

Full Length Research Paper

Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull.Arg., *Boerhavia diffusa* (L) and *Bridellia micranthal* (Hoscht) Baill. used in traditional medicine in Nigeria on *Helicobacter pylori* and four diarrhoeagenic bacterial pathogens

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Extracts of leaves of *Alchornea cordifolia*, *Boerhavia diffusa* and *Bridellia micrantha* were investigated for antibacterial activity against *Helicobacter pylori*, *Salmonella typhi*, *Salmonella enteritidis*, *Shigella flexneri* and Enterohemorrhagic *Escherichia coli* (EHEC). Results showed that the ethanolic extracts of the three plants and aqueous extracts inhibited the growth of all the organisms tested. However, the minimal inhibitory concentration (MIC) ranged between 15.6 and 31.25 mg/ml while the extracts were bacteriocidal at concentration ranging between 31.25 and 250 mg/ml. This indicates that leaf extracts of the three plants are of great potential in treating gastric ulcer and diarrhoea caused by the aforementioned bacteria.

Key words: *Alchornea cordifolia*, *Boerhavia diffusa*, *Bridellia micrantha*, gastric ulcer, diarrhoea.

INTRODUCTION

The pathogenic involvement of *Helicobacter pylori* (Hp) in ulcer diseases was recognized not too long ago (Sunnenberg, 1995; Suzuki and Ishii, 1996). The organism is associated with antral gastritis, duodenal (peptic) ulcer disease (PUD), gastric ulcers and gastric carcinoma (Brooks et al., 2001). According to Nester et al. (2004) the organism survives the acidity of stomach juices by producing a powerful urease. Upon reaching the layer of the mucus, it penetrates the epithelial surface and its toxins incite an inflammatory response. Although about 20% of the infected individuals develop ulcerations, more than 90% of individual with stomach cancers are infected with *H. pylori*. Similarly, diarrhoeagenic organisms including *Salmonella typhi*, *Salmonella enteritidis*,

Shigella flexneri and enterohemorrhagic *Escherichia coli* are of extreme public health importance. *S. typhi* is found only in humans and infection implies contact with an infected person or consumption of contaminated food or water. Typhoid fever remains a global problem with about 16 million cases occurring worldwide annually, resulting in about 600,000 deaths (CIDRAP, 2006). *S. typhi* and *S. enteritidis* are endemic in developing countries including Nigeria. Infectious enteric diseases including acute diarrhoea caused by *Shigella* infection is also rampant in Nigeria and other developing countries where sanitary practices are lacking (Adeleye and Adetosoye, 1993). Similarly, enterohemorrhagic *E. coli* (EHEC) often produce severe illness including bloody diarrhoea (Nester et al., 2004). The organism produces shiga-like toxins that cause the death of intestinal epithelium by interfering with protein synthesis but does not penetrate intestinal epithelial cells.

Due to the persistence of the above bacterial pathogens

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in this environment, successful therapeutic intervention is of paramount importance. Since the available drugs are expensive and have side effects in some cases, herbal preparations used in traditional healthcare systems are popular remedies that are being tried as alternatives. Leaves of *Alchornea cordifolia* are widely used in African traditional medicine. Its uses, including antibacterial effects have been extensively reviewed by Mange et al. (2004). Similarly, *Boerhavia diffusa* extracts have been reported to have the following medically beneficial effects; antifungal (Agrawal et al., 2003), antidiabetic (Pari and Satheesh, 2004), antioxidant (Satheesh and Pari, 2004) and immunomodulatory (Mehrotra et al., 2002). Its antimicrobial potential was also investigated by Abo and Ashidi (1999). The least investigated is *Bridelia micrantha*. Lin et al. (2001) evaluated the bark of the later for anti-diarrhoeal properties.

In all, there had been no previous investigations on the antibacterial activities of extracts of these three plants on *Helicobacter pylori* and a few information exists relating to their antimicrobial effect on diarrhoeagenic bacteria hence the present work was carried out to determine whether these plants can be used to treat ailments caused by these bacterial pathogens.

MATERIALS AND METHODS

Source and collection of leave samples

Leaves samples of the three plants namely: *Alchornea cordifolia* (Ipa), *Boerhavia diffusa* (Ida Odan) and *Bridelia micrantha* (etiponla) were collected from a farm settlement in Sango Ota, Ogun State. *Alchornea cordifolia* leaves were also collected within the University of Lagos environment. All the samples were authenticated by Dr. A. Kadiri of Department of Botany and Microbiology, Faculty of Science, University of Lagos.

Preparation of extracts

The leaves samples were dried at room temperature for 10 days. The completely dried leaves were crushed to coarse powder by grinding. The powder form of each of the leaves was further treated to extract the active ingredients.

Water extract

This was carried out by soaking the powdered leaves in 300 ml of water for 5 days. The solution (powdered leaves material and water) was filtered using Whatman no 1 filter papers. The filtrate of each sample was poured into a beaker and labeled appropriately. The water filtrate were then freeze dried.

