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Antidiarrhoeal Evaluation of Some Nigerian Medicinal Plants Used in Bini Traditional Folk Medicine

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Abstract. Four medicinal plants namely; *Vernonia amygdalina, Psidium guajava, Chromolaena odorata* and *Anarcadium occidentale*, commonly used for the treatment of diarrhoea in Bini traditional folk medicine in Nigeria were tested against *Escherichia coli, Staphylococcus aureus* and *Klebsiella aerogenes*. The leaf extracts of *P. guajava* and *A. occidentale* completely inhibited the growth of all the organisms tested, while *V. amygdalina* inhibited the growth of *K. aerogenes* only. Metronidazole was used as the standard antidiarrhoeal drug. Glycosides were found in all the plant extracts. This study, favours the use of the leaf extracts of *A. occidentale, P. guajava* and *V. amygdalina* for the treatment of diarrhoea in Nigeria.

Keywords: antidiarrhoeal drugs, medicinal plants, Nigeria, Bini folk medicine

Introduction

Nowadays, the value of medicinal herbs in combating diseases is being rediscovered and the herbal medicine trade has become a booming business worldwide. In India, for example, there are 46,000 licensed pharmacies manufacturing traditional remedies, 80% of which come from plants (Alok, 1991). In Africa, many plant species are reported to have medicinal value (George and Pamplona-Roger, 1997). These plant species are used for simple or complex pathological complications to psychological and mental illnesses.

Gastrointestinal disorders are one of the major health problems in developing countries and generally, plants are used in the treatment of diseases, indigenously. In sub-Saharan Africa in 1999, there were 8, 181 deaths per day due to diarrhoea and traditional medicines were often the only affordable and accessible form of healthcare for the majority of this rural African population (Obuekwe and Obuekwe, 2002). Frequently, tannin-containing plants are used to treat diarrhoea. The bark and leaves of cashew (*Anarcadium occidentale*) are a rich source of tannins–a group pf phytochemicals with documented biological activities. Cashew fruit has exhibited antibacterial activity against the Gram- negative bacterium *Helicobacter pylori*, now considered to be the cause of acute gastritis and stomach ulcers (Heirrich *et al.*, 1992). Its effectiveness against leishmanial ulcers has also been documented in the same report. An infusion of the bark and leaves is an astringent and a mouthwash, used in toothache and is given internally in dysentery (Database entry for Cajueiro -*A. occidentale*). The natural rainforest remedy for diarrhoea is standard decoction of leaves and twigs of *A. occidentale* (Indian Mecicinal Plants- *A. occidentale,* 2001). The antiulcerogenic effect acute, subacute toxicity as well as the genotoxic effect of a hydro-ethanolic extract of the cashew (*A. occidentale* L.) leaves have also been investigated (Konan and Bacchi, 2007; Konan *et al.*, 2007).

Psidium guajava (guava) is a common shade tree or shrub in the tropics. The Tikuna Indians decoct the leaves as a cure for diarrhoea (People and Plants online - working paper 1: African Medicinal Plants). Much of its therapeutic activity is attributed to the flavonoids, which have demonstrated some antibacterial activity. Quercetin is thought to contribute to the antidiarrhoeal effect of guava. It is able to relax intestinal smooth muscles and inhibit bowel contractions. The effective use of guava in diarrhoea, dysentery and gastroenteritis can also be related to guava's documented antibacterial properties. Bark and leaf extracts of the tree have exhibited *in-vitro* toxic action against numerous bacteria (Theunissen, 2002).

A range of medicinal plants with antidiarrhoeal properties have been widely used; but the effectiveness of many of the antidiarrhoeal traditional medicines have not been scientifically evaluated. This study investigates the potential antidiarrhoeal properties of the leaf extracts of four Nigerian medicinal plants *P. guajava, A. occidentale, V. amygdalina* and *C. odorata* against *E. coli*, *S. aureus* and *K. aerogenes*.

