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# Antibacterial Activity and Aphrodisiac Potential of the Ethanolic Extracts of *Fadogia agretis* (Sch winffax Hiern) Stem in Male Albino Rats

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**Abstract:** The research is aimed to evaluate the antibacterial activity and aphrodisiac potential of the ethanolic extracts of *Fadogia agrestis* (*rubiaceae*) stem in male albino rats. The antibacterial activity of the extracts were tested on four hospital isolates of *staphylococcus aureus, Escherichia coli, Pseudomonas specie* and *Proteus spp* at a concentration of 500µg using disc diffusion method. For aphrodisiac test, healthy, white male albino rats (*Rattis novergicus*) weighing 270 – 300g aged 5.0 – 5.5 months and female albino rats weighing 150 – 180g aged 3.5 – 5.4 months were used for the study. The male albino rats were orally dosed with 50mg/kg, 100mg/kg and 200mg/kg body weight respectively of the extract at 24hours intervals. Their sexual behaviour was evaluated for 15 minutes each. The plant's extract was found to inhibit *staphylococcus aureus* and *Pseudomonas specie.* Aphrodisiac effect of the extract resulted in significant increase in mount behaviour. The antibacterial effects suggest their possible use for the treatment of infection that showed *in vitro* susceptibility. Also, the increase in the mounting behavior may be responsible for the aphrodisiac effect.

***Keywords:*** *Aphrodisiac potential, ethanolic extracts, fadogia agrestis, albino rats*

## I. Introduction

Aphrodisiac is the word derived from Aphrodite, the Greek goddess of sexual, love and beauty. An aphrodisiac is defined as a food or drug that arouses sexual desire. Many natural substances have been known as aphrodisiacs in Africa and Europe. Sexual relationships are some of the most important social and biological relationship in human life. Male impotence also called erectile dysfunction (ED) is a common medical condition that affects the sexual life of millions of men worldwide. Erectile dysfunction is defined as the persistent inability to obtain and maintain an erection sufficient for naturally satisfactory intercourse. Sexual dysfunction is a serious medical and social symptom that occurs in 10%-52% of men and 25%-63% of women. Erectile dysfunction is adversely affected by diabetes mellitus, antihypertensive, antipsychotic, antidepressant therapeutic drugs. Organic causes of ED like Hypogonadism, hyperprolactinaemia, and neurological disorders. Treatment of ED involves several natural aphrodisiac potentials [1]. Aphrodisiac is also described as any substance that enhances sexual pleasure. Sexual dysfunction caused by various factors such as psychological disorders like Anxiety, depression, stress, fear of sex, neurological disorders, stroke, cerebral trauma, Alzheimer, Parkinson's disease and chronic disorders-diabetes, hypertension, vascular insufficiency, Atherosclerosis, penile disease-phinosis, peyronies, life style-chronic alcohol abuse, cigarette smoking, aging, decrease in hormone level with age. Systemic diseases - cardiac, hepatic, renal, pulmonary, and cancer. Since introduction of sildenafil citrate to treat erectile dysfunction, there has been renewed and vigorous interest in medicinal herbs with folkloric reputation for sexual disorders [2]. Some Hausa aphrodisiac herbal medicines are claimed to be used for the

treatment of infectious diseases, suggesting their antimicrobial activities in addition to the aphrodisiac property.

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection [3, 4, 5, 6]. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections [7].

The possibility of bioactive aphrodisiacs which may be derived from plants, animals or minerals, has been attractive throughout recorded history. Plant species have been used for the treatment of diseases all over the world before the advent of modern clinical drugs. Natural phytochemicals are known to contain substances that can be used for therapeutic purposes or as precursors for the syntheses of novel drugs. Nearly 50% modern drugs are derived from natural products which play an important role in drug development in the pharmaceutical companies. Plants are the most common source of antimicrobial agents [8, 9]. Many aromatic plants have been used traditionally in folkloric medicine, showing inhibition against bacteria, fungi and yeast [10]. Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases [11]. There is an essential need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action.