Ethanol extract

A modification of methanol extraction procedure of Boakye et al. (1977) was adapted. The procedure was similar to the water extraction procedure described above. Powdered leaves material of each of the samples was soaked in 300 ml ethanol for 5 days. The solution (powdered leaves material and water) was filtered using Whatman no 1 filter papers. The filtrate of each sample was pour-

ed into a beaker and labeled appropriately. The ethanol filtrates were placed in a vacuum oven at 40°C (to retain natural state of extracts) and dried for 1 week to evaporate the alcohol. Dried solid extracts were obtained for the three samples.

Phytochemical analysis

Phytochemical tests for tannins, alkaloids, anthraquinone and saponins were carried out using the method described by Akinyemi et al. (2005).

Sources of microorganisms

The test organisms employed for screening antimicrobial activities of the extracts were *H. pylori*, *S. typhi*, *S. enteritidis*, *Shig. flexneri* and enterohemorrhagic *E. coli* (EHEC). All organisms were obtained from Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. *H. pylori* was cultivated on Helicobacter medium containing Brain Heart infusion agar supplemented with laked horse blood and incubated at 37°C microaerophilically for 48 h. Other organisms were sub-cultured into fresh nutrient agar plates 24 h before use.

Antimicrobial assay

Colonies of 3 old days culture of *H. pylori* on Helicobacter medium was suspended in sterile normal saline and the density adjusted to equal MacFarland standard. This was carefully swabbed on fresh *H. pylori* medium (Brain Heart infusion agar supplemented with 10% laked horse blood) in triplicates and allowed to dry before use. Thereafter the water extracts were reconstituted with distilled water while the ethanol extracts were reconstituted with both water and ethanol in ratio 7:3. Holes were perforated with sterile cork borer on the *Helicobacter pylori* plates and 20 μ l of the reconstituted extracts were applied into each hole. Sterile distilled water was used as control. All plates were incubated microaerophilically (using CO₂ incubator) at 37°C for 3 days.

Similar procedure was employed for *S. typhi*, *S. enteritidis*, *Shig. flexneri*, and enterohemorrhagic *E. coli*. But the organisms were plated on Mueller-Hinton agar and incubated for 37°C for 24 h. Zones of inhibition were measured at the end of incubation period in millimeter (mm).

Minimal inhibitory concentration (MIC)

The MIC of the extract was determined for each of the test organisms using the dilution susceptibility tests. 0.5 MacFarland standard of the *H. pylori* was inoculated into fresh *H. pylori* medium as previously described. Thereafter, serial dilutions of the extract were carried out to give the following extract concentration: 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128 respectively. Using the sterile cork borer, holes were perforated on the plates and filled with 20 μ l of each dilution of the extract. All plates were incubated as previously described.

Similar procedure was employed for *S. typhi*, *S. enteritidis*, *Shig. flexneri*, and enterohemorrhagic *E. coli* using Mueller-Hinton agar as the test medium. Incubation was carried out at 37°C for 24 h. Zones of inhibition were measured in millimeters (mm).

Minimal bacteriocidal concentration (MBC)

The lowest extract concentration (above) at which the organism did not recover and grow when transferred into a fresh medium was taken as the MBC.

Table 1. Phytochemical properties of the medicinal plants, *Alchornea cordifolia*, *Boerhavia diffusa* and *Bridellia micrantha*.

Plant	Tannin	Alkaloid	Anthraquinone	Saponin
<i>Alchornea cordifolia</i>	+	+	+	+
<i>Bridellia micrantha</i>	+	-	-	+
<i>Boerhavia diffusa</i>	+	+	-	-

Table 2. Antimicrobial activity (zones of inhibition) of leaf extract of the medicinal plants, *Alchornea cordifolia*, *Boerhavia diffusa* and *Bridellia micrantha*.

Test Bacteria	A (mm)	B (mm)	C (mm)	D (mm)	E (mm)	F (mm)
<i>H. pylori</i>	22	23	0	25	0	28
<i>S. typhi</i>	20	15	0	17	0	13
<i>S. enteritidis</i>	15	17	0	21	0	17
<i>Shig. flexneri</i>	22	22	0	28	0	24
<i>E. coli</i>	0	20	0	25	0	20
Control	0	0	0	0	0	0

A = Water extract of *A. cordifolia*; B = ethanolic extract of *A. cordifolia*; C = water extract of *B. micrantha*; D = ethanolic extract of *B. micrantha*; E = water extract of *B. diffusa*; and F = ethanolic extract of *B. diffusa*.

Table 3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) (mg/ml) of extracts of *Alchornea cordifolia*, *Boerhavia diffusa* and *Bridellia micrantha* on test organisms.

