each crude extract was weighed into a sterile beaker and 30 ml of 50% Dimethyl Sulphoxide (DMSO) was added and stirred. It was then filtered through sterile Whatmann No. 1 filter paper. Subsequently, three concentrations of the aqueous and ethanolic extracts were separately prepared by 10-fold serial dilution of the filtrate to give 1.0%; 0.1% and 0.01% each.*Microbial Cultures and Growth Conditions*

Gram-negative bacteria (20 strains) and Gram-positive bacteria (5 *strains*) from cattle rumen waste, as well as, 69 Gram-negative bacteria strains isolated from vegetable farm irrigation water were used as test organisms in this study. They were obtained from the Microbiology Laboratory in Osun State University, Osogbo. These cultures were first revived in nutrient agar stock and kept in the incubator at 37ºC for 24 hours. They were then sub-cultured on nutrient agar to get pure colonies. The stock cultures were stored on agar slants at 4ºC ± 2ºC for further study.

Assay for Antibacterial Activities of the leaf extracts

Kirby Bauer paper disc diffusion method was used to determine the antibacterial activity of the extracts (Duguid *et al.* 1989). A 25 µl of each of the serially diluted was dispensed onto separate 6-mm blank disks (Becton Dickinson and Company, USA). The crude extract impregnated-disks were dried immediately in an oven, labeled and stored at -20°C in airtight vials without desiccant until used. The test bacterial isolates were grown on Brain Heart Infusion agar plates for 16–18 hours at 35ºC ± 2°C.

Discrete colonies of the test microorganisms were suspended in sterile Ringer's solution with the turbidity adjusted against 0.5 McFarland standard to comprise approximately 1.5 x 10^8 CFU/ml. The inoculum was swabbed on the surface of already poured, set and dried Mueller-Hinton Agar plate (Oxoids Ltd, Basingstoke, Hampshire, UK) using sterile cotton tipped applicators. A sterile forceps was then used to pick each of the extract-impregnated disks and placed on the agar plate containing each of the test bacterial isolates.

Known antibiotic disks active against Gram-negative and Gram-positive bacteria and disks impregnated with Ringer's solution served as positive and negative controls, respectively. The plates were incubated at 35ºC ± 2°C for 16 to 24 hours. The diameter of the zones of inhibition produced by each crude extract on the test isolates were observed and measured in mm and compared with the diameter of the zones of inhibition of positive and negative controls to ascertain antibacterial activities. The results were interpreted as described in the manufacturer's protocol (Inhibition Zone Diameter less than 16 mm showed resistance).

The antibacterial activities of the extracts against the test organisms were compared with those of the antibiotic discs containing Ofloxacin (5µg), Ciprofloxacin (5µg) and Gentamicin (10µg) for Gram-negative bacteria, as well as, Chloramphenicol (10µg), Cotrimoxazole (25µg) and Gentamicin (10µg) for Gram-positive bacteria.

Results

FTIR Characterisation of Crude Extracts of *B. buonopozense* and *N. latifolia*

Aqueous extracts of *B. buonopozense* and *N. latifolia* weighed 17.43g and 21.85g, respectively; while the ethanol extracts weighed 8.82g and 10.49g, respectively. The phytochemical constituents of the leaves were revealed, thus: *B. buonopozense* contained steroids but not anthocyanins, tannins, saponins, glycosides, and terpenoids. *N. latifolia* contained saponins and tannins, but steroids, glycosides and triterpenoids were absent (Table 1).

FTIR revealed the presence and type of vibration, characteristic absorption (cm^{-1}) and the intensity of the chemical groups available in the extract fractions. These chemical groups and bonds were suggestive of the presence of bioactive compounds in the extracts (Tables 2a, b and 3).

Table 1. Preliminary phytochemical screening of the leaf extract

Phytochemical Compound	*B. buonopozense*	*N. latifolia*
Steroids Test: crude extract + 2 ml chloroform + 2 ml conc. H_2SO_4 + acetic acid Observation: greenish colouration	+	-
Saponins Test: crude extract + 5 ml distilled water in test tube + shaken vigorously Observation: formation of stable foam	-	+
Tannins Test: crude extract + 2 ml of 2% $FeCl_3$ solution Observation: blue green or black colouration	-	+
Terpenoids Test: crude extract + 2 ml chloroform evaporated to dryness + 2 ml conc. H_2SO_4 heated for 2 min Observation: grayish colour	-	-
Glycosides Test: crude extract + 2 ml chloroform + 2 ml H_2SO_4 and shaken gently Observation: reddish brown	-	-

Note: '+' sign = present; '-' sign = absent

Table 2a. Functional groups of bioactive organic compounds of *B. buonopozense* leaf extract as revealed by FTIR

