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ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF *Daniella oliveri* AGAINST SOME BACTERIA ASSOCIATED WITH ENTERIC INFECTIONS

[1]O Yahaya, [2]MB Tijjani, [1]OJ Abraham, [1]I. O Umar [1]O. E Miachi and [1]B Usman
[1]Department of Science Laboratory Technology, Federal Polytechnic, Idah, Kogi State, Nigeria
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria
E-mail: ocholiahmed@yahoo.com

ABSTRACT
Daniella oliveri powderd plant materials was extracted using methanol and sterile distilled water. The Agar diffused methods was used to determine the antimicrobial activity of the plant against *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.* The methanolic and water both provide higher yield from different parts of the plant and both showed effectiveness except against *Pseudomonas aureginosa*. Phytochemical screening of the crude extract revealed the presence of tannins, saponins, phenolcs, flavonoids, cardiac glycosides, anthroquinones, and alkaloids and the growth of all bacteria were inhibited though to varying degrees thus justifying their use in traditional medicines in treating enteric infection and other diseases across Africa.
Key words: *Daniella oliveri,* Phytochemicals, methanolic extract, Enteric infections.

INTRODUCTION
The use of plant parts in the treatment of human disease is as old as the disease themselves, and herbal medicine was the major form of medicine in Nigeria. Infectious disease s are major cause of death in developing countries and today according to WHO as many as 80% of world population depends on traditional medicines for their primary health care needs and that 25% of the drugs are based on plants and their derivatives **[1].** Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics which increase the likelihood of being hospitalized and increase the length of stay in the hospital **[2]** and increased use of a particular antibiotic could lead to increased bacterial resistance **[3].** Despite advance orthodox medicines availability, there has been an increased interest in the complementary and alternative medicines particularly by those who have apprehension concerning toxicity and safety of modern drugs [4]. In Nigeria, different plants varieties of plant are used in the treatment of different types of diseases. The roots, stem, bark and leaves of Daniella *oliveri* are used in the treatment of scrotal elephantiasis, dysentery, ring worms, syphilis, typhoid fever, eye sore and ear ache [5]. It was also reported that the stem bark and leaves of *Combretum glutinosum* are used as antipyretic and in the treatment of stomach ache, gonorrhea and typhoid fever and the stem bark of *Daniella oliveri* was used in the treating fever, boil and back ache [6]. *Daniella oliveri* belong to the family Fabaceae. The plants are found in both temperate and tropical regions of the world with 630 genera and species [7]. Substances derived from medicinal plants remain the basis for a large proportion of commercial medications for the treatment of various diseases like heart diseases, high blood pressure, pains, asthma, malaria, typhoid fever, snake bites, arrow poison and other problems. These substances are available in a variety of forms.

Fresh, dried, tablets, capsules, bottled in liquid forms, tea, bath tincture etc are all good as the quality of the raw plant from which there were made [8].

The medicinal usefulness of has been the subject of many numerous chemical and microbiological studies. Some of the reported phytoconstituents of the herds included triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline, and shikimics acids. And some of the other scientific uses include antispasmodic, antiasthmatic, expectorant, anticatarrhal and antisyphilitic [9, 10, 11]. The increasing failure of chemotherapeutics antibiotics resistance exhibited by many pathogenic infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Plants with possible antimicrobial activity should be tested against appropriate microorganisms to confirm their activity and ascertain other parameter associated with it.

MATERIALS AND METHODS

Collection of plant materials

The fresh of *D. oliveri* was collected from Okenya village in Igala-mela/Odolu Local Government Area of Koigi State, Nigeria. The taxonomic al identification of the plant was confirmed by Mrs O.E Miachi.of the Department of Science Laboratory Technology, Federal Polytechnic Idah, Kogi State, Nigeria.

Preparation of Plant materials

The fresh plant was harvested , rinsed with tap water and air dried under shade for 20 days and reduced to coarse powder using the pestle and mortar and then grinded to fine powder using a blender. The powder was stored in an air tight bottle for further use.

Preparation of extract

100g of the powdered sample was soaked in 100ml of methanol and water each contained in a 500 ml sterile conical flask and covered with cotton wool and rapped with aluminum foil after shaken vigorously. The mixture was left to stand overnight at room temperature. The mixture was then filtered using a clean muslin cloth and then whatman paper no.1 filter paper. The percentage extract yield was estimated as dry weight/dry material weight $\times 100$ [12]. For the preparation of dilutions of crude extracts for antibacterial assay, the extracts was reconstituted by dissolving in the extracting solvent and water and further diluted to obtain 400, 200, 100, 50, 25,12.5 mg/ml etc , and maintained at room temperature between 2-8^{o}C.

