Evaluation of the Antibacterial and Antidiarrhoeal Activities of *Heeria Insignis* O. Ktze

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***Heeria insignis* O. Ktze (Anacardiaceae) is an indigenous African shrub used in treatment of diarrhea, venereal diseases, tapeworm, hookworm, schistosomiasis, kidney trouble and for increasing lactation in women after childbirth. The methanol and dichloromethane extracts of the leaves were evaluated for antibacterial activity (using agar-diffusion method) and antidairrheal activity (using isolated rabbit jejunum and castor-oil induced diarrhea in mice). The methanol extract gave higher antibacterial activity than dichloromethane. The order of susceptibility of test**

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microorganisms to methanol extract were *Salmonella typhi*>*Pseudomous aeruginosa*> *Staphylococcus aureus*>*Bacillus subtilis*>*Escherichia coli* which were comparable to standard. The minimum inhibitory concentration of the methanol extract for these microorganisms was also determined. The minimum inhibitory concentration (mg/ml) of methanol extract against microorganisms is; *B. subtilis* (3.9), *S. aureus* (1.95), *E. coli* (62.5), *Ps. aeruginosa* (3.9) and *S. typhi* (1.95). On the isolated rabbit jejunum evaluation, both extracts produced concentration-dependent relation of isolated rabbit jejunum that was not blocked by phentolamine, suggesting that extracts act via mechanisms other than alpha-adrenergic receptor. In the castor oil-induced diarrheoeal test, each extract gave 80% protection at 200 mg/kg, which is comparable to loperamide 2 mg/kg with 80% protection. This finding may explain the use of the plant in diarrhea and bacterial diseases.

Key words: Antibacterial, antidairrhoea, dichloromethane extract, *Heeria insignis*, methanol extract

Diarrhea is a major health worry and sources of malnutrition in developing countries[1]. It is an impaired absorption and hypersecretion syndrome of the gastrointestinal tract[2]. Diarrhea may be brought about by viruses, bacteria, fungi, protozoa, drugs and bacteria endotoxins[3]. In Nigeria, diarrhea remains the major killer disease among children under 5 years, while 7-12 months-old-babies remain the most susceptible[4]. It is estimated that diarrhea causes 4-5 million deaths annually throughout the world. Eighty percent of these deaths are reported in developing countries including Nigeria. To combat the problem of diarrhea in developing countries, the World health organization (WHO) has constituted a diarrhea disease control program aimed at the holistic approach to include all aspects of traditional medical practices, evaluation of health education and preventive approaches[5,6].

In this investigation, *Heeria insignis* O. Ktze (Anacardiaceae) is evaluated for its traditional usage in bacterial and diarrhea infections. It is an indigenous African shrub found extensively in the southern savanna from .Senegal to Niger and Nigeria[7]. This specie has a wide range of healing properties namely: in the treatment of diarrhea and veneral diseases, tapeworm and hookworm, schistosomiasis, kidney diseases and for increasing lactation in women after childbirth[8]. It is known among the Hausas (majority tribe in northern Nigeria) as *Kykenleshi daji*.

The leaves of *Heeria insignis* were collected in Zaria, in December, 2006. This was identified by taxonomical means and authenticated at the herbarium unit of the Department of Biological Science, Ahmadu Bello University Zaria, Nigeria where a voucher specimen number 0015 is available for reference. The air-dried and grounded leaves of the plant were successively extracted first with dichloromethane and latter with 70% methanol at room temperature. The filtrate from the maceration was concentrated and evaporated under normal conditions[9], to give the dichloromethane (yield of 4.6% w/w) and methanol (yield of 7.8% w/w) extract, respectively.

New Zealand rabbits weighing 2.0 kg and Swiss albino mice weighing 18-25 g maintained in the Animal House of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria were used for the experiments. The animals were kept in a well-ventilated area, fed with standard feed (feed masters Ilorin, Nigeria) and water *ad libitum*. This research was carried out in Ahmadu Bello University, Zaria, Nigeria according to the rules governing the use of laboratory animals as acceptable internationally.