Functional Group	Type of Variation	Characteristic Absorption (cm^{-1})	Intensity	Chemical Formula
Bioactive Compound I: in Ethyl Acetate Fraction				
Alcohol	Stretch, free	3363	Strong, broad	O-H
Alkane	Stretch	2976	Medium	C-H
Alkane	Stretch	2927	Medium	C-H
Alkene	Stretch	1647	Variable	C=C
Aromatic	Stretch	1457	Medium, weak, multiple bands	C=C
Amine	Bend	1086	Medium, weak	C-N
Ether	Bend	1045	Strong	C-O
Alcohol	Stretch	877	Strong	C-O
Alkane	Bend	669	Variable	-C-H
Bioactive Compound II: in chloroform fraction				
Alcohol	Stretch	3283	Strong, broad	O-H
Alkene	Stretch	3006	Medium	=C-H
Alkane	Bend	2916	Strong	C-H
Aldehyde	Bend	2849	Strong	=C-H
Aldehyde	Stretch	1740	Strong	C=O
Amide	Stretch	1653	Strong	C=O
Aromatic	Bend	1617	Strong	C=C
Aromatic	Stretch	1541	Medium, weak, multiple bands	C=C
Aromatic	Stretch	1459	Medium, weak, multiple bands	C=C
Amine	Stretch	1377	Medium, weak	C-N
Ester	Stretch	1263	Medium, weak, two bands or more	C-O
Ester	Stretch	1239	Medium, weak, two bands or more	C-O
Ester	Stretch	1161	Medium, weak, two bands or more	C-O
Alcohol	Stretch	1097	Strong	C-O
Ether	Bend	1032	Strong	C-O
Phosphoric Ester	Stretch	926	Medium	P-OR
Aromatic	Bend	736	Sharp	C-H
Aromatic	Stretch	706	Weak	C-H
Bioactive Compound III: in acetone fraction				
Alcohol/ Phenol	Stretch	3341	Strong, broad	O-H
Alkane	Bend	2922	Strong	C-H
Alkane	Bend	2853	Strong	C-H
Ketone	Bend	1744	Strong	C=O
Carboxyl	Stretch	1712	Strong	C=O
Alkene	Stretch	1653	Variable	C=C
Aromatic	Bend	1459	Strong	C=C
Alkane	Bend	1377	Variable	-C-H
Amine	Stretch	1239	Medium	C-N
Amine	Bend	1161	Medium	C-N
Amine	Bend	1114	Medium	C-N
Alkyl Halide	Bend	1041	Strong	C-F
Alkene	Bend	993	Weak	=C-H
Alkene	Bend	851	Weak	=C-H
Alkene	Bend	721	Weak	=C-H

Table 2b. Functional groups of bioactive organic compounds of *B. buonopozense* leaf extract as revealed by FTIR cont'd

Functional Group	Type of Variation	Characteristic Absorption (cm^{-1})	Intensity	Chemical Formula
Bioactive Compound IV: in dichloromethane fraction				
Aromatic	Stretch	3006	Weak	C-H
Alkane	Bend	2924	Strong	C-H
Alkane	Bend	2853	Strong	C-H
Carbonyl	Bend	1742	Strong	C=O
Alkane	Bend	1459	Variable	-C-H
Alkane	Stretch	1377	Variable	-C-H
Acid	Bend	1265	Medium	C-O
Amine	Bend	1239	Weak	C-N
Amine	Bend	1162	Medium	C-N
Ester				
Ester	Bend	1095	Two bands, weak	C-O
Alkene	Bend	738	Strong	=C-H
Alkene	Bend	706	Weak	=C-H
Aromatic	Stretch	3006	Weak	C-H
Alkane	Bend	2924	Strong	C-H
Alkane	Bend	2853	Strong	C-H
Carbonyl	Bend	1742	Strong	C=O
Alkane	Bend	1459	Variable	-C-H
Alkane	Stretch	1377	Variable	-C-H
Acid	Bend	1265	Medium	C-O
Amine	Bend	1239	Weak	C-N
Amine	Bend	1162	Medium	C-N
Ester	Bend	1118	Two bands, weak	C-O
Ester	Bend	1095	Two bands, weak	C-O
Alkene	Bend	738	Strong	=C-H
Alkene	Bend	706	Weak	=C-H
Bioactive Compound V: in ethanol fraction				
Alcohol	Stretch	3336	Strong, broad	O-H
Alkane	Stretch	2899	Weak	C-H
Alkane	Bend	2976	Strong	C-H
Alkane	Stretch	2927	Strong	C-H
Aromatic	Stretch	1451	Weak, multiple bands	C=C
Aromatic	Stretch	1420	Weak, multiple bands	C=C
Aromatic	Stretch	1381	Weak, multiple bands	C=C
Amine	Bend	1272	Weak	C-N
Amine	Bend	1086	Medium	C-N
Alcohol	Stretch	1054	Two bands, weak	C-O
Ether	Bend	1045	Strong	C-O
Alkene	Bend	877	Strong	=C-H

FTIR spectra generated for fractions corresponding to the spots observed in TLC analysis of the eluates of the aqueous and ethanol extracts of *B. buonopozense* and *N. latifolia* leaves revealed the chemical groups and bonds present (Figures 1, 2 and 3).

Antibacterial Activities of Aqueous and Ethanol Extracts

Tables 4 and 5 show the results of susceptibility of the test bacterial isolates to the aqueous and ethanol extracts of *B. buonopozense* and *N. latifolia* Sm. *vis-a-vis* the standard antibiotics used. Cattle rumen waste Gram-negative and Gram-positive bacteria screened did not show sensitivity to the aqueous and ethanol extracts of *B. buonopozense* and *N. latifolia*. The vegetable farm irrigation water Gram-negative and Gram-positive bacteria also showed resistance to them (0-13.5 mm Inhibition Zone Diameter for the aqueous extract of *B. buonopozense* and 0-11.5 mm Inhibition Zone Diameter for the aqueous extract of *N. latifolia*).

