Advanced Journal of Microbiology Research Vol. 2015
Available online at http://internationalscholarsjournals.org/journal/ajmr

Short Communication

Evaluation of antibacterial activity of some medicinal plants on common enteric food-borne pathogens

A. O. Ajayi* and T. A. Akintola

Department of Microbiology, Adekunle Ajasin University, P.M.B. 01, Akungba-Akoko, Ondo State, Nigeria.

Accepted 22 January, 2014

Enterobacteria are often responsible for various gastrointestinal foods borne infection in humans especially in the developing countries. *Cassia occidentalis*, *Croton zambensicus* and *Newbouldia leavis* known as 'Ewe ori esi', Ajekobale and Akoko respectively in Yoruba are three plants whose leaves are used in combination by boiling to treat gastrointestinal and diarrhea illnesses among indigenes of Akungba-Akoko, Ondo State, Nigeria. The leave extracts from these medicinal plants were screened *in vitro* in the laboratory for their antibacterial activity against two prominent enteric bacteria, that is, *Escherichia coli* and *Salmonella typhimurium* using the agar disc diffusion method. The tyndalized leave extract of *C. zambesicus* showing antibacterial inhibition zone of 4 and 2 mm against *S. typhimurium* and *E. coli* respectively exhibited highest activity during the study than the autoclaved samples and other plant sources tested independently or combined, showing that the combinations of the extract samples do not exhibit synergistic effects. *C. zambesicus* can however be optimized clinically for chemotherapeutic control of these food-borne enteric infections.

Key words: Antimicrobial, enterobacteria, evaluation, food -borne, pathogens, plants.

INTRODUCTION

Enteric bacteria are major causes of food-borne illnesses and gastrointestinal problems in the developing countries and human beings around the world. The most common agents are *Escherichia coli* and *Salmonella typhimurium* but *Shigella* species and other species of *Salmonella* also have been implicated in a significant number of cases (Stainer et al., 1987). Symptoms of food borne illnesses range from stomach upset to more serious symptoms including diarrhea, vomiting, abdominal cramps and fever. In some people, especially children, haemolytic uremic syndrome (HUS) can occur from infection by a particular strain of *E. coli* 0157:117 and can lead to kidney failure and death (NIH, 2003).

Vehicle of transmission of these etiologic agents are mainly food and water. Many disease-causing organisms of medical importance have developed resistance to antibiotics. Palombo and Sample (2001) suggests there is a distinct and constant need for safe and more efficient therapeutic agent. A way out of reducing antibiotic resistance and adverse effects on host is the employment of antibiotic resistance inhibitors of plant origin (Nostro et al., 2000; Kim et al., 1995). Chariantly et al., (1999) stated that plant–derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in them as sources of agents to fight microbial diseases. Aburjai et al., (2001) also confirms folkloric accounts in literatures on the use of variety of plant preparations for the treatment of infections.

Clinically, antibiotics produced by soil microorganisms and higher plants have been known sources of antibiotics (Trease et al., 1972) . According to Charles and Simmon (1992), the use of medicinal plants also contributes significantly to primary health care in various parts of the world.

Cassia occidentalis, *Croton zambensicus* and *Newbouldia leavis* known as 'Ewe ori esi', Ajekobale and Akoko respectively in Yoruba are three plants whose leaves are used in combination by boiling to treat gastrointestinal and diarrhea illnesses. Similar crude

*Corresponding author. E-mail: jidet02@yahoo.com.

Table 1. Some biochemical characteristics of enterobacteria used.

Biochemical Test	*Salmonella typhi*	*Escherichia coli*
Cultural characteristics on EMB agar	Dark smooth colony	Green metallic sheen
Gram stain	-	-
Shape	Shortrod	Shortrod
Catalase test	+	+
Citrate utilization	+	-
Lactose fermentation	-	+
Indole production	-	+
Motility	+	+
Presumptive confirmation of test isolates	+	+

extracts of the leaves of these plants were used both independently and in combinations to investigate their *in vitro* antibacterial activity against *E. coli* and *S. typhimurium.*

Enteric bacteria belong to the family enterobacteriaceae, which are large group of Gram negative, peritrichously flagellated or non-flagellated straight rods with simple nutritional requirements. They grow best under aerobic condition but also ferment carbohydrates by an anaerobic pathway (Prescott et al., 2008). Enterobacteria include *coli* forms like *E. coli* and other Gram-negative enteric flora, non-fermenters that are non-lactose fermenting or slow lactose fermenting bacterial which could be either normal flora or regular pathogens also forms part of the enteric organisms (Talaro and Talaro, 1996).

