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SHORT COMMUNICATION

Revalidation of antibacterial and aphrodisiac activities of *Fodogia agrestis* in Kano state, Nigeria

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ABSTRACT

Fodogia agrestis is a medicinal plant widely for its reported antibacterial and aphrodisiac activities. Revalidation of its known antimicrobial and aphrodisiac activities holds promise for dual usage as antibacterial and aphrodisiac agent. **Aim:** To validate the use of *Fodogia agrestis* as antibacterial and aphrodisiac agents in Kano state, Nigeria. **Materials and Methods:** Antibacterial activity of the ethanolic extract of the *Fadogia agrestis* was tested on four standard bacterial isolates at a concentration of 500µg using agar diffusion method. The aphrodisiac activities of ethanolic extract of the plant were evaluated in male albino rats. The rats were given oral doses of 50mg/kg body weight, 100mg/kg and 200mg/kg of extract at three hour intervals respectively. Their mounting behaviors were evaluated. Aphrodisiac activity of the extract was compared with that of Sildenafil (Viagra). Phytochemical analysis and acute toxicity tests were done as well (LD₅₀) **Results:** The ethanolic extract from *Fadogia agrestis* contains glycosides and carbohydrates, steroids, saponins, resins and flavonoids Extracts of the plant resulted in significant increase in mount frequency up to 9 attempts/15 min in albino rats at a minimum concentration of 50mg/kg. The mount activity doubled with doubling of extract concentration. Extract of *Fadogia agrestis* showed similar activity with Sildenafil (Viagra). Extract of *Fadogia agrestis* inhibited only *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. **Conclusion** Antibacterial and aphrodisiac effects of *Fadogia agrestis* plant extracts in rats is hereby revalidated. Future studies should outline the minimal inhibitory concentration and clinical significance of extract. **Key words:** Antibacterial and aphrodisiac property, *Fadogia agrestis*, Albino rats, Acute toxicity and Viagra

Introduction

Ethno medicinal practice involves the use of plant parts or herbs for the treatment of various disease conditions. In line with the practice of herbal medicine in many communities all over the world, the Hausa communities in the Northern part of Nigeria also practices traditional medicine as a system of preventing, curing or eliminating a physical, psychological, psychiatric or social disease. Aphrodisiac may be defined as any substance that enhances sex drive or sexual pleasure and heightens the sense of sight, touch, smell, taste and hearing. Herbal aphrodisiacs are plants, stem barks, or roots that are used singly or in combination in order to enhance libido. It can be used in food or meat.

According to WebMD (1), People take *Fadogia agrestis* to treat erectile dysfunction, impotence, increase sex drive,

Improve athletic performance, and support *Fadogia agrestis* is also used to treat malaria. *Fadogia agrestis* is also reported to be popular among athletes and body builders as an alternative to anabolic steroids, because it may increase sexual behaviors and raise the level of the male hormone testosterone. But no one knows if *Fadogia agrestis* has these effects in people. Overall, there isn't enough information to know how *Fadogia agrestis* might work for any medical condition. Male impotence or erectile dysfunction (ED) is a significant problem that may contribute to infertility. There has been a worldwide increase in the incidence of erectile dysfunction, probably due to old age and other risk factors such as the presence of chronic illnesses (e.g. heart diseases, hypertension and diabetes mellitus), smoking stress, alcohol, drug abuse, caffeine and sedentary life styles. Erectile dysfunction may be defined as the consistent inability to

achieve an erectile sufficient for the purpose of satisfactory sexual intercourse or the ability to ejaculate, or both.

The rate at which many disease and associated complications emerge and/or re-emerge especially is unfathomable especially in communities where such diseases were thought to have been eradicated or reduced to barest minimum. The increasing interest in herbal local alternatives to imported drugs are due to so many reasons which may include but not limited to: the reported containment of severe acute respiratory syndrome (SARS) in China (2), reported 8% use in African populations for different health problems (3-4), and it's expected role in global health. The expected role of herbal medicine in changing global is seen by the huge support in China, India, Nigeria, the United States of America (USA) and WHO (3) respectively. China launched a safety research programme focusing on herbal medicine injections from traditional Chinese medicine (5). South Africa included the need for investigating traditional medicines within its national drug policy (6). The Association for the Promotion of Traditional Medicine (PROMETRA), based in Dakar, Senegal, is "dedicated to preserving and restoring African traditional medicine and indigenous science" (7). Nigeria has a national committee on traditional medicine with the expressed desire to boost Nigeria's market share of traditional medicine (8). Industries have also invested millions of US dollars looking for promising medicinal herbs and novel chemical compounds (9-10). The high scale of investment in herbal research reflects genuine public, industrial and governmental interest in this area (11). Since the antibacterial and aphrodisiac activities have been reported (12) in rats under separate experimental condition, the aim of this study was to revalidate the use of *Fodogia agrestis* as antibacterial and aphrodisiac agents in Kano state, Nigeria.