A preliminary phytochemical study was carried out on the leaf using standard methods[9,10]. Antibacterial activity was carried out using agar-diffusion method. MIC was conducted using serial dilution method while antidiairrhoeal property was evaluated using rabbit jejunum and castor oil models.

Antibacterial susceptibility test of standardized log phase cells (10^6 cells/ml) of *Bacillus subtilis* NCTC 8326 B76, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25722, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* (clinical isolate in Zaria, Nigeria) against selected antibacterial agents namely amoxicillin (25 µg), amoxicillin-clavulanic acid (20/10 µg), tetracycline (30 µg), ofloxacin (30 µg), nalidixic acid (30 µg), co-trimoxazole (25 µg), nitrofurantoin (30 µg), and *Heeria insignis* methanol extract (100 mg/hole) was determined using the official microbiological protocol of agar diffusion method[11,12].

Standardized overnight cultures of test bacterial cells was used to seed melted Murller Hinton Agar

(MHA) at 45^0 and poured into sterilized plate in triplicates aseptically. These were allowed to solidify. Antibiotic discs were then aseptically placed at reasonable equidistant on the seeded MHA plates and allowed to stand for one hour. For the methanol extract of *Heeria insignis*, each seeded plate of test bacteria was bored with 6.0 mm diameter sterile cork borer. The base of the hole sealed with 1.0 ml of the plant extract and allowed to stand for one hour, for proper diffusion. These plates were then incubated at 37^0 for 18 h. The diameter of zone of inhibition produced by each antibiotic disc or plant extract was measured (using metric rule) and the result interpreted[13].

In determining the MIC, graded concentration of the extract were prepared and mixed with melted (45^o) Mueller Hinton Agar in triplicates using doubling dilution method aseptically. The plates were then inoculated with 0.1 ml of 10^6 cells/ml of log-phase washed cells of test bacteria. Negative and positive controls were also set up. The plates were incubated at 37^o for 18 h. The lowest concentration that showed no visible growth was considered as the MIC of the plant extract against the test bacteria[14].

A modified method as described by Agunu[15] was used. A 24 h fasted rabbit was scarified by a blow on the head and exsanguinated. Segments of the jejunum (2-3 cm) were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with tyrode solution of composition (in mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 1.3, $NaHCO_3$ 12.0, $MgCl_2$ 0.5, $NaPO_4$ 0.14 and glucose 5.5. The tissues were mounted in a 25 ml organ bath containing tyrode solution maintained at 37 ± 1.0^o and aerated with air. An equilibrium period of 60 min was allowed during which physiological solution was changed every 15 min. Effects of acetylcholine (2.0×10^{-3}–3.2×10^{-8} g/ml), methanol extract (1.6×10^{-4}–3.2×10^{-3}), and dichloromethane extract (8.0×10^{-6}–1.6×10^{-3}) were investigated non-cumulatively. The contact time for each concentration was 1 min, which was followed by washing the tissue three times. The tissue was allowed to rest for 15 min before the next edition. At 2.0 and 4.0 mg/ml dose of extract, the effect of phentolamine (PTA) on tissue responses was also determined. Responses were recorded isometrically using Ugo Basile microdynamometer 7050 and maintained at sensitivity of 2 and paper speed of 24 mm/min.

The mice were fasted for 12 h prior to the commencement of the experiment and were randomly divided into six groups of five mice each. The mice in the first group received normal saline i.p while mice in the second and third groups received 100 and 200 mg/kg orally of methanol extract while the fourth and fifth groups received 100 and 200 mg/kg orally of dichloromethane extract. The sixth group received loperamide 2 mg/kg orally. After 30 min of administration of the extract, castor oil (0.2 ml/mouse) was given intragastrically. The animals were placed in individual cages over clean filter paper. Three hours after caster oil challenge, the cages were inspected (by an observer unaware of the particular treatment) for the presence of the characteristic diarrhea[16] and the percentage protection calculated[17]. The result was analyzed using chi-square.