The Inhibition Zone Diameter for most of the isolates that were sensitive to antibiotics ranged between 17-38 mm. All the Gram-negative isolates obtained from cattle rumen waste and vegetable farm irrigation water were sensitive to three antibiotics Ofloxacin, Ciprofloxacin and Gentamycin used as control having acceptable inhibition

Table 3. Functional groups of bioactive organic compounds in Ethyl Acetate fraction of *N. Latifolia* leaf extract as revealed by FTIR

Functional Group	Type of Variation	Characteristic Absorption (cm^{-1})	Intensity	Chemical Formula
Bioactive Compound I: in ethyl acetate fraction				
Alcohol	Stretch	3649	Weak	O-H
Alcohol	Stretch, free	3267	Strong, sharp	O-H
Carboxylic acid	Stretch	2980	Variable	O-H
Alkyl	Stretch	2926	Variable	C-H
Amine	Stretch	1086	Medium, Weak	C-N
Alkene	Stretch	1647	Variable	C=C
Aromatic	Stretch	1457	Medium, weak, multiple bands	C=C
Ether	Stretch	1045	Strong	C-O
Alkene	Bend	875	Sharp	=C-H
Bioactive Compound II: in ethyl acetate fraction				
Alcohol	Stretch	3345	Strong	O-H
Alkene	Bend	2974	Sharp	=C-H
Alkyl	Stretch	2888	Medium	-C-H
Alkene	Stretch	1647	Variable	C=C
Aromatic	Stretch	1449	Medium, weak, multiple bands	C=C
Aromatic	Stretch	1420	Medium, weak, multiple bands	C=C
Alkane	Bend	1381	Variable	-C-H
Sulfone	Stretch	1326	Asymmetric	S=O
Acid	Stretch	1272	Strong	C-O
Ether	Stretch	1085	Strong	C-O
Ether	Stretch	1045	Strong	C-O
Alkene	Bend	879	Strong	=C-H
Bioactive Compound III: in ethyl acetate fraction				
Alcohol	Stretch	3326	Strong, broad	O-H
Alkane	Bend	2976	Strong	O-H
Alkane	Bend	2927	Medium	O-H
Alkene	Stretch	1647	Variable	C=C
Aromatic	Stretch	1451	Medium, weak, multiple bands	C=C
Aromatic	Stretch	1420	Medium, weak, multiple bands	C=C
Alkane	Bend	1381	Variable	-C-H
Amine	Stretch	1325	Medium, weak	C-N
Amine	Stretch	1272	Medium, weak	C-N
Alcohol	Bend	1086	Strong	C-O
Alkyl Halide	Bend	1043	Strong	C-F
Alkene	Bend	877	Strong	=C-H

zone diameter range. The Gram- positive bacteria *Corynebacterium diphtheriae* and *Arcanobacterium haemolyticum* were sensitive to Gentamycin, Chloramphenicol and Cotrimoxazole, while *Lactobacillus sp.* and *Listeria monocytogenes* were resistant to the antibiotics - Chloramphenicol, Cotrimoxazole and Gentamycin used as control.

Discussion

A large number of plants produce secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, steroids, saponins, tannins, glycosides and quinines used as active ingredients in pharmaceuticals, cosmetics and pesticides. Thus, the present study confirms that Traditional African Herbal Extracts (TAHEs) - aqueous extracts of *B. buonopozense* and *N. latifolia* leaves - are important repositories of these phytonutrients and can find use for bacterial control and thus for bioconservation of environmental biomass.

Tannins are inhibitory to structural carbohydrate-fermenting bacteria (e.g. *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*) (McSweeney *et al.* 2001). They inhibit these bacteria and consequently reduce the release of soluble sugars from the plant cell wall matrix, which would be a source of carbon for *E. coli* (Flint *et al.,* 2008). Tannins have antimicrobial properties against several bacterial pathogens. However, tannin-resistant strains of certain pathogens may arise.

As pathogens can be present in cattle manure, this may inadvertently affect human and environmental health (Berard *et al.*, 2009). Phytochemical-rich plants (*B. buonopozense* and *N. latifolia*) may serve to reduce the viability of this group of bacteria whose habitat is the rumen of cattle.

