African Journal of Biotechnology Vol. 11(42), pp. 10008-10014, 24 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1049
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

In vitro susceptibility testing of *Yersinia* species to eight plant extracts and three natural antimicrobial agents

Owhe-Ureghe, U. B.[1]*, Ogiehor, S. I.[2] and Ehwarieme, D. A.[1]

[1]Department of Microbiology, Delta State University, Abraka, Delta State, Nigeria.
[2]Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Accepted 28 September, 2011

The antimicrobial effects of the ethanolic and aqueous extracts of the leaves of *Cymbopogon citratus* (DC) Stapf, *Acalypha hispida* Forsk, *Vernonia amygdalina* Del, *Occimum gratissimum* L., *Sida acuta* Burm, F., *Coffea arabica* L., seeds of *Carica papaya* L., fruit juice of *Citrus aurantifolia* (Christim) Swingle, wild honey, processed honey (Laser brand) and processed coffee (Nescafe) on *Yersinia pesudotuberculosis, Yersinia enterocolitica* 0:3, *Yersinia enterocolitica* 0:8*, Yersinia Kristensenii* 0:11, 23, *Yersinia intermidia* 0:52, 53, and *Yersinia intermidia*-like bacteria were evaluated. In this study, only processed honey did not inhibit the test organisms, while other extracts investigated show varying zones of inhibition. The zones of inhibition obtained from the aqueous extract ranged from 6.0 mm for *V. amygdalina* Del against *Y. enterocolitica* 0:03 to 17.7 mm for *Citrus aurantifolia* (christim) Swingle against *Y. pesudotuberculosis* , while the zones of inhibition obtained for ethanolic extract ranged from 6.6 mm for *V. amygdalina* Del against *Y. enterocolitica.* 0:3 to 17.6 mm for processed coffee against *Y. pseudotuberculosis.* The minimum inhibitory concentration (MIC) of the various antimicrobials ranged from 0.0034 µg/ml for *Y. enterocolitica* 0:3 to 0.00155 µg/ml for *Y. intermedia* 0.52, 53, using dilution techniques. The MIC compares with that of commercial antibiotics and therefore suggest that these extracts could be used in the effective management of yersiniosis, in this era of bacterial multiple drug resistance to commonly used antibiotics. However, further studies to determine the shelf life, appropriate dosages and/or whether there are any contraindications for those who may use these extracts as medication is recommended.

Key words: Plant extracts, natural antimicrobial agents, anti-*Yersinia* species agent.

INTRODUCTION

All over the world, people depended on herbs for the treatment of various ailments before the advent of modern medicine (Geelhoed et al., 1994). Man's use of herbs for treatment of various diseases dates back thousands of years.

According to Sofowora (1993), there are about 149 plants that are commonly used in traditional medicine in Africa. Sofowora's list may not be exhaustive because there are many plants that are yet to be discovered or may have not been documented. Herbs are used in the treatment of some bacterial, fungal and viral infections (Quereshi et al., 1988). Plant extracts have been in use as antiseptics and disinfectants. The most widely used include alcohols, chlorine containing compound, iodine preparations, phenols compounds (resins tannins, alkaloids and terpene), inorganic and organic mercurial and silver preparations, quaternary ammonium compounds, boric acid, oxidizing agents and aldehyde derivatives. In Nigeria, the leaves of *Kalanchoe crenata* have been used either on their own or in combination with other ingredients to treat headache, general debility, small pox and convulsion in children and adults (Soyinka, 1986). In East Africa, a decoction of the boiled roots of *Kalanchoe lanceolata* is taken in small quantities for the treatment of gonorrhoea and as a vermifuge (Kokwaro, 1976). Osuinde and Esiovwa (1998) reported the

*Corresponding author. E-mail: owheureghe@yahoo.com.

antibacterial activity of some species (*Piper guineese*, L. and *Piper umbrellatum, L*) on some Gram negative bacteria. Antimicrobial activities of extracts of *Spondias mombin,* L., *Carica papaya*, L. and *Viscum album* L. against some Gram positive and Gram negative beacteria have been documented (Osuinde and Isibor, 1998). The scientific bases of the use of *Citrus aurantifolia* (lime) juice in most Nigerian homes as a herbal remedy for supurative wound infections, pimples and gastrointestinal disorders has been documented (Oboh et al., 1995).