Materials and Methods:

Plant materials were collected from Kano and Kaduna States of Nigeria between July and December 2004. The plants were identified by experienced Botanist using standard identification protocols, at the Biological Science Department Bayero University Kano, Nigeria, and authenticated by comparison with Voucher specimen at the Herbarium of Bayero University, Kano Nigeria.

Active ingredients of air-dried and ground plants were extracted using the procedure described by Fatope, (13). Forty grams of plant materials were percolated in 400ml of different solvents of 95% ethanol, ethyl acetate, chloroform, and pet ether at room temperature for seven days. The percolate was filtered to remove solids and then evaporated to dryness on a laboratory rotary evaporator. The crude extracts of the plants materials were carefully labeled, weighed and stored at 40°C in a refrigerator for later analysis. What man No. 1 filter paper (of 6mm diameter) were punched out with the aid of a perforator and placed in Bijou bottles. These were then sterilized by autoclaving at 121°C for 15 minutes. The discs were allowed to cool and stored. The negative control disc that were to serve as

controls for the antimicrobial sensitivity testing were prepared by adding 2ml of ethanol onto sterile discs in a Bijou bottle (each disk is capable of absorbing 0.01ml of solution).

The microorganisms used in this study were standard strains of *Staphylococcus aureus*, *Escherichia coli*, *pseudomonas aeruginosa* and *Proteus spp* stored at the microbial bank of the Microbiology Department, Bayero University, Kano state, Nigeria. They were supplied for this study by same Department of Microbiology after due ethical protocols and re-identification using standard Microbiological methods to ensure purity while ruling out contamination. Each organism was maintained in axenic form in triplicate. Within two weeks before disposal. The slants were incubated at 36°C for 24 hours. The slants were used for 3 weeks of experimentation before new ones were prepared.

A pure stock culture stored in the laboratory was microscopically studied to confirm their morphology, as well as behavior to gram stain test using standard procedure. Also the susceptibility of these isolates to multi disc was established following the disk-diffusion protocol described by Clinical laboratory Standard Institute. The protocol used for disc diffusion is the popular Kirby-Bour method modified by the clinical laboratory standard institute and the disc selection protocol was according to the first line of antibiotics commonly prescribed for the treatment of diseases associated with selected organisms (14-15).

The isolates were then purified and kept in the departmental laboratory for research purpose. Two colonies of each of the clinical isolates such as *Staphylococcus aureus*, *Escherichia coli*, *pseudomonas aeruginosa* and *Proteus spp* were picked and inoculated into 10ml of nutrient broth in test tubes. These were then incubated at 37°C overnight (18hours) in ambient air and the resulting turbid broth culture was then standardized to a turbidity of 0.5 McFarland using standard methods. The agar diffusion method as described by Kirby & Bauer et al, (17) was employed for the bioassay procedure. In this pour-plate process, 20ml Nutrient Agar plates (Sterling, UK) were flooded with 1ml each of the standardized inoculums and the excess inoculum was decanted.

The inoculated plates were then air-dried and filter paper discs containing the crude ethanolic extracts of the plant samples at 500m concentrations were then arranged and pressed firmly to the inoculated agar surface with the use of a sterile pair of forceps. The impregnated discs were sufficiently spaced out to prevent overlapping of the zones. Each disc was also kept away at least 15mm from the edge of the Petri-dish to avoid contamination and to get enough diameter during result reading. All the plates were allowed a diffusion time of thirty minutes which is a time for the extracts to diffuse in to the agar medium before the incubations. The plates were then inverted and incubated air at 37°C for 18-24 hours. The zone of inhibition diameters of the semi-confluent growth were measured with the aid of a meter ruler to the nearest mm (with respect to each isolate

and concentration). The following standard keys were employed. 0/mm indicates no effect. Diameter <8.0mm (zone of inhibition) indicates low sensitivity, Diameter >8.0mm (zone of inhibition) indicates high sensitivity

Test for aphrodisiac activity

Healthy, male albino rats (*Rattis Nevergicus*) weighing 270g-300g, aged 5.0-5.5 months and female albino rats weighing 150g-180g, aged 3.5-4 month were used for the study and obtained from a small animal holding unit of department of pharmacology, ABU Zaria, Nigeria. They were kept in well ventilated condition (28°C – 31°C. Photo period: 12h natural light and 12 hours dark, humidity 50% - 55%) with free access to rat pellets and tap water.

Mount was operationally defined as the male assuming the copulatory position but failing to achieve intromission. To quantify mounting behavior, non-oestrous female rats were paired with malestrated with single dose of the extracts (50mg/kg. 100mg/kg o.p). Animals were observed for 3 hours and behavior were scored using standard methods. Males were placed individually in a cage. After 15 minutes of acclimatization a non-oestrous female was introduced in to the area. The numbers of mounts were recorded during 15 minutes observation period at the start of 1st hours. Then the female was separated for 75 minutes as before at the 3rd hours the female was separated again for 45 minutes then reintroduced for 15 minutes for the last 3 hours. All the experiments were performed at 26-27°C.