The phenolic and anthocyanin contents of agro-economical plants as well as the presence of phytochemicals such as tannins serve to lower the shedding of these microorganisms which form a part of the normal

Table 4. Antibacterial activity of B. buonopozense leaf extracts (indicated as inhibition zone diameter [mm])

Bacterial Isolate	Diversity	Water Extract (%) 1	0.1	0.01	Ethanol Extract (%) 1	0.1	0.01	Standard Antibiotics		
Cattle Rumen Waste Gram Negative Bacteria								**OFL**	**CPR**	**GEN**
Bacillus cereus	1	0	0	0	0	0	0	22	22	20
Chryseomonas luteola	4	0	0	0	0	0	0	22-28	23-28	18-22
Klebsiella oxytoca	1	0	0	0	0	0	0	32	30	24
Providencia rettgeri	4	0	0	0	0	0	0	24-30	24-31	20-25
Pseudomonas aeruginosa	4	0	0	0	0	0	0	17-30	17-30	14-24
Pseudomonas fluorescens	1	0	0	0	0	0	0	28	30	20
Shigella dysenteriae	2	0	0	0	0	0	0	26-29	28-30	20-28
Stenotrophomonas maltophilia	3	0	0	0	0	0	0	28-30	28-30	20-22
Cattle Rumen Waste Gram Positive Bacteria								**CHL**	**COT**	**GEN**
Corynebacterium diphtheriae	2	0	0	0	0	0	0	22-30	14-26	20-25
Arcanobacterium haemolyticum	1	0	0	0	0	0	0	25	26	24
Lactobacillus sp.	1	0	0	0	0	0	0	0	0	10
Listeria monocytogenes	1	0	0	0	0	0	0	0	0	10
Vegetable Farm Irrigation Water Gram Negative Bacteria								**OFL**	**CPR**	**GEN**
Chryseomonas luteola	1	6.5	8	7.25	0	0	0	16	24	24
Citrobacter diversus	7	0-11	0	0-5.5	0	0	0	22-32	23-34	20-26
Citrobacter freundii	6	0	0	0	0	0	0	24-34	24-32	13-30
Enterobacter aerogenes	1	0	0	0	0	0	0	26	24	22
Escherichia coli	8	0	0	0	0	0	0	20-30	20-30	20-24
Klebsiella pneumonia	6	0	0	0	0	0	0	22-38	24-35	16-26
Proteus sp.	4	0-7.5	0	0-3.75	0	0	0	24-26	25-30	20-24
Proteus vulgaris	2	0	0	0	0	0	0	22-25	22-24	20-24
Pseudomonas sp.	20	0-13.5	0	0-6.75	0	0	0	18-32	20-34	14-30
Salmonella typhi	3	0	0	0	0	0	0	20-24	22-24	18-20
Serratia fonticola	5	0	0	0	0	0	0	20-36	25-32	13-22
Shigella sonnei	4	0	0	0	0	0	0	26-35	27-32	21-25
Vibrio cholera	1	0	0	0	0	0	0	30	32	20
Yersinia sp.	1	0	0	0	0	0	0	32	32	28

Note: OFL- Ofloxacin (5µg), CPR-Ciprofloxacin (5µg), GEN-Gentamicin (10µg), CHL-Chloramphenicol (10µg), COT- Cotrimoxazole (25µg)

Figure 1. Characteristic FTIR absorption frequency of functional group of biaoctive organic compounds I, II and III in Ethyl Acetate fraction of N. latifolia leaf extract