Antibacterial activity of the ethanolic extract of *Daniella oliveri*, *Annona segagalensis* and *Mitragyna sipulosa* against some Gram positive and Gram negative bacterial species have also been reported by Olukemi and Kandakai-Olukemi (2004). The antimicrobial effect of the aqueous and ethanolic extracts of *Breynia nivosus, Ageratum conysoides* and the combination of herbal preparations on *Streptococcus mutans* has also been established in South Eastern Nigeria (Amadi et al., 2007). Studies on the antimicrobial properties of spices, herbs and their components have been documented earlier with a sustained interested to date (Oboh et al., 1995; Ejechi, 1996; Asagba et al., 2004; Amadi et al., 2007).

Yersiniosis is an infectious disease caused by bacteria of the genus *Yersinea.* Most yersiniosis infections among humans are caused by *Yersinea enterocolitica* (Jones, 2003). Infection with *Y. enterocolitica* occurs mostly among young children and is believed to be contracted by consumption of poorly cooked meat products, unpasteurized milk and water contaminated by the bacteria (Jones, 2003). Symptoms of infection depend on the age of person infected. Common symptoms in children are fever, abdominal pain and diarrhea. Bacteremia may develop in infants. In older children and adults, right-sided abdominal pain and fever may be the predominant symptom, and may be confused with appendicitis. Treatment often requires aggressive antibiotic therapy involving ciprofloxacin, chloramphenicol, ampicillin and polymycin (Collins, 1996). Unfortunately, there is paucity of information regarding home remedies or alternative therapy for yersiniosis, especially in this era of emergence of drug resistance. The susceptibility of *Yersinia* species to various plant extracts and other natural antimicrobial agents, except *C. aurantifolia* (Oboh et al*.,* 1995) have not been extensively studied.

The purpose of this study was to ascertain the susceptibility of *Yersinia* species to extraction of various plants and natural antimicrobial agents and their possible use for the treatment of yersiniosis in this environment.

MATERIALS AND METHODS

Bacterial strains

The *Yersinia* species (*Y. pseudotuberculosis, Y. enterocolitica*.0:3, *enterocolitica* 0:8, *Y. kristensenii* 0:11, 23, *Y. intermedia* 0:52, 53 and *Y. intermedia*-like bacteria 0:52, 53) used for this investigation were obtained from the laboratory of Prof. D. E. Agbonlabor of the Department of Microbiology, Ambrose Alli University, Ekpoma, Nigeria.

Plants and other natural antimicrobials

The fresh leaves of *C. citratus* (DC) Stapf, *A. hispida* Forsk, *V. amygdalina* Del, *O. gratissimum* L., *S. acuta* Burm, F., *C. offea arabica* L., seeds of *C. papaya* L., and fruits of *C. aurantifolia* (Christim) Swingle were collected from a forest in Ujemeh-Ekpoma, Edo State and Abraka in Delta State, Nigeria. These plants were identified by Dr. S. M. Ayodele of Botany Department, Kogi State University, Nigeria. The processed honey (Laser brand) and coffee (Nascafe brand) were bought from a supermarket at Abraka, Delta State, Nigeria, while the wild honey was bought from persons who marketed it from a forest at Okene, Kogi State, Nigeria.

Preparation of extracts

The *C. aurantifolia* (Christim) Swingle fruit were washed with sterile distilled water, and the bark disinfected with 70% methanol and rinsed thoroughly in sterile distilled water. The fruits were aseptically peeled to remove the rinds. They were then sliced and the juice mechanically released into sterile flat bottom flask under aseptic conditions. The fluid was then subjected to centrifugation at 5000 rpm. The supernatant (raw extract) was then plated into blood agar plates and incubated at room temperature (28±2°C) for 18 to 24 h under aerobic conditions to assure sterility. A part of the juice obtained was diluted with sterile distilled water to give ½ dilutions as previously described by Oboh et al. (1995).

The leaves or seeds of other plants were air dried in the laboratory for two weeks and blended into fine powders with a domestic blender (coffrets Blender Standard 3). These were treated separately with 95% ethanol, and hot sterile distilled water in the ratio of 1:2 (w/v). The mixtures were allowed to stand with occasional stirring at room temperature (28±2°C) for 24 h, after which they were filtered and then concentrated by evaporation to dryness in a water bath of 40°C as previously described by Osuinde and Esiovwa (1998). These extracts were also diluted to obtain ½ concentration. A portion of the neat processed and wild honey was also diluted to obtain ½ dilution. While in the case of processed coffee (Nescafe brand) 1 g was diluted 1:100 (W/V) to obtain the neat solution, and further diluted to ½ concentration.