Fresh leaves of the plants. *P. guajava, A. occidentale, V. amygdalina* and *C. odorata* were collected locally from Benin City, Nigeria.

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Preparation of extracts for microbiological analysis. Decoctions of the fresh leaves of the plants were made by pounding 1 g of the leaf using sterile mortar and pestle and extracting in 200 ml distilled water to get 5 mg/ml concentration. One ml of each decoction was pipetted and used for *in vitro* agar diffusion assay. Fresh leaves of the plants were also dried in an oven at a temperature of 40 °C for two days. The leaves were ground into fine powder and 150 g of the powdered leaves of the plants were extracted with 2.5 l of ethanol using Soxhlet extractor and concentrated using rotary evaporator.

Cultures of *E. coli, S. aureus* and *K. aerogenes* were obtained as clinical isolates from a reference hospital in Benin City, Nigeria. Overnight cultures of the organisms were obtained by sub-culturing into test tubes containing 10 ml of sterile nutrient broth (Oxoid). Viable counts of the organisms using the standard plate method were 2.4×10^3, 2.16×10^4 and 2.8×10^3 for *E. coli, S. aureus* and *K. aerogenes*, respectively. Using agar diffusion method, 100, 150, 200 and 250 mg/ml concentrations of the plant extracts were pipetted onto plates containing 1 ml of overnight cultures of the test organisms. Overnight cultures were used to ensure that the organisms were at their maximum growth. The strains were maintained on nutrient agar slants and stored in the refrigerator and transferred to fresh slants every two weeks. Cultures were usually transferred to fresh medium before use.

Results were recorded after 24, 48 and 72 h. Metronidazole was used as the standard drug in concentrations 100, 150, 200 and 250 mg/ml. Triplicate tests were made and mean was calculated. The minimum inhibitory concentrations (MICs) for various extracts active against the test organisms were determined using the double strength dilution method.

Phytochemical tests. Phytochemical analyses of the plant extracts were assayed to determine the active chemical compounds. For glycosides, 0.1 g of powdered dried leaves of *A. occidentale* was added to 20 ml distilled water in a beaker. This was boiled gently for 2-3 min, filtered hot and cooled. One ml of the filtrate was tested with Fehling's solutions A and B and the result noted. Five ml of dil. sulphuric acid was added to the remainder of the filtrate and boiled for 3-5 min and later filtered. Filtrate was made slightly alkaline with sodium bicarbonate. Fehling's solutions A and B were added and the mixture boiled. A change in colour from deep blue to green, yellow or red indicated a positive reduction test (red precipitate of cuprous oxide). This test was repeated for *V. amygdalina, P. guajava* and *C. odorata.*

For saponins, 0.1 g of powdered leaves of *A. occidentale* was added to 20 ml distilled water in a beaker and boiled gently for 3 min, filtered hot and cooled. Five ml of the filtrate was shaken vigorously with water and the result was noted. Five ml of dil. sulphuric acid was added to the remainder of the filtrate and boiled gently for 3-5 min, cooled and filtered. Residue was washed into a test tube with 2 ml distilled water, shaken vigorously and the result was recorded. Filtrate was made alkaline with sodium bicarbonate, Fehling's solution was added and boiled. Result was also noted. The test was also repeated for *V. amygdalina, P. guajava* and *C. odorata.*

For tannins, 2 g of powdered leaves of *A. occidentale* was added to 50 ml of distilled water in a beaker, boiled for 5 min, filtered and made up to 50 ml volume. Two drops of test solution and one drop of aqueous ferric chloride were then added to 10 ml distilled water. To 5 ml of test solution, 5 ml of 0.25% ferric ammonium citrate solution and 0.5 g sodium acetate were added, boiled and cooled. A purple/blackish bulky precipitate, insoluble in hot water or blue solution indicated the presence of pseudo tannins. Five ml of the precipitate was further diluted with 10 ml of distilled water and 10 ml of 10% aqueous lead acetate solution was added. Appearance of a precipitate indicated the presence of tannins. These tests were repeated for the other plant materials to be tested.