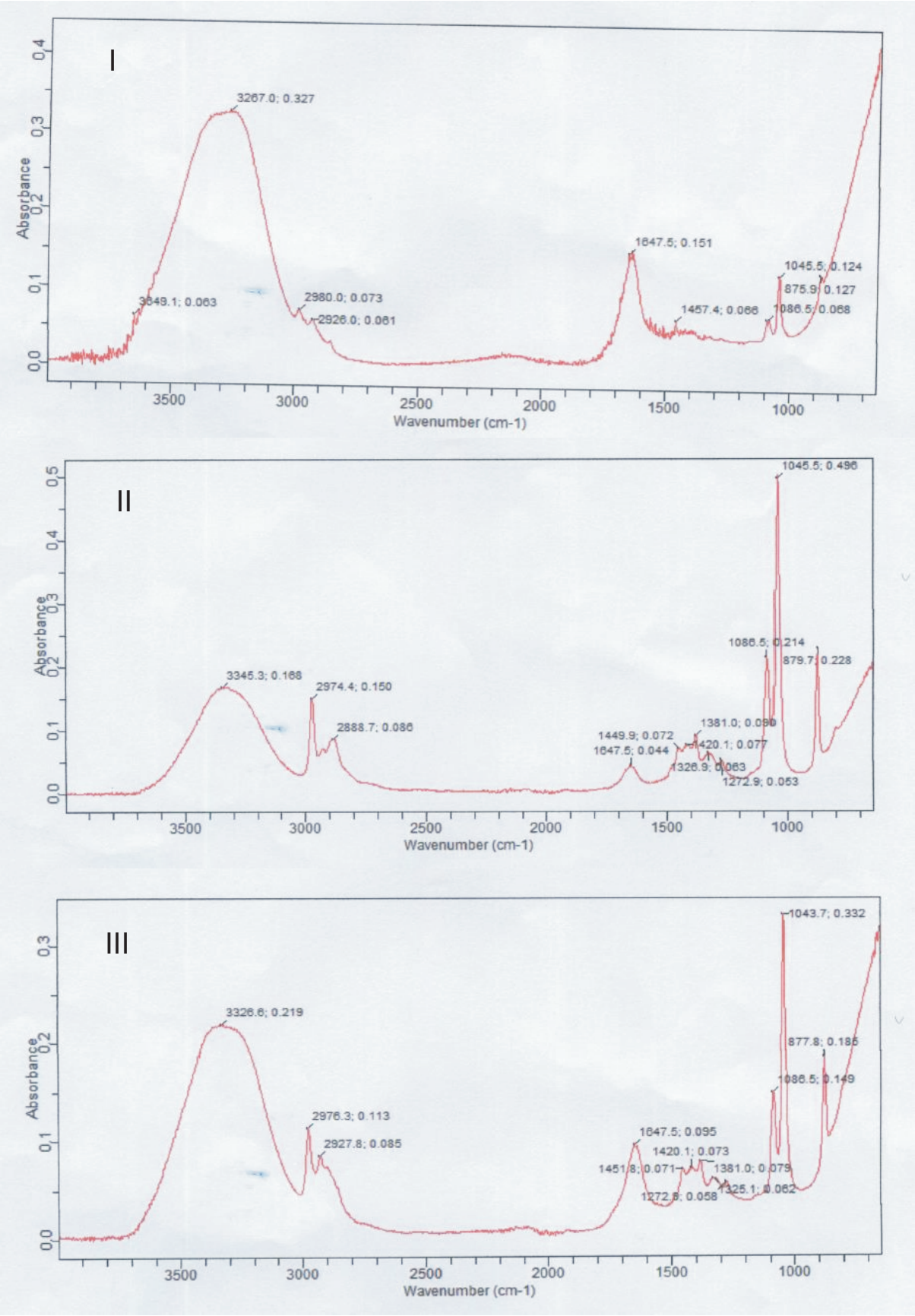

Figure 2. Characteristic FTIR absorption frequency of functional group of biaoctive organic compounds I, II and III in Ethyl Acetate fraction of B. buonopozense leaf extract. I - compound in ethyl acetate fractionII - compoun in chloroform fraction; II - compound in acetone fraction

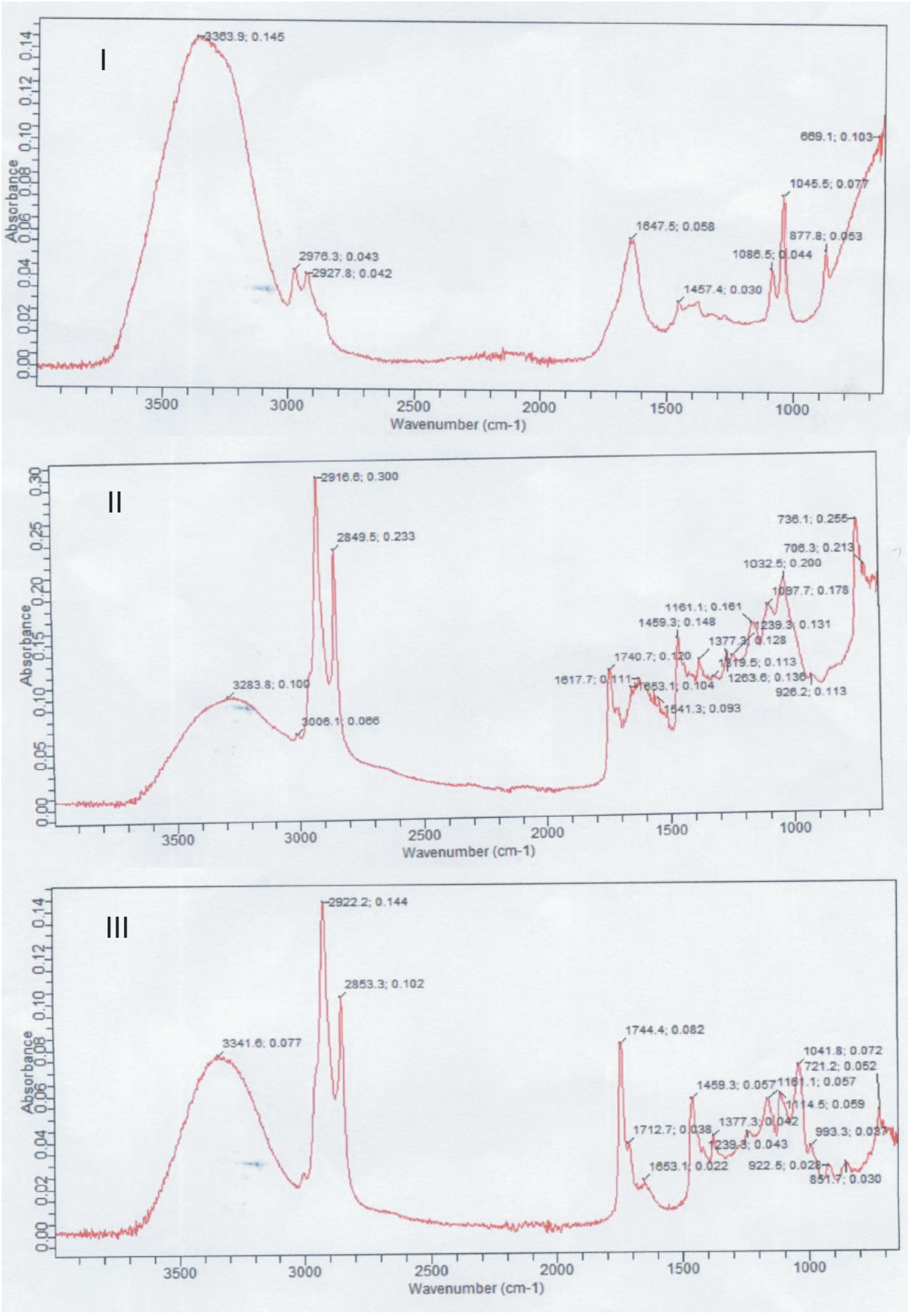

Figure 3. Characteristic FTIR absorption frequency of functional groups of biaoctive organic compounds IV and V of *B. buonopozense* leaf extract. IV - compound in dichloromethane fraction; V - compound in ethanol fraction