Test for antimicrobial activity

The disk diffusion method (Oboh et al., 1995) were adopted to test the *Yesinia* species sensitivity to the various prepared extracts and dilutions (wild honey, processed honey and processed coffee) used in this study. 18 to 24 h old broth cultures of the test organisms were diluted to give a final innocula of 1.10×10^7 CFU/ml. The surface of the sterile Mueller-Hinton agar (oxoid) plates was swabbed with the respective diluted sterile cultures to ensure confluent growth. 100 sterile disks from Watmam NO 1 filter paper (5 mm in diameter) were soaked in 1 ml of the neat and ½ dilution of each extract in sterile screw-capped bottles for 60 min.

With the aid of sterile forceps, the impregnated disks were then placed on the surface of the seeded agar plates at a spacing of 2 cm and press down gently to ensure full contact with the inoculated medium. The plates were left on the bench for about 60 min to allow for diffusion into the medium and were incubated aerobically at room temperature (28±2°C) for 18 to 24 h. Thereafter, the diameter of the zones of inhibition were measured and recorded.

Table 1. The inhibitory effect of neat ethanolic plant extracts and some natural antimicrobials against *Yersinia* sp.

Extract	Diameter of zones of inhibition (mm)					
	YPS	YE 0:3	YE 0:8	YK 0:11, 23	YI 0:52, 53	YIL 0:52, 53
Cymbopogon citratus (DC) staft leaves	12.7	16.5	10.4*	14.4	11.0*	13.7
Acalypha hispidia Forsk leaves	13.7	15.3	12.3	11.5	10.7*	13.6
Vernonia amygdalina Del leaves	17.3	6.6*	11.3	13.7	12.3	10.6*
Occimum gratissimum L leaves	16.0	16.0	14.0	14.0	15.0	15.3
Sida acuta Burm F. leaves	13.5	13.1	11.6	12.8	12.5	12.2
Carica papaya L leaves	13.2	13.0	12.0	12.5	12.0	12.1
Coffee Arabica L. Leaves	12.3	16.0	8.7	13.8	14.0	15.0
Processed coffee (Nascafe)	17.6	16.1	15.7	14.0	15.3	15.0

YPS = *Yersinia pseudotuberculosis*; YE 0:3 = *Yersinia enterocolitica* 0:3; YE 0:8 = *Y. enterocolitica* 0:8; YK0:11, 23 = *Yersinia Kristensensii* 0:11, 23; YI0:52, 53 = *Yersinia intermdia* 0:52, 53; YIL0:52, 53 = *Y. intermedia*-like bacteria 0:52, 53; * = bacteriostatic effect; no asterisk = bactericidal effect.

Table 2. The inhibitory effect of ½ dilutions of the extracted ethanolic plant and natural antimicrobials against *Yersinia* sp.

Extract	Diameter of zones of inhibition (mm)					
	YPS	YE 0:3	YE 0:8	YK 0:11, 23	YI 0:52, 53	YIL 0:52, 53
Cymbopogan citratus leaves	0	13.3	7.0*	11.5	0	10.0*
Acalypha hispidia Forsk leaves	7.1*	10.7*	5.4*	8.0*	6.3*	9.4*
Vernonia amygdalina Del leaves	0	5.8*	10.0*	10.0*	0	8.0*
Occimum gratissimum L leaves	10.0	7.3*	12.3	10.8*	11.0*	12.6
Sida acuta Burm F. leaves	12.0	10.5*	10.5*	10.5*	10.0*	8.5*
Carica papaya L leaves	10.5*	10.0*	9.0*	10.0*	10.0*	9.5*
Coffee Arabica L. leaves	0	7.0*	7.6*	9.0*	0	6.0*
Processed coffee (Nascafe)	12.0	10.3*	11.2*	12.6*	12.4	13.0

YPS = *Yersinia pseudotuberculosis;* YE 0:3 = *Yersinia enterocolitica* 0:3; YE 0:8 = *Y. enterocolitica* 0:8; YK0:11, 23 = *Yersinia Kristensensii* 0:11, 23; YI0:52, 53 = *Yersinia intermdia* 0:52, 53; YIL0:52, 53 = *Y. intermedia*-like bacteria 0:52, 53; * = bacteriostatic effect; no asterisk = bactericidal effect.