Preliminary phytochemical study on the leaves reveals the presence of tannins, flavoniods, saponins, cardiac glycosides and steroids. Alkaloids and anthraquinones are absent. The methanol extract gave higher antibacterial activity than dichloromethane extract. The order of susceptibility of test microorganisms to methanol extract were *Salmonella typhi*>*Pseudomonas aeruginosa*>*Staphylococcus aureus*>*Bacillus subtilis*>*Escherichia coli* which were comparable to standard (Table 1). The methanol extract at 100 mg/hole had greater zone of inhibition compared to standard drugs used except ofloxacin on *S. typhi*. Similarly, only ofloxacin and nitrofurantion gave higher activity on *E. coli* compared to the methanol extract.

The MIC of the methanol extract for the microorganisms were also determined (Table 2). The MIC (mg/ml) of methanol extract against microorganisms is; *B. subtilis* (3.9), *S. aureus* (1.95), *E. coli* (62.5), *Ps. aeruginosa* (3.9) and *S. typhi* (1.95). The extract is particularly very effective against *S. aureus* and *S. typhi* both with MIC of 1.95.

On the isolated rabbit jejunum evaluation, both extracts produced concentration-dependent relaxation of isolated rabbit jejunum up to a point. At doses of 0.5 to 4.0 mg/ml, extracts exhibit increased relaxation of rabbit jejunum. However at 8.0 mg/ml, there was no increase in tissue relaxation compared to 4.0 mg/ml (Table 3). Similarly activity of both extracts was not blocked by pentolamine, suggesting that extracts

TABLE 1: SUSCEPTIBILITY PROFILE OF TEST BACTERIA AGAINST ANTIBIOTICS AND *HEERIA INSIGNIS*

Antibacterial	Diameter zone of inhibition (mm)				
	B.subtilis	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*S. typhi*
Amoxicillin (25µg)	26.5±0.4	16.5±0.2	0.0	2.1±0.2	23.0±0.7
Amoxicillin-clavulanic acid (20/10µg)	20.5±0.2	10.5±0.01	0.0	29.0±0.5	30.0±0.45
Gentamicin (10 µg)	27.0±0.2	31.0±0.1	0.0	29.0±0.5	30.0±0.45
Tetracycline (30 µg)	20.5±0.2	31.0±0.1	0.0	20.5±0.2	27.0±0.3
Ofloxacin (30 µg)	45.0±0.2	36.0±0.2	21.5±0.2	41.0±0.8	39.0±0.4
Nalidixic acid (30 µg)	30.5±0.2	0.0	0.0	29.0±0.4	30.5±0.2
Co-trimoxazole (25 µg)	20.5±0.2	0.0	0.0	17.0±0.04	27.0±0.5
Nitrofurantoin (300 µg)	28.5±0.2	32.5±0.25	17.0±0.02	31.5±0.5	30.5±0.25
Heeria insignis methanol extract (100mg/hole)	26.0±0.5	27.5±0.65	16.5±0.65	29.0±0.4	35.0±0.75

Ps. aeruginosa = *Pseudomonas aeruginosa* ATCC 27853, *B. subtilis* = *Bacillus subtilis* NCT 8326 B76, *S. aureus* = *Staphyllococcus aureus* ATCC 25923, *E. coli* = *Escherichia coli* ATCC 25722, *S. typhi* = *Salmonella typhi* (clinical isolate), Cork borer diameter = 6.0mm

TABLE 2: MIC OF *HEERIA INSIGNIS* METHANOL EXTRACT AGAINST TEST BACTERIA

Test bacteria	MICs of *H. insignis* methanol extract
B. subtilis	3.90
Staph. aureus	1.95
E. coli	62.50
Ps. aeruginosa	3.90
S. typhi	1.95

act via mechanisms other than alpha-adrenergic receptors.

In the castor oil-induced diarrhoeal test, the methanol extract gave 60% protection at 100 mg/kg, which increased to 80% at 200 mg/kg. However, dichloromethane gave 80% protection at 100 mg/kg, which did not increase with increase in dosage (200 mg/kg) and is comparable to loperamide (2 mg/kg) with 80% protection (Table 4). Extracts significantly ($P<0.05$) protected mice from diarrhea compared to control.