Enteric infections remain a leading cause of childhood mortality in developing countries. It has been reported that enteric pathogens are the most frequent cause of diarrhea illnesses that account for an animal mortality rate of 5 million people worldwide (Bennish, 2004). And prominent pathogenic enterics include *Salmonella, Shigella* and strains of *E. coli* (Talaro and Talaro, 1996).

MATERIALS AND METHODS

Microorganisms used

Pure cultures *Escherichia coli* and Salmonella typhimurium were obtained from the University College Hospital, University of Ibadan and the School of Medical Laboratory Science, Obafemi Awolowo Teaching Hospital, Ile-Ife respectively. The organisms were further identified and maintained in stock culture at 4°C.

Culture media

Tryptone soy agar, Eosin methylene blue (EMB) agar and Nutrient agar were generally used during the study. Tryptone soy agar was used for the antimicrobial tests and EMB cultures distinguish the lactose- fermenting bacteria, which produces dark colonies, or green metallic sheen (as exhibited by most E. coli species), while non-lactose fermenters appeared colourless. Nutrient agar was routinely used for stock cultures and subculturing.

Plant materials screened

The plants used for the study includes *C. occidentalis*, *C. zambesicus* and *N. laevis* wild crafted locally in Akungba-Akoko township, Ondo State Nigeria. The leaves from this plant sources were washed with clean water and 50 grams of the leaves were milled or blended with 500 ml distilled water with the aid of an electric blender.

The blended samples were divided into two portions and sterilized separately. First at 121°C for 15 min and the second portion was tyndalized for 3 days at 70°C for 30 min each day. It was then concentrated at 70°C in the oven after sterilization.

In vitro antimicrobial test

The disc agar diffusion described by Nostro et al. (2000), Xavier et al. (2003) and Ajayi (2006) was used to determine growth inhibition pattern of bacteria by the plant extracts. A loopful of the test organisms from broth cultures was diluted and evenly spread by streaking on the Tryptone Soy Agar plates under aseptic condition.

Paper disc impregnated with the sterilized plant extracts and other different concentrations of extracts were placed with the aid of sterile forceps at equidistant on the plating culture. Discs dipped in sterilized distilled water served as control. The samples were incubated at 37°C for 24 h and examined for zones of growth inhibi - tion and the diameter of these zones were measured in millimeters.

RESULT

The strains of *S. typhi* and *E. coli* used were identified by standard microbiological method as shown in Table 1. The antimicrobial pattern of the plant extracts studied was demonstrated in Table 2, whereby *C. occidentalis* and C. *zambiensis* had higher activity of 3 and 4 mm zone of antimicrobial activity respectively during the study compared with other samples tested with lower values. The highest value of 4 mm zone of antibacterial inhibition demonstrated by *C. zambesicus* outweighs the use of the combined plant extracts of *C. occidentalis, C. zambesicus* and *N. leavis* with 2 mm zone of inhibition.

In Table 3, the result shows that most of the plants extracts were less effective against the strain of *E. coli* tested except, *C. zambesicus* which had the highest antibacterial inhibition zone of 2 mm. The tyndalized

Table 2. Antibacterial activity of plant extracts against *Salmonella typhi*

Plant extracts	Zones of inhibition (*S. typhi*)			
	Autoclaved		Tyndalized	
	S.D.T	D.D.T	S.D.T	D.D.T
Cassia occidentalis	++	+++	-	+
Croton zambensicus	+	+++	++	++++
Newbouldia leavis	+	++	+	++
C.C.N	++	++	++	++
Distilled water	-	-	-	-

Legend: +: 1, ++: 2, +++: 3, ++++: 4 mm
S.D.T: Single disc test D.D.T: Double disc test Extract – C.C.N is the combination of *C. occidentalis, C. zambensicus N. leavis*

Table 3. Antibacterial activity of plant extracts against *E. coli*

Plant extracts	Zones of inhibition (*E. coli*)			
	Autoclaved		Tyndalized	
	S.D.T	D.D.T	S.D.T	D.D.T
Cassia occidentalis	-	++	-	-
Croton zambensicus	++*	++	++*	++
Newbouldia leavis	-	-	-	-
C.C.N	-	-	++*	++
Distilled water	-	-	-	-

Legend: +: 1, ++: 2, +++: 3, ++++: 4 mm
*: Faint zone of inhibition, S.D.T: Single disc test D.D.T: Double disc test. Extract – C.C.N is the combination of *C. occidentalis, C. zambensicus N. leavis*

combined extract similarly showed the same effect under this context.