Determination of MIC of the antimicrobial agents

The tube method of Cowan (1985) was used to determine the MIC of the test antimicrobial against the *Yersinaia* species. Sterile nutrient both (oxoid) was used as the diluents/growth medium in this study. An aliquot (0.5 ml) of the sterile nutrient broth was dispensed into seven sterile tubes plugged with non-absorbent cotton wool under aseptic condition. An equivalent amount of the plant extracts and natural antimicrobials was added into the first and second tubes. They were gently agitated and 0.5 ml aliquot was transferred from the second tube into the third. This was also agitated gently and the serial dilution repeated till the sixth tube and 0.5 ml amount was discarded from the sixth tube. The seventh tube served as negative control. Thereafter, 0.5 ml of the *Yersinia* species (1.10×10^7 to 1.40×10^7CFU/ml) was added to each tube to bring the volume of each tube to 1 ml. This was repeated for all the test organisms. The set-up was incubated at 28±2°C for 24 h. The MIC was recorded as the lowest concentration (highest dilution) of the extract and natural antimicrobials that inhibited visible growth (that is, no turbidity).

RESULTS

Results represented in Tables 1 to 6 shows that the eight plant extracts, the processed coffee (Nascafe brand), wild honey and processed honey possessed antibacterial activity, with the aqueous extracts being significantly less inhibitory than the ethanolic extracts. The five species (or six strains) of *Yersinia* showed varying degrees of sensitivities to the various antimicrobials except the processed honey. The zones of inhibition of the ethanolic extract of *C. citratus* (DC) Staft against the test organisms ranged from 10.4 to 16.5 mm (Table 1) while its aqueous extracts gave 9.0 to 13.0 mm zones of inhibition (Table 3). The zones of inhibition of the ethanolic extract of *A. hispida* Forsk against the test organisms ranged from 10.7 to 15.3 mm, while its aqueous extracts gave 10.0 to 12.9 mm zones of inhibition. The zones of inhibition of the ethanolic extract of *V.amygdalina* Del against the test organisms ranged from 6.6 to 17.3 mm (Table 1), while its aqueous extract gave 6.0 to 13.0 mm zones of inhibition. The zones of inhibition of the ethanolic extract of *O. gratissimum* against the test organisms ranged from 14.0 to 16.0 mm (Table 1), while its aqueous extract gave 11.7 to 15.3 mm (Table 3) zones of inhibition. The zones of inhibition of

Table 3. Diameter of zones of inhibition of plant (mm) [crude neat hot water] against *Yersinia* sp.

Extract	Diameter of zones of inhibition (mm)					
	YPS	YE 0:3	YE 0:8	YK 0:11, 23	YI 0:52, 53	YIL 0:52, 53
Cymbopogan citratus leaves	10.5*	13.0	12.1	12.0	9.0*	12.0
Acalypha hispidia Forsk leaves	12.0	12.9	11.8	11.0*	10.0	12.5
Vernonia amygdalina Del leaves	13.0	6.0*	13.0	12.0	11.5	11.5
Occimum gratissimum L leaves	14.7	11.7	12.7	13.0	15.3	13.0
Sida acuta Burm F. leaves	12.8	12.7	11.0*	11.5	11.6	11.6
Citrus aurantifolia fruit juice	17.7	14.3	15.8*	15.2	16.2	15.4
Carica papaya seeds	12.7	12.0	12.0	12.0	11.8	11.5
Coffee Arabica leaves	12.7	11.3	12.3	13.6	14.0	13.5
Processed coffee (Nascafe)	15.0	15.0	15.0	14.0	15.0	14.0
Natural honey	13.0	11.3	12.3	12.8	12.7	12.5
Processed honey	0	0	0	0	0	0

YPS = *Yersinia pseudotuberculosis;* YE 0:3 = *Yersinia enterocolitica* 0:3; YE 0:8 = *Y. enterocolitica* 0:8; YK0:11, 23 = *Yersinia Kristensensii* 0:11, 23; YI0:52, 53 = *Yersinia intermdia* 0:52, 53; YIL0:52, 53 = *Y. intermedia*-like bacteria 0:52, 53; * = bacteriostatic effect; no asterisk = bactericidal effect.

Table 4. Diameter of zones of inhibition of half diluted planted extract crude neat hot water (1/2 (1/2) against *Yersinia* sp.