To determine the effects of *Fodogia agrestis* on mounting behavior, five class were used (2) Dimethylsulfoxide, groups 2,3,4 were given plant extract and group five was given standard Viagra. Each of the three rats in group I served as a control, and each rat were taken for the study and fed with the required dosage i.e. 50mg/ml, 200mg/ml and 500mg/ml. All extracts were dissolved in Dimethylsulfoxide just before oral administration. The first group received Dimethylsulfoxide water only and served as control. Groups II, III, and IV were given the extracts of *Fodogia agrestis* at the above concentration the last group V were given (Sildenafil citrate 10mg/kg) and served as a standard.

To determine general short term lethal dose of the extract, the animals were divided into 4 groups, each containing 3 rats. Group1 animals served as control and received distilled water in an identical manner. The group II, III, and IV were given extract at different concentrations according to the Lorke, (16) method using 3 animals for each extract. The animals were observed continuously for 1 hours for any gross behavior changes or death and intermittently for the next 6 hour and then again at 24 hours after administration.

Result and discussion

Table 1; Bacterial species and their corresponding zone diameter against tested plant extract of *Fadogia agrestis*

Bacteria used	S. aureus	E. coli	Proteus sp	P. aeruginosa
Zone diameter	8 mm	0.0mm	0.0mm	18 mm

Fadogia agrestis were tested for antibacterial activity at 500µg disc potency on selected hospital isolates. *Staphylococcus aureus* was found to be sensitive to extracts *F. agrestis*. *Escherichia coli* and *Proteus mirabilis* were also effective against *F. agrestis* extract tested. *Pseudomonas aeruginosa* showed that highest sensitivity to *F. agrestis* in the control there is no activity for all of the extracts.

Toxicity test

Table 2. Investigation of the toxicity of *Fadogia agrestis* in male rats in mg/kg body weight.

Extract	1 st dose mg/kg	Mortality	2 nd dose mg/kg	Mortality
<i>Fadogiaagrestis</i>	10mg	No	1600m	No
	100mg	death	g	death
	1000m	No	2900m	No
	g	death	g	death
		No	5000m	No
		death	g	death

Lork (16) method was used for the toxicity test for the Albino rats.

From experiment, it was observed that the three doses (50, 100, and 200mg/kg) of plant extracts tested impacted lower mounting behaviour on the Albino rats than did the standard. The maximum activity was observed during the 2nd hour, the tested extract exhibited lower mounting on the female rat when measured 15minutes after administration of *Fadogia agrestis* of the three doses, the higher mounting activity was exhibited by 100mg/kg and 200mg/kg per dose respectively. The mounting behavior of the second rat increased when challenged with different doses of test substance which varied from 50mg, 100mg, and 200mg respectively.

In spite of the spectacular advances made in the field of synthetic organic drugs, interest in the microbiology, chemistry and pharmacology of medicinal plants and plant products has increased tremendously in recent years. This may probably be because almost 80 percent medicines of the world used daily are directly or indirectly obtained from plants. This increasing interest is being constantly demonstrated by frequent occurrence of national and international conferences, workshop and seminars here and there on medicinal plants. This large quality of modern drugs comes from less than 15 percent of the plants which are known to have been investigated pharmacologically. Antibacterial activity of the extracts, were tested at 500mg/ml *fadogia agrestis* showed effect of 8mm zone of inhibition against *Staphylococcus aureus* and 18mm zone against *pseudomonas mirabilis*. While no effect on *E. coli* and *Proteus spp.* Toxicity of the test extracts, acute toxicity studies of the extracts were carried out in order to evaluate the safety of the plants as well as the effects for the produced by a single dose of extracts given during a 24 hour period. The acute toxicity of the crude ethanol extract of *Fadogia agrestis*

was determined and the median lethal dose (LD50) was calculated.

The ethanol extract of *Fodogia agrestis* did not show any mortality when different doses were administered at 10mg, 100mg and 1000mg evening the second doses administered i.e. 600mg, 2900mg and 5000mg *Fodogia agrestis*, it was found that no gross behavioral changes were observed. According to Lorke (17) above 5000mg/kg doses are of no practical interest. A statistical analysis using one-way analysis of variance (ANOVA) to account for the different treatments complemented with unpaired t-test were as follow for *Fodogia agrestis* at 5% level of significance indicated that the number of mounts is independent of the concentration meaning that at any given dose it can serve the same purpose. The standard drug containing Sildenafil citrate at 5% level of significance showed that there was no significant different ($p < 0.05$) in the number of mount. The standard has similarities at 5% level of significance with the first extract that is *Fodogia agrestis* based on the doses given at different concentration.

In conclusion: The reported (12) antibacterial activities of extracts of *Fodogia agrestis* plants from Kano State Nigeria and reported aphrodisiac effect are hereby re-validated in Kano state, suggesting their possible use for the treatment of urogenital infections caused by respective organisms that showed in-vitro susceptibility while still exerting its effects as aphrodisiac agent on rat model. Clinical trial on human subject is recommended

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