For flavonoids, 1 g of the powdered leaves of *A. occidentale* was extracted with water by heating on a water bath, cooled and filtered. To 5 ml of the filtrate, 1 ml of sodium hydroxide was added. A yellow solution, which became colourless on addition of concentrated hydrogen chloride, indicated the presence of flavonoids. This was repeated for other plant materials to be tested.

Results and Discussion

Antidiarrhoeal screening of the aqueous extracts of the fresh leaves of four plants showed no activity. After 24 h contact time, 100, 150, 200 and 250 mg/ml of the ethanolic leaf extracts of *A. occidentale, P. guajava* and *V. amygdalina* exhibited activity against the three test organisms; namely, *S. aureus, E. coli* and *K. aerogenes.* The ethanolic leaf extract of *C. odorata* at the same concentrations was active only aginst *K. aerogenes* after 24 h (Table 1).

After 48 h contact time, the ethanolic leaf extracts of only *A. occidentale* and *P. guajava* had activities at the same concentrations as in 24 h contact time. *V. amygdalina* was active at 200 mg/ml concentration, against *S. aureus* only and after 48 h at 250 mg/ml concentration against the 3 test organisms. Further more, after 72 h contact time, the ethanolic leaf extracts of *A. occidentale* and *P. guajava* retained their activities against the 3 test organisms at the same concentrations as after 24 and 48 h contact time. However, with

Table 1. Antibacterial screening of the leaf extracts of four Nigerian medicinal plants

Concentration of ethanolic leaf extracts (mg/ml)	Growth of test organisms								
	K. aerogenes			*E. coli*			*S. aureus*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Anarcadium occidentale									
100	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-	-
Psidium guajava									
100	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-	-
Vernonia amygdalina									
100	-	+	+	-	+	+	-	+	+
150	-	+	+	-	+	+	-	+	+
200	-	+	+	-	+	+	-	+	+
250	-	-	-	-	-	+	-	-	+
Chromolaena odorata									
100	no activity at all concentrations tested			no activity at all concentrations tested			no activity at all concentrations tested		
150									
200									
250									
Metronidazole (control)									
100	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-	-

- = no growth (total inhibition of test organism); + = growth (no inhibition)

V. amygdalina, the activity was recorded against only *K. aerogenes* after 72 h contact time and none against *E. coli* and *S. aureus* (Table 1).

Phytochemical screening of the leaf extracts revealed the presence of glycosides and tannins in *A. occidentale*; glycosides, saponins, tannins and flavonoids in *P. guajava*; glycosides, tannins and saponins in *V. amygdalina* and glycosides, tannins and flavonoids in *C. odorata* (Table 2).

The minimum inhibitory concentrations (MICs) of the leaf extracts of *A. occidentale* having antidiarrhoeal activities were 40.02 mg/ml; 22.84 mg/ml and 46.69 mg/ml, those of *P. guajava* were 33.35 mg/ml, 39.97 mg/ml and 53.36 mg/ml, against *K. aerogenes*, *E. coli* and *S. aureus*, respectively, whereas, leaf extract of *V. amygdalina* was active against only *K. areogenes*, in the concentration of 66.67 mg/ml.

Metronidazole was used as the standard antidiarrhoeal drug with MIC of 0.67 mg/ml; 0.571 mg/ml and 0.67 mg/ml for *K. aerogenes*, *E. coli* and *S. aureus*, respectively (Table 3).