Extract	<i>H. pylori</i>		<i>S. typhi</i>		<i>S. enteritidis</i>		<i>Shig. flexneri</i>		EH <i>E. coli</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A	150	300	150	300	-	-	18.75	150	-	-
B	47.5	95	23.75	95.0	95	190	11.875	23.75	23.75	95
D	40.0	8.0	5.0	10.0	20	40	2.5	5.0	20.0	40.0
F	31.25	62.5	15.625	31.25	62.5	125	7.8125	15.625	31.25	125

A = Water extract of *A. cordifolia*; B = ethanolic extract of *A. cordifolia*; D = ethanolic extract of *B. micrantha*; and F = ethanolic extract of *B. diffusa*.

RESULTS AND DISCUSSION

The results of the phytochemical analysis of the plant extracts are given in Table 1. Extracts of *A. cordifolia* had all the bioactive agents present, that is, tannins, alkaloids, anthraquinone and saponin. Extracts of *B. micrantha* had tannins and saponins present while extracts of *B. diffusa* had tannins and alkaloids present. The result of the antimicrobial assay of the three leaves extracts against *H. pylori*, *S. typhi*, *Shig. flexneri*, *S. enteritidis* and EHEC is shown in Table 2. Both water and ethanolic extract of *A. cordifolia* showed activity against *H. pylori* and the other bacteria screened, whereas activity was recorded in the ethanolic extract of *B. micrantha* and *B. diffusa*, but not in their water extracts. A summary of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the extract is shown in Table 3. Water extract of *A. cordifolia* was found to be both bacteriostatic and bacteriocidal for *H. pylori* at concentrations of 150 and 300 mg/ml respectively whereas other

extracts showed similar effects at higher concentration. Similar trend was observed for all other bacteria screened.

A. cordifolia was found to contain all the bioactive compounds screened namely, tannins, alkaloids, anthraquinone and saponin. There have been several reports on the natural occurring plant chemicals found in these plants. These include steroids, sap phenols, flavonols, flavones, tannins, xanthenes and alkaloids (Ogunlana and Ramstad, 1975). The phenolic acids are gallic acid, ellagic acid, protocatechic acid (Lamikanra et al., 1990; Banzouzi et al., 2002) while the flavonoids include quercetin, hyperin and guaijaverin (Ogunbamila and Samulesson, 1990; Ajali, 2000). Similarly Ferreres et al. (2005) identified 10 phenolic compounds in leaf and root samples of *B. diffusa*. These include rhamnoside, quercetin, robinobioide etc. This current study also revealed that *B. micrantha* leaves contain tannins and saponins. This is in agreement with previous works.

All three plant extracts exhibited varying degrees of

activity against *H. pylori*, *S. typhi*, *Shig. flexneri*, *S. enteritidis* and EHEC. However *A. cordifolia* was found to be most active. The leaf ethanolic extracts was active against all the bacterial isolates and the water extract showed similar activity with the exception of EHEC. Similarly the ethanolic extracts of *B. diffusa* and *B. micrantha* showed activity on all the organisms while the water extracts showed no activity at all probably because the active ingredients are less soluble in water. The antibacterial activity of properties of *A. cordifolia* has been extensively studied. Igbenegu et al. (2007) reported that it was active against multiresistant *S. aureus*. Earlier Okeke et al. (1999) had shown that it was very active against seventy four bacterial strains studied *in vitro*. Other workers (Ogunlana and Rainstad, 1975; Kambu, 1990; Ebi, 2000; Ajali, 2000) had made similar observations. This current finding is in line with the earlier reports.

The MIC against *H. pylori* was found to be in the range of 4.7 - 15 mg/ml while the MBC was in the range of 9.5 – 30 mg/ml in our study. This figure compare favourably with the findings of Pesewu et al. (2007). It must be noted that this plant is adjudged to be anti-inflammatory (Manga et al., 2004) is also used to cure arthritis, rheumatism, fungal infections, infertility and many other ailments (<http://www.rain-tree.com>).

On the contrary, fewer studies have been carried out on *B. diffusa* while reports on the medicinal value of *B. micrantha* are even fewer. Our study however revealed that ethanolic extracts of the leaves of both plants inhibited *H. pylori* and other diarrhoeagenic organisms studied. Lin in et al. (2001) had reported that methanolic extracts of *B. micrantha* (Bark) demonstrated weak inhibitory activities against *Shig. flexneri* and *S. plesiomonas*, and reduces diarrhoeic episodes in experimental models of diarrhoeic rats. Also Abo and Ashidi (1999) had reported significant inhibitory activity of *B. diffusa* against pathogenic bacterial strains studied in Ibadan, Nigeria.

Based on our findings the ethanolic extracts of leaves of *A. cordifolia*, *B. diffusa* and *B. micrantha* can be useful in the treatment of duodenal ulcers caused by *H. pylori* as well as diarrhoeal infections caused by *S. typhi*, *Shig. flexneri*, *S. enteritidis* and enterohaemorrhagic *E. coli*. Similarly the aqueous extracts of *A. cordifolia* could be effective in treating most of the above health conditions.

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