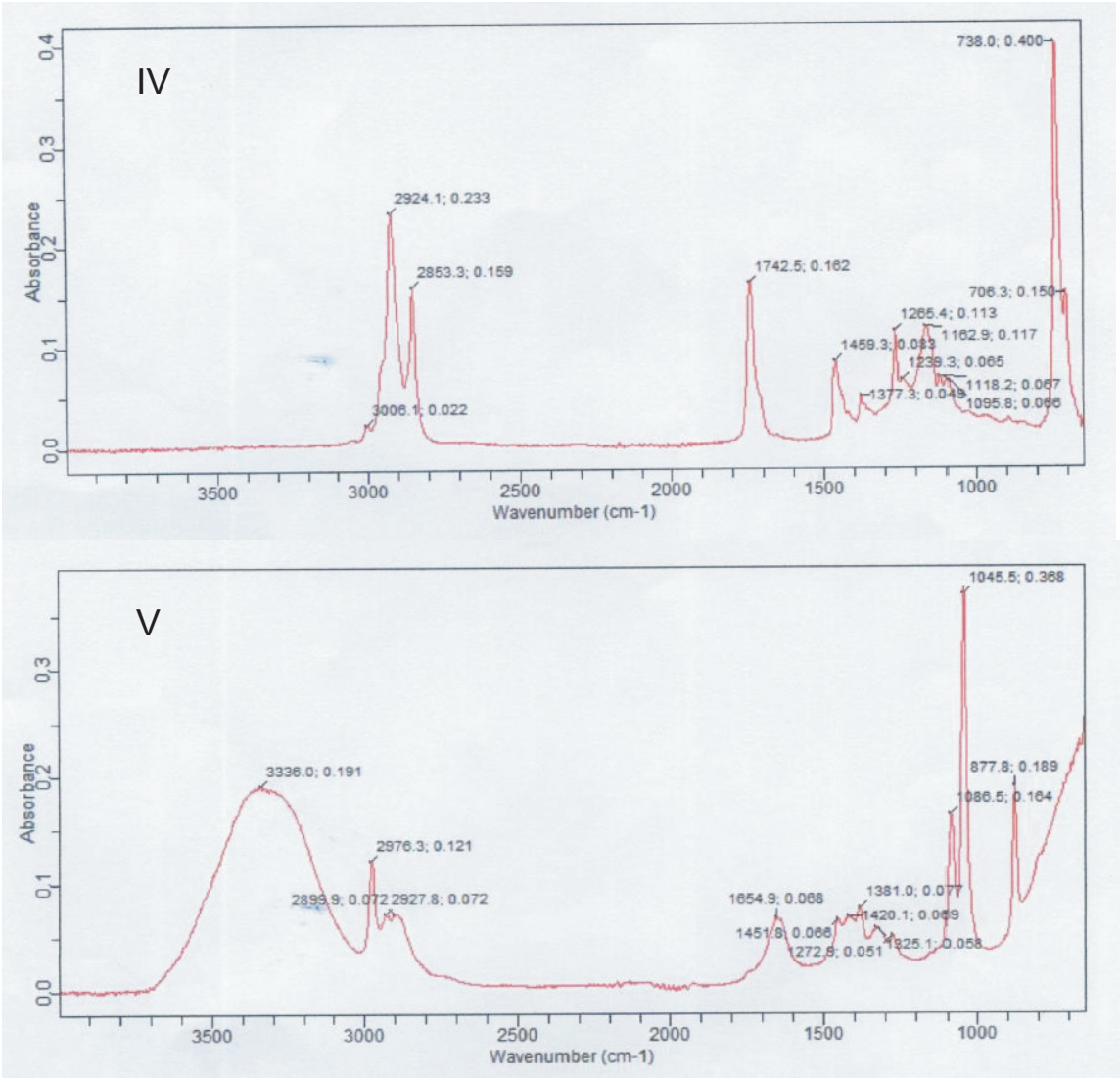

Table 5. Antibacterial activity of N. latifolia leaf extract (indicated as inhibition zone diameter [mm])