*Fadogia agrestis,* a shrub with a yellowish stem and leaves of 1-3 feets high, is one of the several plants commonly used in the management of erectile dysfunction [12]. It belongs to family *Rubiaceae* and can be seen in Nigerian region and is claimed to be an aphrodisiac helpful in management of erectile dysfunction. Identification of phytochemical constituents reported the presence of alkaloids and saponins as major and anthraquinone and flavonoids as minor constituents. Aqueous extract of *Fadogia agrestis* at doses 18 mg/Kg, 50 mg/Kg and 100 mg/Kg BW/Day were tested in male rats for sexual behaviour parameters and testosterone level. At all doses significant change in sexual behavior parameters was noted as there was increase in mounting frequency and intromission frequency, and reduction in mounting latency and intromission latency along with prolonged ejaculatory latency. Serum testosterone level was found to increase in a dose dependent manner. Therefore, this plant finds place in the list of potent aphrodisiacs available for further animal and clinical trials [13].

This study was carried out to determine the antimicrobial property of this Hausa aphrodisiac plant. Male impotence or erectile dysfunction is a significant problem that contributes to infertility [14].

### I.1. Mechanism in aphrodisiac potentials

Sexual desire is regulated by the central nervous system which integrates tactile, olfactory and mental stimuli [15]. Some aphrodisiac provide a burst of nutritional value improving the immediate health of the consumer and improving libido. Some have more specific physiological affects but are not psychologically active. They may affect blood flow; increase duration of sexual activity by numbing the genital area while others are made up of compounds that are psychopharmacological by stimulating sexual arousal. This category includes a wide range of neurotransmitters, hormones, pheromones and drugs that interfere with the normal function of these molecules. This category is most difficult to study because knowledge of both sexual arousal and the mechanisms of the psychoactive properties of drugs are limited [16].

### I.2. Side effects of sexual dysfunction treatments

These include drowsiness, insomnia, headaches, nasal congestion, headaches, dizziness, tachycardia and weight

### I.3. **Aims and objectives**

This study is aimed at determining the antibacterial activities and aphrodisiac potential of the ethanolic extract of *Fadogia agrestis* with the objectives of evaluating the aphrodisiac properties of *Fadogia agrestis* and the antimicrobial sensitivity test of the plant.

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## II. Materials and Methods

### II.1. Plant materials

Plant material was obtained from Kurmi market in Kano city, Nigeria and was identified in the Biological sciences department of Bayero University, Kano. They were authenticated by comparison with voucher specimen at the Herbarium of the department.

### II.2. Extraction and fractionation

Active ingredients of air-dried and ground plant samples were extracted using the procedure [16]. 40g of the plant material was percolated in 400ml of 95% ethanol at room temperature for seven days. The percolate was filtered to remove solids and then evaporated to dryness on a laboratory rotary evaporate. The crude extracts of the plant materials were carefully labeled, weighed and stored at 40$^{o}$C in a refrigerator for the analyses.

### II.3. Preparation of the sensitivity discs

Whatman No.1 filter paper of 6mm diameter was punched out with the use of a perforator and placed in Bijou bottles. These were then sterilized by autoclaving at 121$^{o}$C for 15 minutes. The discs were allowed to cool and were stored.

### II.4. Antimicrobial screening test culture

The microorganisms used in the study were *staphylococcus aureus, Escherichia coli, Pseudomonas specie, Proteus spp* for determining the Minimum Inhibitory Concentration (MIC) of the plant extracts. The ethanolic extracts were tested for their effect against the growth of pathogenic bacteria by disc diffusion method [17]. The extract at four different concentrations viz., 18 mg/Kg, 50 mg/Kg and 100 mg/Kg were employed for antimicrobial activity. The antibiotic discs, oxacillin (10µg) and tetracycline (10µg) served as positive control for bacteria. The bacteria tested were inoculated into nutrient agar and PDA medium respectively. After the incubation period of 24 hours at a temperature of 35°C, four colonies isolated from these media were inoculated on 4ml of nutrient broth and incubated for 2 hrs. at 35°C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller-Hinton agar medium were streaked separately with these microbial suspensions of bacteria. Sterile filter paper discs impregnated with 18 mg/Kg, 50 mg/Kg and 100 mg/Kg extracts and control discs were applied over the culture plates. After equilibrium at 4°C, the plates were incubated overnight at 37°C and the diameter of any resulting zones of inhibition was measured. Triplicates were maintained for all these experiments.

### II.5. Bioassay procedure

The agar diffusion method [18, 19] was employed for the bioassay procedure. In this, pour-plate process, 20m NA plates (Sterlin, UK) were flooded with 1ml each of the standardized inoculum and then decanted off. The inoculated plates were then air-dried and filter paper discs containing the crude ethanolic extracts of the plant samples at 500µg concentrations were then arranged and pressed firmly to the inoculated agar surface with the aid of a sterile pair of forceps. The impregnated discs were sufficiently spaced out to prevent overlapping of the zones. Each disc was kept away at 15mm from the edge of the petri-dish. All the plates were allowed a pre-diffusion time of 30 minutes for the extract to diffuse into the agar medium before the incubations. The plates were then inverted and incubated in ambient air at 37$^{o}$C for 18 hours.