Table 2. Phytochemical analysis of the ethanolic extract of four medicinal plants

Plant	Compounds found
Psidium guajava	glycosides, saponins, tannins and flavonoids
Anarcadium occidentale	glycosides and tannins
Vernonia amygdalina	glycosides, tannins and saponins
Chromolaena odorata	glycosides, tannins and flavonoids

The results of this study show that the leaf extract of *A. occidentale* (cashew) possesses antidiarhoeal activity. This correlates with works of other investigators (Database entry for Cajueiro - *Anarcadium occidentale*; Indian Medicinal Plants, *Anarcadium occidentale*, 2001; Konan and Bacchi, 2007; Akinpelu, 2001). Today in Peruvian herbal medicine, cashew leaf tea called 'casho' is employed as a common diarrhoeal remedy. Methanolic bark extract of *A. occidentale* has also been found to exhibit *in vitro* antimicrobial activity

Table 3. Minimum inhibitory concentrations (MICs) of the plant extracts having antidiarrhoeal activities

Plant extracts (mg/ml)	*K. aerogenes*	*E. coli*	*S. aureus*
Anarcadium occidentale	40.02	22.84	46.69
Psidium guajava	33.35	39.97	53.36
Vernonia amygdalina	66.67	-	-
Metrinadizole (standard drug used as control)	0.67	0.571	0.67

against 13 of 15 microorganisms tested at a concentration of 20 mg/ml (Akinpelu, 2001). An essential oil obtained by steam distillation of the leaves of *A. occidentale* acted as a tranquilizer and analgesic and had mild antibacterial and antifungal activities (Indian Medicinal Plants: *Anarcadium occidentale*, 2001).

Psidium guajava also possesses antidiarrhoeal activities. Its bark and leaf extracts have been found to possess *in vitro* toxic action against numerous bacteria (People and Plants online-working paper1: African Medicinal Plants). In several studies, guava had shown significant antibacterial activity against such common diarrhoea-causing bacteria as *Staphylococcus*, *Shigella*, *Bacillus*, *Salmonella*, *Escherichia coli*, *Clostridium* and *Pseudomona*s (Theunissen, 2002). The effectiveness of *P. guajava* as an antidiarrhoeal agent has been confirmed; the methanolic extract is the only agent showing significant inhibitory activity against the growth of *Salmonella* and *Shigella* species and enteropathogenic *E. coli* (Caceres *et al*., 1993).

In this study, *V. amygdalina* showed antidiarrhoeal activity on *K. aerogenes* at 250 mg/ml after 72 h contact time and not on the other test organisms even though antidiarrhoeal activity of the plant has been indicated in the reports of other investigators (Akinpelu, 1999; Obaseiki-Ebor *et al*., 1993). Phytochemical analyses of the leaf extracts revealed the presence of glycosides, tannins, saponins and flavonoids. Medicinal plants had earlier been screened for groups of phytochemical compounds with antibacterial and antiamoebic activities and were found to contain tannins, alkaloids, saponins, flavonoids, steroids and/or triterpenes and reducing sugars (Otshudi *et al*., 2000).

The search for new active chemical compounds in high biological diversity regions has become a challenge to modern pharmaceutical industries. The history of drug discovery implies that the ethnobotanical approach is the most productive of plant surveying methods (Cox and Balick, 1994). The antiulcerogenic effect and acute toxicity of hydroethanolic extract from the cashew (*A. occidentale* L.) leaves have been investigated by Konan and Bacchi (2007) and the extract was found to inhibit gastric lesions induced by HCl/ethanol in female rats and its a methanolic fraction (257.12 mg/kg) reduced gastric lesions by 88.20%. Thus the plant is likely to contain the active principle with antiulcer effect.

In conclusion, the ethanolic extracts of the medicinal plants investigated in this study were found to possess good antidiarrhoeal potential and supported the claims made in Bini folklore medicine. The study suggests that the use of an effective and economical herbal formulation based on such extracts could be beneficial against certain bacterial infections.

Further studies on these plants are required to identify and isolate the specific glycosides that are responsible for the antidiarrhoeal activities with a view to producing new antidiarrhoeal drugs from them.

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