Extract	Diameter of zones of inhibition (mm)					
	YPS	YE 0:3	YE 0:8	YK 0:11,23	YI 0:52,53	YIL 0:52,53
Cymbopogan citratus leaves	8.6*	10.0*	9.5*	10.0*	7.0*	8.0*
Acalypha hispidia Forsk leaves	11.0	10.5*	10.5*	9.7*	9.0*	10.5*
Vernonia amygdalina Del leaves	11.6	0	12.0	11.5	10.0*	7.5*
Occimum gratissimum L leaves	9.0*	8.0*	10.5*	11.0*	11.5	10.8*
Sida acuta Burm F. leaves	10.5*	10.5*	8.0*	4.0*	10.3	6.0*
Citrus aurantifolia fruit juice	14.5	12.8	12.8	12.5	12.9	13.2
Carica papaya seeds	10.6	10.0*	10.0*	10.5*	10.0*	9.0*
Coffee Arabica leaves	11.0*	9.0*	10.5*	12.0	12.0	10.0*
Processed coffee (Nascafe)	16.8	10.3*	14.8	12.6	13.8	12.6
Natural honey	10.1*	11.0*	11.2	10.1*	10.6	11.0
Processed honey	0	0	0	0	0	0

YPS = *Yersinia pseudotuberculosis;* YE 0:3 = *Yersinia enterocolitica* 0:3; YE 0:8 = *Y. enterocolitica* 0:8; YK0:11, 23 = *Yersinia Kristensensii* 0:11, 23; YI0:52, 53 = *Yersinia intermdia* 0:52, 53; YIL0:52, 53 = *Y. intermedia*-like bacteria 0:52, 53; * = bacteriostatic effect; no asterisk = bactericidal effect.

the ethanolic extract of *Sida acuta* Burm F. against the test organisms ranged from 11.6 to 13.5 mm, while its aqueous extracts gave 11.00 to 12.8 mm zones of inhibition (Table 3). The zones of inhibition of the ethanolic extract of *Carica papaya* L. seed against the test organisms ranged from 12.0 to 13.2 mm (Table 1) while its aqueous extracts gave 11.5 to 12.7 mm zones of inhibition (Table 3). The zones of inhibition of the ethanolic extract of *Coffee arabica* L. leaves against the test organisms ranged from 8.7 to 16.0 mm, while its aqueous extracts gave 11.3 to 14.0 mm zones of inhibition. The zones of inhibition of the ethanolic solution of processed coffee (Nescafe brand) against the test organisms ranged from 14.0 to 17.6 mm (Table 1), while its aqueous extracts gave 14.0 to 15.0 mm zones of inhibition (Table 3). The zones of inhibition of *C. aurantifolia* (Christim) Swingle fruit juice ranged from 14.3 to 17.7 mm. Wild honey gave zones of inhibition from 11.3 to 13.0 mm, while processed honey did not show any zone of inhibition.

Tables 5 and 7 show the sensitivity pattern of the six *Yersinia* sp. to the various plant extracts and natural antimicrobials used in this study. Processed honey did not show any inhibitory effect on all the test organisms. *Y. pseudotuberculosis* was sensitive to all the neat ethanolic extracts, while its sensitivity to the various aqueous extracts was similar except that for *C. critratus* (DC) stapf, which was bacteriotatic. The inhibitory effects of both ethanolic and aqueous extracts of *V. amygdalina* Del was bacteriostatic to *Y. enterocolitica* . while other

Table 5. Susceptibility pattern of *Yersinia* sp. to the various antimicrobial agents (ethanolic extracts).

Extract	YPS	YE 0:3	YE 0:8	YK 0:11, 23	YI 0:52, 53	YIL 0:52, 53
Cymbopogan citratus leaves	+	+	-	+	-	+
Acalypha hispidia Forsk leaves	+	+	+	+	-	+
Vernonia amygdalina Del leaves	+	-	-	+	+	-
Occimum gratissimum L leaves	+	+	+	+	+	+
Sida acuta Burm F. leaves	+	+	+	+	+	+
Citrus aurantifolia fruit juice	+	+	+	+	+	+
Carica papaya seeds	+	+	+	+	+	+
Coffee Arabica leaves	+	+	-	+	+	+
Processed coffee (Nascafe)	+	+	+	+	+	+

YPS = *Yersinia pseudotuberculosis*; YE = *Y. enterocolitica*; YE = *Y. enterocolitica*; YK = *Y. Kristensensii*; YI = *Y. intermdia*; YIL = *Y. intermedia*-like bacteria; + = sensitive; - = resistant.

Table 6. Susceptibility pattern of *Yersinia* sp. to the various natural antimicrobial agents (water extracts).