### II.6. Measurement of zones of inhibition diameter

The zone of inhibition diameters of the semi-confluent growth were measured with the aid of a metre rule to the nearest mm, with respect to each isolate and concentration. The following keys as demonstrated:

| | |
|---|---|
| 0/mm indicates | No effect |
| Diameter < 8.0mm zone of inhibition | Indicates low sensitivity |
| Diameter > 8.0mm zone of inhibition | Indicates high sensitivity |

**II.7. Phytochemical screening**

The screening was done on the day of collection using the procedures [20]. This was done by macerating 50 g of the fresh plant material in a sterile mortar and pestle separately with water and ethanol. The extract was filtered using muslin cloth and the process repeated until all soluble compounds had been extracted, as judged by loss of color of the filtrate. Extract was evaporated to dryness on water bath at 45°C and further dried to a constant weight at the same temperature in a hot-air oven. A portion of the residue was used to test for the presence of tannins, alkaloids, saponins, anthraquinones, reducing sugar, and cardiac glycosides.

**II.8. Test for alkaloids**

0.5 g of both ethanolic and aqueous extract was stirred separately with 5 ml of 1% aqueous hydrochloric acid on a steam bath. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and was observed for the formation of white precipitation or turbidity [21].

**II.9. Test for saponins**

The ability of saponins to produce frothing in aqueous solution was used as preliminary screening test for the compound. 0.5 g of both extract was added and mixed with Fehling's solution and then 5% of sodium trioxocarbonate solution was later added. The mixture was then boiled and observed for frothing or pink precipitation formation [21].

**II.10. Test for tannins**

0.5 g of both aqueous and ethanolic extracts was stirred separately with 1 ml of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue black precipitate was taken as evidence for the presence of tannins [22].

**II.11. Test for anthraquinones**

0.5 g of both extracts was boiled with 1 ml of 10% sulphuric acid and filtered. 2.5 ml of benzene was added to the filtrate while hot and shaken. The benzene layer was separated and half its own volume, 10% ammonia solution was added. This was observed for the formation of a pink color in the lower ammonia phase [22].

**II.12. Cardiac glycosides**

About 100 mg of the extracts was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a desoxy sugar characteristic of cardenolides [22].

**II.13. Test for reducing sugar**

0.5g of both extracts was treated with few drops of Fehling's solution, heated and observed for blue-black color precipitation formation [23].

**2.14 Aphrodisiac Activity Test**

Healthy, white male albino rats (*Rattis novergicus*) weighing 270 – 300g aged 5.0 – 5.5 months and female albino rats weighing 150 – 180g aged 3.5 – 5.4 months were used for the study and all were obtained from a small animal holding unit of the department of pharmacology, ABU Zaria, Nigeria.

### II.14. Mounting behaviour test

Mount is operationally defined as the male assuming the corpulatory position but failing to achieve intromission. To quantify mounting behaviours, non-oestrous female rats were paired with males treated with single dose of the drugs. (50 mg/Kg, 100 mg/Kg and 200 mg/Kg O.P.). Animals were observed for 3 hours and behaviours were scored as described by [24]. Males were placed individually in a cage. After 15 minutes of acclimatization, a non-oestrous female was introduced into the arena. The number of mounts was recorded during 15 minutes observation period at the start of 1$^{st}$ hour. Then, the female was separated for 75 minutes. Again, she was re-introduced and the number of mounts was observed for 15 minutes as before. At the 3$^{rd}$ hour the female was separated again for 45 minutes then re-introduced for 15 minutes for the last 3 hours. All the experiments were performed at room temperature of 26 – 27$^{o}$C.

## III. Results and Discussion

### III.1. Statistical analysis

The microbiological activity of the ethanolic extracts were assessed using the statistical analysis of one-way analysis of variance (ANOVA) to count for the different treatments complementation and unpaired t-test indicate that *Fadogia agrestis* at 5% level of significance indicated that the number of mounts is independent of concentration, meaning that at any given dose it can serve the same purpose.