Bacterial Isolate	Diversity	Water Extract (%)			Ethanol Extract (%)			Standard Antibiotics		
		1	0.1	0.01	1	0.1	0.01			
Cattle Rumen Waste Gram Negative Bacteria								**OFL**	**CPR**	**GEN**
Bacillus cereus	1	0	0	0	0	0	0	22	22	20
Chryseomonas luteola	4	0	0	0	0	0	0	22-28	23-28	19-22
Klebsiella oxytoca	1	0	0	0	0	0	0	32	30	24
Providencia rettgeri	4	0	0	0	0	0	0	24-30	24-31	20-25
Pseudomonas aeruginosa	4	0	0	0	0	0	0	17-30	17-30	14-24
Pseudomonas fluorescens	1	0	0	0	0	0	0	28	30	20
Shigella dysenteriae	2	0	0	0	0	0	0	26-29	28-30	20-28
Stenotrophomonas maltophilia	3	0	0	0	0	0	0	28-30	28-30	20-22
Cattle Rumen Waste Gram Positive Bacteria								**CHL**	**COT**	**GEN**
Corynebacterium diphtheriae	2	0	0	0	0	0	0	22-30	14-26	20-25
Arcanobacterium haemolyticum	1	0	0	0	0	0	0	25	26	24
Lactobacillus sp.	1	0	0	0	0	0	0	0	0	10
Listeria monocytogenes	1	0	0	0	0	0	0	0	0	10
Vegetable Farm Irrigation Water Gram Negative Bacteria								**OFL**	**CPR**	**GEN**
Chryseomonas luteola	1	0	0	0	0	0	0	16	24	24
Citrobacter diversus	7	0	0	0	0	0	0	12-32	12-34	10-26
Citrobacter freundii	6	0	0	0	0	0	0	24-34	28-32	12-30
Enterobacter aerogenes	1	0	0	0	0	0	0	26	24	22
Escherichia coli	8	0	0	0	0	0	0	24-30	20-30	20-22
Klebsiella pneumonia	6	0-11.5	0	0	0	0	0	12-38	12-35	11-28
Proteus sp.	4	0	0	0	0	0	0	22-26	26-30	20-22
Proteus vulgaris	2	0	0	0	0	0	0	20-24	28	18-20
Pseudomonas sp.	20	0	0	0	0	0	0	18-30	20-34	14-30
Salmonella typhi	3	0	0	0	0	0	0	20-22	22-24	18-20
Serratia fonticola	5	0	0	0	0	0	0	20-36	26-32	10-28
Shigella sonnei	4	0-6	0-6	0	0	0	0	26-35	27-32	21-22
Vibrio cholera	1	10.5	0	0	0	0	0	30	32	20
Yersinia sp.	1	11.5	0	0	0	0	0	28	26	16

Note: OFL - Ofloxacin (5µg), CPR- Ciprofloxacin (5µg), GEN- Gentamicin (10µg), CHL- Chloramphenicol (10µg), COT - Cotrimoxazole (25µg)

fauna of the cattle rumen. The extracts tested in this study did not have antimicrobial activity on the different strains and species of the bacteria; thus making *B. buonopozense* and *N. latifolia* candidates for forage in the hope that they will serve to reduce the shedding of the potentially pathogenic bacteria in cattle without affecting animal production.

By identifying functional groups present in the bioactive compounds of the leaf extracts of *B. buonopozense* and *N. latifolia* Sm, we can suggest that new supplements can be formulated from the plants to fortify cattle feed thereby reducing bacteria shedding in cattle, which might affect economic vegetables.

The absolutely no inhibitory effect of the ethanol extracts of both plant leaves on the entire tested bacterial isolates might be because the major bioactive compounds were not extracted into the organic solvent (Agyare *et al.*, 2006). Hence, the method and solvent of extraction should be carefully selected (Okoli and Iroegbu, 2004; Mujeeb *et al.*, 2014). As only the fresh vegetable irrigation water Gram-negative bacteria showed certain (though not protective) sensitivity to only the aqueous extract of both plants, it suggests that steroids in *B. buonopozense* as well as saponins and tannins which were confirmed to be present in *N. latifolia* might be quantitatively too low to elicit antimicrobial activity (Maitera *et al.*, 2011).

The lack of susceptibility of *the studied bacteria* to the ethanol extracts could also be as a result of possible inherent resistance gene against a wide range of both antibiotics and non-antibiotic antimicrobial agents due to the permeability barrier afforded by their outer membranes (Lino and Deogracious, 2006). The aqueous extracts of the plants displayed wide Inhibition Zone diameters and higher Minimum Inhibitory Concentration values against all test bacteria compared to ethanol extracts of the leaves, albeit below the cut-off value of 16mm IZD. It was so, maybe because the bioactive components are water-soluble (El-Mahmood *et al.*, 2008).

Conclusion

The leaves of B. buonopozense and N. latifolia screened for phytochemical constituents seemed to have the potential to act as sources of useful products to improve the health of husbandry herbivorous animals and in turn vegetable cultivation farms. The findings suggested that *B. buonopozense and N. latifolia* leaves could be potential sources of natural agents that hold great promise for use in organic farming system and bioconservation as reduction in bacteria shedding from cattle rumen would mean protection for economic vegetables in farms where manure and compost are used.

It appears that *B. buonopozense and N. latifolia* leaf extracts do not possess antimicrobial activity against a panel of bacteria of public health and environmental importance. However, possession of certain bioactive compounds which are known to pave inhibitory properties against bacteria is encouraging and may provide useful lead molecules which could qualify for the synthesis of new broad spectrum antibiotics.