Extract	YPS	YE 0:3	YE 0:8	YK 0:11,23	YI 0:52,53	YIL 0:52,53
Cymbopogan citratus leaves	-	+	+	+	-	+
Acalypha hispidia Forsk leaves	+	+	+	-	-	+
Vernonia amygdalina Del leaves	+	-	+	+	+	+
Occimum gratissimum L leaves	+	+	+	+	+	+
Sida acuta Burm F. leaves	+	+	-	+	+	+
Citrus aurantifolia fruit juice	+	+	+	+	+	+
Carica papaya seeds	+	+	+	+	+	+
Coffee Arabica leaves	+	+	+	+	+	+
Processed coffee (Nascafe)	+	+	+	+	+	+
Natural honey	+	+	+	+	+	+
Processed honey	-	-	-	-	-	-

YPS = *Yersinia pseudotuberculosis*; YE = *Y. enterocolitica*; YE = *Y. enterocolitica*; YK = *Y. Kristensensii*; YI = *Y. intermdia*; YIL = *Y. intermedia*-like bacteria; + = sensitive; - = resistant.

extracts and dilutions were bactericidal. *Y. enterocolitica* 0:8 was sensitive to all the ethanolic extract except that of *C. citratus* (DC) stapf, *V. amygdalina* Del and *C. arabica* L., while it was not sensitive to the aqueous extracts of *Sida acuta* Burm F. but was sensitive to other extracts. Except for the aqueous extract of *A. hispida* Forsk, *Y. Kristensenii* 0:11, 23 was sensitive to all the extracts (that is, both ethanolic and aqueous) used in this study. The sensitivity pattern of *Y. intermedia* 0:52, 53 to both ethanolic and aqueous extracts of the antimicrobials used in this study were similar. It was sensitive to all except *C. citrates* (DC) stapf, and *A. hispida* Del. *Y. intermidia-like* bacteria 0:52, 53 was sensitive to all ethanolic extracts except that of *V*. amy*gdalina* Del, while it was sensitive to all the aqueous extracts.

The initial concentrations of the extracts and their MIC of the various extracts against the respective *Yersinia* sp. are presented in Tables 7 and 8. For the ethanolic extracts (Table 7), the MIC ranged from 0.0034 µg/ml (for *Y. intermedia* 0:52, 53 to 0145µg ml). The MIC for the aqueous extracts ranged from 0.0040 to 0.0165 µg/ml for all the *Yersinia* sp., except for *Y. pseudotuberculosis* and *Y. intermedia* 0:52, 53, which ranged from 0.0040 to 0.0145 µg/ml and 0. 038 to 0.0165 µg/ml, respectively.

DISCUSSION

The *in vitro* sensitivity of many bacterial agents of human and animal diseases to various plant extracts have been reported all over the world. Although *in vitro* zones of inhibition observed with one drug cannot be compared with those obtained with another antimicrobial agent, due to the differences in the rate of diffusion through agar gel among other factors (Barry and Thornberry, 1983); zones observed for most of the extracts used in this investigation compares favorably with those of standard zones for known organisms (Oboh et al., 1995).The result of this study suggest that, except for the processed honey, all other antimicrobials used, could be employed as a remedy in the management of gastrointestinal or other disorders associated with *Yersinia* sp. However,

Table 7. The concentration of the ethanolic extracts (µg/ml) and their respective MIC (µg/ml) against *Yersinia* sp.

Extract	Concentration of the extracts and MIC					
	YPS	**YE 0:3**	**YE:08**	**YK 0:11, 23**	**YI 0:52, 53**	**YIL 0:52,53**
A.C.L	0.028 (0.007)	0.028 (0.0035)	0.028 (-)	0.028 (0.0035)	0.028 (-)	0.028 (0.0070)
A.H L	0.027 (0.0070)	0.027 (0.0034)	0.027 (0.0135)	0.027 (0.0135)	0.027 (-)	0.027 (0.0070)
V.A.L.	0.031 (0.0039)	0.031(-)	0.031(-)	0.031 (0.0078)	0.031 (0.055)	0.031(-)
O.G.L.	0.029 (0.0036)	0.029 (0.0036)	0.029 (0.0036)	0.029 (0.0036)	0.029 (0.0036)	0.029 (0.0036)
S.A.L.	0.028 (0.0070)	0.028 (0.0070)	0.028 (0.0140)	0.028 (0.0140)	0.028 (0.0140)	0.028 (0.0140)
C.P.S.	0.029 (0.0073)	0.0073 (0.029)	0.029 (0.00145)	0.029 (0.0145)	0.029 (0.0145)	0.029 (0.0145)
C.A.L.	0.028 (0.0140)	0.028 (-)	0.028 (-)	0.028 (0.0070)	0.028 (0.0035)	0.028 (0.0035)
P.C.	0.032 (0.040)	0.032 (0.0040)	0.032 (0.0040)	0.032 (0.0040)	0.032 (0.0040)	0.032 (0.0040)