Microorganisms

The bacteria used were clinical isolates obtained in the department of Science Laboratory Technology, Federal Polytechnic Idah, Kogi State, Nigeria. The bacteria were maintained at a temperature between 2-8 oC. Standardization of culture was carried out according to Baker and Thomsberg [13] and the National committee for clinical Laboratory Standards [14] by suspending an 18hrs culture into sterile Universal bottles containing nutrient broth. Normal

saline was gradually added so as to compare the turbidity to Mcfarland Standard of 0.5 which corresponds approximately 1.0 $\times 10^8$ cfu/ml.

Determination of antimicrobial activity

The method described by Abubakar, [11] was used. Briefly, 1.0ml of 18hrs culture of each bacterium was adjusted to 1.0$\times$ 10^8 cfu/ml was spread into a sterile plate so as to achieve a confluent growth. 3ml of molten nutrient agar at 45^{o}C was added to each plate and rocked for even spread and proper mixing of the bacteria and the agar. The plates were allowed to solidify and 6mm in diameter and 2.5mm in deep were bored on the surfaces of the agar medium using a sterile cork borer. 0.5ml of the reconstituted extract at concentration of 100mg/ml was placed into one of the holes and 0.5ml of pure sterile distilled water was pipette into a hole as negative control while 26.7 µg ml was used as positive control. The plates were allowed to stand for 1 hr for proper diffusion of the extract and then incubated at 37oC for 24 hrs and the zones of inhibitions were measured to the nearest mm. the mean of the plates were taken.

RESULTS AND DISCUSSION

The percentage yield is higher for the methanolic extract of the stem bark (16.8%) while water has more yields from the leaves stem (9.2%). The phytochemical characters of the medicinal plant investigated are summarized in table 2. Alkaloids, tannins, flavonoids, terpenoids and cardiac glycosides were present in both the leaves and the stem bark. The antimicrobial activity of crude extract of *Daniella oliveri* were evaluated by measuring the diameters of the zones of growth inhibition on the tested bacteria species and the results are presented on table 3. The entire test organisms were susceptible to *Daniella oliveri* extract though to varying degree. The methanolic extract showed the highest level of inhibition on Salmonella typhi and E.coli while the aqueous extract shoed no response.

Table 1. Percentage yeld of the crude Extract of *Daniella oliveri*

Plant parts	Extraction solvent	Raw plants Powder (g)	Extracted plant powder (g)	Percentage yield (%).
Leaves	Methanol	120	3.2	2.7
	Water	120	11.0	9.2
Stem bark	Methanol	120	20.1	16.8
	water	120	8.6	7.2

Table 2. Photochemical constituents of 1 *Daniella oliveri*

	Stem bark		**Leaves**	
Phytochemicals	Methanol	Aqueous	Methanol	Aqueous
Saponnins	+	+	+	+
Alkaloids	+	+	+	+
Phenolics	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	+	+	+	+

Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Anthroquinones	+	+	+	+

Key : (+) positive (-) Negative

Table 3. Antibacterial activity of *Daniella oliveri*

	Zone of inhibition diameter (mm)				
	Stem bark		**Leaves**		
Organism	Methanol	Aqueous	Methanol	Aqueous	Gentamycin
Salmonella typhi	9 .0	8.0	2.0	-	12.0
Pseudomonas aeruginosa	8.0	6.0	5.0	8.0	9.0
Staphylococcus aureus	6.0	8.0	8.0	6.0	12.0
Escherichia coli	8.0	5.0	5.0	-	12.0

The yield when compared with the results obtained by Owolabi Etal., [15] 10.74% for water extract of Senna obtusifolia and Daugar et al., [16] 52% water and 3.78% ethanolic extract, it is relatively small. Factors like age of the plant and the polarity of the solvent used may affect the yield. Water and methanol seems to be a good a good solvent for this plant which supports the use of water and alcohol as traditional solvent.

The phytochemical screening of the crude extract of the plant showed that the leaves and stem bark were rich in some bioactive components as seen in table 2. Alkaloids, flavonoids, tannins, and saponins. They were known to show medicinal activity as well as exhibiting physiological activity [17] and exhibit anti-inflammatory, anti-oxidant and membrane stabilizing property [18]. The presence of terpenoid has been reported to be useful in herbal medicines [19]. These phytochemicals also have some strong antimicrobial significance against some potential enteric pathogens [20]. The presence of Alkaloids in significant quantities may be used as antimalarial, analgesics and stimulants [21]. The other phytochemicals present in the plants are known to inhibit tumor growth, treatment of intestinal disorder lke dyarrhoae and dysentery, tennin are used in treating wounds, sprains, bruises and arresting bleeding [22, 23]. Studies carried out by Ijel et al., [24] indicated the possibility that the use of plant extract in high doses could lead to toxic injury to kidney which may interfere with renal tubular functioning and could induce acute renal failure. This study shows that some plants show much promise in the development of in the development of phytomedicines with great antimicrobial properties as seen presently with the traditional medical practitioners.

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