Future studies will aim to purify the bioactive compounds in the *B. buonopozense and N. latifolia leaf extracts to confirm their individual biocontrol efficacy against bacteria of public health and environmental significance, using higher concentrations on the isolates. The studied plants, B. buonopozense and N. latifolia, are part of the regular diet of cattle, there is pr*obability that the bacteria in this study are naturally endowed to resist the bioactive components in these plant due to their constant exposure. These bacteria might have developed resistance to the bioactive compounds in the plants over time. There is need to search for the resistance genes in the bacterial flora of the cattle rumen waste and vegetable farm irrigation water in a separate study.

References

Agyare C., Mensah A. Y. and Osei-Asante S. (2006). Antimicrobial activity and phytochemical studies of some medicinal plants from Ghana. Boletin Latino americano y del Caribe de plants medicinales y aromaticas. Sociedad Latinoamericana de fitoquimica, Santiago, Chile, pp 113-117.

Berard N. C., Holley R. A., McAllister T. A., Ominski K. H., Wittenberg K. M., Bouchard K. S., Bouchard J. J. and Krause D. O. (2009). Applied and Environmental Microbiology 75:1074–1079. Potential to reduce *Escherichia coli* shedding in cattle feces by using sainfoin (*Onobrychis viciifolia*) forage, tested *in vitro* and *in vivo*.

Chung H., Pamp S. J., Hill J. A., Surana N. K., Edelman S. M., Troy E. B., Reading N. C., Villablanca E. J., Wang S., Mora J. R., Umesaki Y., Mathis D., Benoist C., Relman D. A., Kasper D. L. (2012). Gut immune maturation depends on colonization with a host- specific microbiota. Cell 149(7): 1578-1593.

Dominguez-Bello M. G., Costello E. K., Contreras M., Magris M., Hidalgo G., Fierer N., et al. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 107: 11971–5.

Duguid J.P., 1989. Staphylococcus: cluster-forming gram-positive cocci. In: Collee, J.G., Duguid, J.P., Fraser, A.G., Marmion, B.P. Eds.: Mackie and Mccartney. Practical Medical Microbiology, 13th ed. Churchill Livingstone, UK, pp. 303–316.

El-Mahmood A. M, Doughari, J. H. and Chanji, F. J. Scientific Research and Essay 3(3): 102-105.

Flint H. J., Bayer E. A., Rincon M. T., Lamed R. and White B. A. (2008). Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat. Rev. Microbiol. 6: 121-131.

Handelsman J. (2004). Metagenomics: Application of Genomics to Uncultured Microorganisms. Microbiol Mol Biol Rev. 68(4): 669–685.

Lino A. and Deogracios O. (2006). The *in vitro* antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steanotaenia araliacea* – Ugandan Medicinal plants. Afr. Health Sci. 6(1): 31-35.

Lotz M., Gutle D., Walther S., Menard S., Bogdan C., Hornef M. W. (2006). *Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. J Exp Med 203: 973-984.*

Maitera O. N., Khan M. E. and James T. F. (2011). Phytochemical analysis and the chemotherapeutics of leaves and stembark of

Nauclea latifolia grown in Hong, Adamawa State Nigeria. Asian Journal of Plant Science and Research 1 (3):16-22.

McSweeney C. S., Palmer B., McNeill D. M. and Krause D. O. (2001). Microbial interactions with tannins: nutritional consequences for ruminants. Anim. Feed Sci. Technol. 91:83-93.

Neu J. and Rushing J. (2011). Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin Perinatol. 38:321–31.

Nuding S., Fellermann K., Wehkamp J. *et al.* (2007). Reduced mucosal antimicrobial activity in Crohn's disease of the colon. Gut 56: 1240–1247.

Okoli A. S., Iroegbu C. U. (2004). Evaluation of extracts of *Anthocleista djalonensis*, *Nauclea latifolia* and *Uvaria afzalii* for activity against bacterial isolates from cases of non-gonococcal urethritis. J. Ethnopharmacol. 92(1): 135-44.

Olasupo N. N., Fitzgerald D. J., Gasson M. J., Narbad A. (2003). Activity of natural antimicrobial compounds against *Escherichia coli* and *Salmonella enterica* serovar *typhimurium*. Lett. Appl. Microbiol. 37: 448–451.

Sofowora A. (1993). Medicinal plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York. Pp. 34-36.

Mujeeb F., Bajpai P. and Pathak N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. BioMed Research International 2014: 1-11.

Sonnenburg E. D., Zheng H., Joglekar P., Higginbottom S. K., Firbank S. J., Bolam D. N., Sonnenburg J. L. (2010). Specificity of polysaccharide use in intestinal *Bacteroides* species determines diet-induced microbiota alterations. Cell 141: 1241–1252.

Tiwari G., Tiwari R., Pandey S., Pandey P. (2012). Promising future of probiotics for human health: Current scenario. Chron Young Sci 3: 17-28.

How to cite this article:

Akinde S.B., Ajayi E.I.O., Awojide O.I., Azeez O.K., Osunsanmi K.O., Olabiran O.A. and Fowosire A.A (2016). FTIR Characterisation and *in vitro* Activities of *Bombacopsis buonopozense* and *Nauclea latifolia* Leaf Extracts against Bacteria Isolated from Cattle Rumen Waste and Vegetable Farm Irrigation Water. UNIOSUN J. Sciences. 1(1): 38-46