(-) = Not determined because the zones of inhibition was less than 11.5 mm; MIC in parenthesis.
ACL= *Cymbopogan citratus* leaves; AHL =*Acalypha hispidia* leaves; VAL = *Vernonia amygdalina* leaves; OGL = *Occimum gratissimum* L leaves; SAL = *Sida acuta* leaves; CAF = *Citrus aurantifolia* fruit juice; CPS = *Carica papaya* seeds; CAL = *Coffee Arabica* leaves; PC = processed coffee (Nescafe); NH = natural honey.

Table 8. The concentration of the ethanolic extracts (µg/ml) and their respective minimum inhibition concentration (MIC) (µg/ml) against *Yersinia* sp.

Extract	Concentration of the extracts and MIC					
	YPS	**YE 0:3**	**YE:08**	**YK 0:11, 23**	**YI 0:52, 53**	**YIL 0:52,53**
A.C.L	0.030 (-)	0.030 (0.0075)	0.030 (0.0150)	0.030 (0.015)	0.030 (-)	0.030 (0.015)
A.H L	0.029 (0.0145)	0.029 (0.0145)	0.029 (0.0145)	0.029 (-)	0.029 (-)	0.029 (0.0145)
V.A.L.	0.033 (0.0083)	0.033 (-)	0.033 (0.0083)	0.033 (0.0165)	0.033 (0.0165)	0.033 (0.0165)
O.G.L.	0.032 (0.0040)	0.032 (0.016)	0.032 (0.016)	0.032 (0.008)	0.032 (0.0040)	0.032 (0.0080)
S.A.L.	0.031 (0.0078)	0.031 (0.0078)	0.031(-)	0.031 (0.0155)	0.031 (0.0155)	0.031 (0.0155)
C.F.A.	0.032 (0.0040)	0.032 (0.0040)	0.032 (0.0040)	0.032 (0.0040)	0.032 (0.0040)	0.032 (0.0040)
C.P.S.	0.033 (0.0083)	0.033 (0.0165)	0.033 (0.0165)	0.033 (0.0165)	0.033 (0.0165)	0.033 (0.0165)
C.A.L.	0..030 (0.0075)	0.030 (0.015)	0.030 (0.015)	0.030 (0.0075)	0.030(0.0038)	0.30 (0.0075)
P.C.	0.033 (0.0041)	0.033 (0.0041)	0.033 (0.0041)	0.033 (0.0041)	0.033 (0.0041)	0.033 (0.0041)
N.H.	0.029(0.0073)	0.029 (-)	0.029 (0.0145)	0.029(0.0145)	0.029 (0.0145)	0.029(0.0145)

(-) = Not determined because the zones of inhibition was less than 11.5 mm; MIC in parenthesis.
ACL= *Cymbopogan citratus* leaves; AHL =*Acalypha hispidia* leaves; VAL = *Vernonia amygdalina* leaves; OGL = *Occimum gratissimum* L leaves; SAL = *Sida acuta* leaves; CAF = *Citrus aurantifolia* fruit juice; CPS = *Carica papaya* seeds; CAL = *Coffee Arabica* leaves; PC = processed coffee (Nescafe); NH = natural honey.

more clinical trials are necessary to ascertain the safety, or otherwise of these extracts. The non-inhibitory effect exhibited by the processed honey compared to the bactericidal potential of the wild honey suggests that the actual active components could have been removed in the course of processing.

The results presented in Tables 1 and 3 indicate that ethanolic extract has more inhibitory potential than the aqueous extracts of the various plant materials used in this study, however, the zones of inhibition shown by the aqueous extracts compared favourably with those of the ethanolic extracts, therefore, individuals who could not withstand alcoholic drinks could use hot water extracts of these plants as a remedy for yersiniosis.