The average of the zones of inhibition of the plant extracts against the test organisms were calculated and subjected to the t-tests to confirm the interpretation. If it falls within 0.00mm it is described as inactive. If the average inhibition diameter falls between 1.00 – 8.00mm it is described as a region of low activity and these were tested whether they were significantly greater than the test value of 8.00mm by calculating the p values. If the p values were less than 0.05, then the plant extract was partially active or else inactive. If the average zone of inhibition diameter was more than 8.00mm, it is regarded as active. These were then tested whether they were significantly greater than the test value of 8.00mm by calculating the p values. If the p values were less than 0.05, then the plant extract was active or else partially active. If the average zone of inhibition diameter was much higher than 8.00mm, it is regarded as highly active. These were then tested whether they were significantly greater than the test value of 8.00mm by calculating the p values. If the p values were less than 0.05, then the plant extract was highly active or else active. The results of the phytochemical screening of the plants extracts are tabulated below.

***Table 1**: Result of phytochemical screening of the plant extract.*

| **Phytochemical constituents** | **Aqueous extract** | **Ethanolic extract** |
|---|---|---|
| Cardiac glycosides | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Anthraquinones | + | + |
| Alkaloid | + | + |
| Reducing sugar | + | + |

Key: + = Present

***Table 2**: Minimum inhibitory concentration (MIC)*

| **Tested organisms** | *S. aureus* | *E. coli* | *Proteus spp* | *P. spp* | Average |
|---|---|---|---|---|---|
| **Concentration** (µg/ml) | 188.34 | 50.37 | 180.75 | 51.62 | 06.50 |

***Table 3**: Result of antibacterial activity of ethanolic extract of Fadogia agrestis against test organisms compared with two antibiotics*

| **Concentration** | **Tested organisms (Zone of inhibition (mm)** | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *Proteus spp* | *P. spp* | Control | Average |
| *Fadogia agrestis* extract (500µg/ml) | 08 | 00 | 00 | 18 | 00 | 06.50 |
| Oxacillin (10 µg) | 27.54 | 21.26 | 29.66 | 25.41 | 00 | 25.98 |
| Tetracyclin (10 µg) | 25.07 | 23.61 | 26.05 | 26.16 | 00 | 25.22 |

**Key:** 00 = no effect

It is so observed that the higher the concentration of the plant extract, the greater the diameter of the clearing zones observed. The plant extracts demonstrated a partially active, active and very active antibacterial activities against the tested bacteria.

The mean zone of inhibition demonstrated by the plant extract at 80 mg/ml was 06.50 mm which can be interpreted as active against all the test organisms and this was comparable to the mean zone of inhibition by oxacillin (25.98 mm) and tetracyclin (25.22 mm) as the positive controls used.

The mean zone of inhibition of the extract was 06.50 mm at the highest concentration of 80 mg/ml used against all test organisms. However, compared to the one of the positive controls, the antibiotics tetracycline (10 µg/disc) and oxacillin (10 µg/disc) gave a very active antibacterial activity with an average zone of inhibition of 25.22 and 25.98 mm respectively each against all the test organisms.

The plant extract was not active against *E. coli* and *Proteus spp* at the highest concentration of 80 mg/ml. The overall average of zones of inhibition for all concentrations of the extract used was 06.05 mm. Statistical analyses confirmed that it has very active antibacterial activity against *Pseudomonas spp* and *S. aureus.*

The positive control tetracyclin (10 µg/disc) gave an average diameter of zone of inhibition of 25.22 mm with the highest (26.16 mm) against *Proteus spp* 26.05 mm, *S. aureus* with 25.07 and the least *Escherichia coli* with 23.61mm.

The other positive control, Oxacillin (10 µg/disc) gave an average diameter of zone of inhibition of 25.98 mm with the highest of 29.66 mm against *Proteus spp,* followed by 27.54 mm against *S. aureus,* 25.41 mm against *P. spp* aand the least, 21.26 mm against *Escherichia coli*.

Results of the phytochemical screening are shown in Table 1. This table indicates that the physiologically active constituents present in the plant extracts with antibacterial properties include alkaloids, steroids, saponins, tannins, reducing sugars, anthraquinones and flavonoids compounds.

The lowest concentration of a drug that prevents the growth of an organism *in vitro* is called the minimum inhibitory concentration usually obtained by serial dilution test tube preparations in Mueller Hinton broth and inoculated by the test organisms. The results of the MIC determination as shown in Table 2 revealed that two test organisms, gram negative *Escherichia coli* (188.34 µg/ml) and *Proteus spp* (180.75 µg/ml) scored higher than the gram positive organisms followed by *P. spp* (51.62 µg/ml) and then *Escherichia coli* (50.37 µg/ml).