DISCUSSIONS

C. occidentalis and *C. zambensicus* exhibited active anti-microbial activity against the enterobacteria tested that is, *S. typhi* and *E. coli* than N. leavis and the combination of the three plant sources. However the *C. zambensicus* was most active when tyndalized before use showing the 4 and 2 mm zone of inhibition against *S. typhi* and *E. coli* respectively, which was the highest during the study. Ashbir and Ashenati (1999) demonstrated that excessive heating like that experienced during sterilization at 121°C for 15 min during this study could affect some thermo-labile biological active substances in plants. Based on this and result obtained in this study, the tyndalized plant extracts could be much more relevant for use as antimicrobial agent to maintain the plant ingredients activity.

Synergistic effect experienced in combination of some plant sources for use as antimicrobial agent described by Farnsworths et al. (1966), does not work out effectively in this study because the combined extract showed 2 mm zone of microbial inhibition which is lower value compared with that from *C. zambensicus* alone (Table 2). Thus, the use of this single plant source is recommended for use against the combined usage practiced among the indigenes of Akungba-Akoko. The trial of the double disc test gives much more distinct result for the test.

Future research on this study can however concentrate on optimizing clinically the chemotherapeutic processes of *C. zambensicus* for treatment and control of these common food-borne enteric infections like typhoid in Akungba-Akoko and other parts of the country where the plants can be easily cultivated or otherwise commercialized for pharmaceutical purpose.

REFERENCES

Ajayi AO (2005). Larvicidal and antimicrobial activity of some medicinal plants commonly grown in Nigeria. J. Res. Biosci. 2(1): 72-76.

Ashebir M, Ashenafi (1999). Assessment of the antibacterial activity of some traditional medicinal plants on some food-borne pathogens. Ethiop. J. Health Dev. 13(3): 211-216

Banish M (2004). Micronutrients and enteric infections in African Children. Int. Cent. For Trop. Dis. Res. pp 3- 9

Chariantly CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BP (1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. J. Ethnopharmacol. 64: 265-270

Charles DJ, immon JE (1992). A new geranoil chemotype of *Ocimum gratissimum* . J. Essential Oil Res. 4(3): 231-234

Farnsworths NR, Henry IK, Svogoda GH, Blomster RN, Yates MJ, Eular KL (1966). Biological and phytochemical evaluation of plants.1. Biological Test procedures and Results form Two Hundred Accessions. Lloydia. 25: 101-122

Kim H, Park SW, Park JH, Moon KH, Lee CV (1995). Screening and isolation of antibiotic resistance inhibitions from herb materials 1. Resistance inhibition of 21 Korean plants. Nat. Prod. Sci. 1: 50-54

NIH (2003). Bacteria and foodborne illness. NIH publication No. 04-4730. National Institute of Health, U.S.A. pp. 1-5.

Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA (2000). Extraction methods and bioauthography for evaluation of medicinal plant antimicrobial activity. Letters in Appl. Microbiol. 30 (5): 379-385

Palombo EA, Semple SJ (2001). Antibacterial activity of traditional Australian medicinal plants. J. Ethnopharm. 77: 151-157

Prescott ML, Harley PJ, Klein AD (2008). Microbiology. (7th Edition). Wm. C. Brown Publishers. U.S.A. pp 557-561.

Stainer RY, Ingraham JL, Wheelis ML, Painter PR (1987). General Microbiology. 5th ed. Macmillan Press. pp 21-40.

Talaro K, Talaro A (1996). Foundation in Microbiology. 2nd ed. Wm. C.

Trease G, Evans W (1972). Pharmacognosy, University Press, Aberdeen, Great Britain. p. 161-163.

Xavier TF, Senthilkuma S, Okoh S, Sebastinraj J (2003). Antibacterial activity of Malaxis Rheedii Sw. an ornamental orchid of shvaroy region of Eastern Ghat, S. India. Sci. Focus. 5: 82-88.