The generally low MIC and the sensitivity of the *Yersinia* sp. to these natural occurring antimicrobials compares with commercial antibiotics. This implies that these extracts (Tables 7 and 8) could be effectively used in the treatment of any disease or disorders associated with *Yersinia* sp.Furthermore, like commercial antibiotics, the results of this study (Table 6) suggests that these extracts could be used to successfully separate organisms in the genus *Yersinia,* into species and thus could serve as epidemiological makers. Moreover, results obtained in this study revealed a large number of plants (herbs) and common antimicrobials in this environment that could be effectively used in the management of yersiniosis. The taxonomic significance of these plant extracts on *Yersinia* sp. has also been revealed. Further studies are recommended to increase the list of possible plants (herbs) that could be used in the treatment of yersiniosis.

REFERENCES

Amadi ES, Oyeka CA, Onyeagba RA, Igbogu OC, Okoli I (2007). Antimicrobial screening of *Breynia nivosu* and *Ageratum conyzoides* against Dental caries organisms. J. Biol. Sci. 7(2): 354-358.

Asagba SO, Owhe-Ureghe UB, Falodun A, Okoro F(2004). Evaluation of the toxic effect of *Mansoia altisima* extract after short term oral administration of rats. West Afr. J. Pharmacol. Drug Res. 20(1&2): 53-57.

Barry AL, Thornsberry C (1983). Susceptibility Tests: Diffusion procedure J. Clin. Pathol. 19: p. 492.

Collins FM (1996). *Pasteurella* and *Francisella* In: Barron's Medical Microbiology. 4th edition. University of Texas Medical Branch. ISBN 0-9631172-1-1.

Cowan ST (1985). Manual For The Identification Of Medical Bacteria. 3rd Edn. University Press Cambridge, p. 237.

Ejechi BO (1996). Growth inhibition of *Pseudomonas aeruginosa* and *Enterococcus faecelis* by crude extractives of *Mansonia altisima* timer saw dust. Curr. Microbiol J. 32: 297-298

Geelhoed GW, Willix Jr. RD, Jean B (1994). Natural Health Secrets from around the world. Short Tower books Publishers, Florida.

Jones TF (2003). 'From pig to pacifier': chitterling-associated yersiniosis outbreak among black infants. Emerg. Infect. Dis. 9(8): 1007-1009.

Kokworo JD (1976). Medical Plants of East Africa. East African literature Bureau, Nairobi, pp. 47-62.

Lauea LZ (1987). Herbs and Spices. J. Fd. Sci. 68: 102-104.

Nakhaei M, Mahboobeh F, Malekzadeh F, Khaje-Karamoddin M, Ramezani M (2006). *In vitro* Anti *Helicobacter pylori*. Effects of sweet basil (*Occimum basilicum* L.) and purple Basil *(Occimum basilicum Var purpurascens)*. Pak. J. Biol. Sci. 9(15): 2887-2891.

Oboh PA, Agbonlahor DE, Ekundayo AO, Owhe-Ureghe UB (1995). Antibacterial activity of *Citrus aurantifolia* (lime) juice against some Gram positive and Gram negative bacteria. Nig. Ann. Nat. Sci. 3: 1-9.

Olukemi MA, Kandakai-Olukemi YT (2004). Antibacterial activity of the ethanolic extract of Daniella Oliveri Annona senegalensis and Mitragyna stipulosa. Nig. J. Microbiol. 18(1&2): 235-240.

Osuinde ML, Esiovwa HK (1998). Antibacterial activity of *Piper guineense* L. and *Piper umbellatum* L. on some Gram negative bacteria. J. Mod. Lab. Sci. 7: 78-82.

Osuinde ML, Isibor ON (1998). *In vitro* activities of extract *Spondias mombin L. Carica papaya L.* and *Viscum album L*. J. Med. Lab. Sci. 7: 78-82.

Quereshi S, Qadri NM, Mirza M, Armed S, Tariq P, Bukhari AQS (1988). Antimicrobial property of Ral. A herbal remedy for many skin diseases Kar. Univ. J. Sci. 16: 10-107.

Shelef LA, Naglik DA, Dogen DW (1980). Sensitivity of some common food borne bacteria to the spices sage, rosemary and all spices. J. Fd. Sci. 45: 102-1044.

Sofowora A (1993). Medical Plants and Traditional Medicine in Africa. 2nd edn. Spectrum Books Ltd., Ibadan, Nigeria, pp. 9-25.

Soyinka F (1986). Clinical application of plants. In: The State Medicinal Plant Research in Nigeria. Sofowora (ed). Nig. Soc. Pharmacognosis, p. 89-96.