### III.2. Discussion

Many people rely on herbal medicines for healthcare [25] possibly because the other treatment options available are becoming more expensive and often carry gender side effects. There should be scientific dissemination of information on the therapeutic efficacy of these plants. The ethanolic extract of *Fadogia agrestis* stem has been in use by many people in our local communities as a means of treating sexual inadequacy and stimulating sexual vigour as well as treatment of the isolate of *Staphylococcus aureus* and *Pseudomonas spp* that showed *in vitro* susceptibility.

The antimicrobial activity of the extract at 500µg/ml showed effect of zone of inhibition against *Staphylococcus aureus* with 8mm and 18mm zone against *Psedomonas euroginosa.* No effect on *E. coli* and *Proteus spp.* The antimicrobial activity of the plants' extracts is due to the presence of the secondary metabolites present in them. It has been observed that the higher the concentration of the plant extract, the greater the diameter of the clearing zones observed.

The aphrodisiac potential of 50mg, 100mg and 200mg of the extract for 15minutes and observation for mounting behavior results in significant increase in the indices of sexual vigour. The mounting frequency indicates the aphrodisiac potential of *Fadogia agrestis* stem extract.

In this study, a marked effect on the sexual behaviour mounting compared with standard and control, is an indication of stimulation in the desired component of sexuality. Apart from the desire that is essential for initiation of sex, penile tumescence and rigidity as well as accessory muscles that help in providing penile rigidity and ejaculation are dependent on testosterone for normal rigidity and ejaculation for normal sexual activity.

The antibacterial activity of the plant extracts used in this study might be similar to antibiotics or antibacterial drugs that either inhibit the growth or kill pathogenic microorganisms since a partially active, active and very active antibacterial activities of the plant extracts against the test organisms has been obtained. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA [26]. Moreover, the mode of action of steroids which was present in the plants with antibacterial property is that they directly damage cell membranes. This indicates the loss of viability that maybe caused by the steroids' damage to the membranes with the loss of cytoplasmic constituents [27].

Anthraquinone which is present in the plant is a member of the quinone family. They are a good source of free radicals, and they are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function. Probable targets in the microbial cell are surface- exposed adhesions, cell wall polypeptides and membrane bound enzymes. Quinones also render substrates unavailable to the microorganism [28].

The plant extracts with antibacterial activity was tested positive for saponins. The antibacterial activity of saponins may be attributed to their interactions with membrane sterols, especially the pore-formation on membranes [29]. The interaction between saponins and membranes is complicated as it depends on the composition of the target membrane, the type of side chain, and the nature of the aglycone to which these are attached [30].

Tannins which were present are water-soluble polymeric phenolics that precipitate proteins16. It was documented that tannins can be toxic to filamentous bacteria [31]. The mechanisms for tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation. Condensed tannins have been known to bind to cell walls of ruminal bacteria, preventing growth and protease activity. One of their molecular actions is to complex with proteins through non-specific forces of hydrogen bonding, hydrophobic forces, and covalent bond formation.

Secondary metabolites are molecules produced by organisms especially plants that have no obvious role for normal growth and development. They are produced in small amounts and may be widespread or restricted to particular plants groups or species, or even plant parts. The general groups of secondary metabolites produced by plant include cardiac glycosides [32]. These secondary metabolites usually enhance plant's protection against predation and competition, and are responsible for use of plants in traditional medicines.

The MIC tests revealed that the plant extracts was effective against the tested microorganisms inhibited at a lower concentration of 62.5 µg/ml, an MIC of 62.5 µg/ml for *E. coli*. The results of this study were consistent with previous studies as the gram negative organisms showed greater resistance to other extracts from sponges and from honey [33]. This is because the gram negative organisms are surrounded by more complex cellular envelope, and therefore exhibit more permeability barrier so that they can afford greater resistance to many antibiotics [34].

## IV. Conclusion

The results of this study provide evidence to support acclaimed potential of *Fadogia agrestis* as antimicrobial medicinal plant and as aphrodisiac in traditional medicine. It has also provided scientific evidence as to its purported aphrodisiac effect. The aqueous and the ethanolic extracts were found to contain the essential phytochemicals. However, the results suggest that the plant extracts that gave antibacterial properties may act as alternative to chemical or synthetic bactericides since it was proven that they can control or inhibit the growth of pathogenic organisms.

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