Plants have long being in use by man in maintaining health and wellbeing, Worldwide, there is increase in the use of medicinal plants both in developed and developing countries. *Heeria insignis* is a medical plant used in Nigeria for various purposes including skin diseases and diarrhea. The antibacterial investigation shows that the methanol extract produced significant zone of inhibition against test bacteria in the order *Salmonella typhi>Pseudomonas aeruginosa>Staphylococcus aureus>Bacillius subtilis>Escherichia coli* (Table 1). The extract had highest antibacterial activity (zone of inhibition) against *Salmonella typhi*. At the concentrations used, the methanol leaves extract appears significantly better than standard antibiotics such as amoxicillin, gentamicin, tetracycline, nalidixic acid, co-trimoxazole on *E. coli*. The MICs (in mg/ml) of

TABLE 3: INHIBITORY EFFECTS OF PLANT EXTRACTS COMPARED TO ACETYLCHOLINE ON RABBIT JEJUNUM

Dose (mg/ml)	Percentage responses to plant extracts	
	Dichloromethane extract	Methanol extract
Acetylcholine	62.5	62.5
0.5	12.5	12.0
1.0	11.0	10.5
2.0	8.5	7.0
2.0 with PTA	8.5	7.0
4.0	7.5	6.5
4.0 with PTA	7.5	6.5
8.0	7.5	6.5

TABLE 4: PLANT EXTRACT PROTECTION COMPARED TO STANDARD AND CONTROL

Extract/drug	Dose mg/kg	No. of mice protected	% protection
Normal saline	0.5 ml	0/5	0.0
Methanol extract	100	3/5	60
	200	4/5	80
Dichloromethane extract	100	4/5	80
	200	4/5	80
Loperamide	2	4/5	80

Both extracts and loperamide significantly ($p<0.05$) protected mice against castor oil-induced diarrhea when compared with the control (n=5).

methanol extract against microorganisms (Table 2) are; *B. subtilis* (3.9), *S. aureus* (1.95), *E. coli* (62.5), *Ps. aeruginosa* (3.9) and *S. typhi* (1.95). The MICs, especially for *S. aureus* and *S. typhi* at 1.90 mg/ml, shows the strong antibacterial activity of the plant, as these bacteria are implicated in diarrhoea[3]. On the isolated rabbit jejunum evaluation, both extracts produced concentration-dependent relaxation of isolated rabbit jejunum up to a point. At doses of 0.5 to 4.0 mg/ml, extracts exhibit increased relaxation of rabbit jejunum. However at 8.0 mg/ml, there was no increase in tissue relaxation compared to 4.0 mg/ml. Similarly activity of both extracts was not blocked by pentolamine, suggesting that extracts

act via mechanisms other than alpha-adrenergic receptors.

Flavonoids have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins, and to complex with bacterial cell walls. They can also disrupt microbial membranes[18]. Tannins on the other hand have many human physiological activities such as stimulation of phygocytic cells, host-cell mediated activity, and a wide range of antiinfective actions[19]. Tannins form complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects as well as by covalent bond formation[19,20]. Saponins acts by complexing with the small amount of sterol in the cell membrane, thus interfering with bacteria cell membrane permeability[18].

In the pathophysiology of diarrhea and cholera toxin, prostaglandins are implicated. Extracts of plants that contain flavonoids[18] are known to modify the production of cyclooxygenase 1 and 2 and lipooxygenase[19] thereby inhibiting prostaglandins production. Some plants show antidairrhoea properties by their antimicrobial activities[21,22] or by blocking the eicosanoids (prostaglandins and congeners)[20]. Though, several constituents are present in the leave extract, it's, possible that flavonoids, tannins or steroids are singly or in combination are responsible for the observed effects. The pharmacological effect of loperamide is due to its antimotility and antisecretary properties[23].

WHO[1] recommends that for diarrhea treatment, oral rehydration salt (ORS) is preferable over antibiotics. However, the traditional treatment of diarrhea is less cumbersome, inexpensive and naturally tolerate by the body system. The observed antibacterial activity coupled with the effects on smooth muscle and castor oil-induced diarrhea may justify the usage of the plant leaves in the treatment of diarrhea.

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