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Full Length Research Paper

In vitro evidence of anti-infective activity of crude aqueous extract obtained by boiling ripe stem-bark of *Bridelia ferruginea* Benth

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The prevalence of multiple antibiotics resistance has prompted efforts to explore for more efficacious antimicrobial agents. The extract obtained by boiling the bark of *Bridelia ferruginea* Benth. is used traditionally for treating oral thrush called 'Efu'. For this reason the *in-vitro* effectiveness and range of activities of crude extract obtained by boiling, ripe-stem-bark of *Bridelia ferruginea* have been carried out. The extract was tested against various microorganisms collected from clinical specimens. Bacterial isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and *Candida albicans,* and other *Candida* species were tested for susceptibility using 0.5 McFarland turbidity standards of pure isolates on Mueller Hinton agar and Sabouraud dextrose agar. Ditches of 6 mm diameter were made on the media and 0.1 mls (9 drops) of the extract was added to each. For bacterial isolates, 3 or 4 standard antibiotic discs were added for comparison. The zone of inhibition around the extract ranged between 10 - 24 mm in diameter for bacterial and 14 - 25 mm for *Candida* (fungus). These results led to validate the activity both for fungal and bacterial agents of the crude extract obtained by boiling of the ripe stem bark of *B. ferruginea* as used traditionally.

Key words: *Bridelia ferruginea*, boiling, fresh, stem bark, *Candida*, bacteria.

INTRODUCTION

The prevalence of multiple antibiotics resistance developed by microorganisms against the available synthetic antibiotics has increased astronomically in the last decade (Jones and Pfaller, 1998; Shears, 1993; Hart and Kariuki, 1998). The rate of development of resistance to both the old and newer drugs calls for active search for more effective as well as affordable anti-infective agents.

This problem has prompted tremendous effort to explore for more potent antimicrobial agents, especially of natural origin to contrast this resistance. Literature reports and ethnobotanical records suggest that plants have tremendous potentials in the pharmaceutical industry as important sources of new compounds for antimicrobial drugs syntheses (Sofowora, 1982).

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Herbal preparations have been found to be useful in treating some conditions but the extent of use is often met with a major set back from the fact that no scientific evidence is available for many of such preparations.

A large proportion of the population of African countries still rely on the use of local herbs in the management of many ailments ranging from surgical to medical either infectious and non-infectious, with different degree of success or claim of benefitial responses.

The extract of the bark of *Bridelia ferruginea* has been used for 'Efu' in many parts of Nigeria where some people use it as gargle for oral candidiasis or orally to clean the infection that may be in the gastrointestinal tract and in blood, (the 'internal Efu') this may be assumed, in medical term to mean systemic fungal infection or candidaemia.

B. ferruginea Benth. is the commonest savannah *Bridelia*. It is usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition. Its common

names are Kirni Kizni (Hausa); Marehi (Fulani); Iralodan/Ira (Yoruba); Ola (Igbo); Kensange abia (Boki). Its habitat is the savannah, especially in the moister regions extending from Guinea to Zaire and Angola. The tree is 6 - 15 m high, up to 1.5 m in girth and bole crooked branching low down. The bark is dark grey, rough and often marked scaly (Rashid et al., 2000).

The bark in Obbo-Ile (Ekiti) is called 'Eepo Ura', the young branches of the tree is used locally as chewing stick, to produce sharp feelings in the oral mucosa, while the fresh stem-bark can be chewed and the saliva (juice mixed with saliva) swallowed for cleaning of the mouth, the throat and the gut from "Efu" (Thrush). The stem bark is also boiled or pound in mortar and the extract used for treating individuals with debilitated illness that has been observed to develop "black-tongue" and throat known as "Efu dudu" as a result of the current illness. Most of the treated people have been found to feel better after drinking the extract.

The earliest inspiration to perform a laboratory experiment on this extract to validate on scientific basis the use of the extract was in 2004 when the chief author used it to treat herself, after having used oral Mycostatin^{R} for oral thrush which has become recurrent, and got better without any recurrence for over twelve months.

Recently it was observed that another individual who had persistent infection despite repeated courses of oral Nystatin and oral Fluconazole (Diflucan^{R}) 150 mg statum dose for vaginal candidiasis got a complete cure within one week of topical application of this preparation and had no recurrence. While another patient with oropharyngeal candidiasis who initially received repeated courses of oral Nystatin for fourteen days per course but with very minimal relief and later recurrence also took the extract as gargle and 5 mls orally twice daily and had complete cure of the candidiasis after one week and has not had a recurrence in the last two years.

These latest evidences spur the initiation of the *in-vitro* testing to confirm scientifically the activity of the crude extract obtained by boiling of the bark of *B. ferruginea* (Euophorbiaceae) in the management of candidiasis and other infections.

In the course of writing up the findings of this experiment, it was found from literature that several works have been carried out from different parts of the world on different parts of the tree- *B. ferruginea.* The activities of various products from *B. ferruginea* from the literature include anti-inflammatory (Olajide et al., 2003), Trypanocidal (Ekanem et al., 2008) antidiabetic (Iwu, 1983; Onukwo et al., 1996), antibacterial and antifungal (Irobi et al., 1994; Muanza et al., 1994; Jose and Kayode, 2009; Talla et al., 2002) to mention a few, while the form of extraction also varied widely.

B. ferruginea has also diverse uses such as antiemetic, anti-inflammatory, antidiarrhoeal, antimalarial, antinoceptic, antiviral and several others as reported in the review of its phytochemical and ethnopharmacology by Ngueyem et al. (2009). The bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle "ogun efu" (Orafidiya et al., 1990), while Kolawole et al. (2007) reported its potential for water treatment.

The current study was carried out to establish whether the crude aqueous extract obtained by boiling fresh, ripe-stem-bark of *B. ferruginea* is actually effective *in vitro*, and also to determine the range of anti-infective activities of the extract in the form consumed traditionally in the area. This work is intended to serve as the beginning of an intense search into other related facts in the activity of *B. Ferruginea* and its combination with other herbs and fruits as used traditionally.

MATERIALS AND METHODS

Plant materials

1. Fresh ripe stem-bark (the ripeness is determined by redness of the bark when peeled from the tree) of *B. ferruginea*, family Euophorbiaceae was collected by the author from the trees in Obbo-Ile, Ekiti Local Government Area of Kwara State, Nigeria.

2. Preparation and extraction of the bark –The scaly rough part of the bark was scrapped-out so that the more fleshy part could be exposed and later cut into small pieces to fit into available pot (a kettle can also be used). To the pot was added approximately, 1.2 kg of the minced bark and 1.5 L of tap-water and the mixture was heated to boiling for about 2 h and was allowed to cool. The extract was strained and then put in bottles and stored at room temperature and later in the refrigerator prior to use.

Collection, isolation, purification and identification of pathogenic organisms

The source of all the organisms used in this experiment is from specimens from patients attending University College Hospital, Ibadan, whose specimens were sent to the Medical Microbiology and Parasitology Laboratory for microscopy culture and sensitivity.

Bacterial organisms collected are from various specimens ranging from urine, blood, and swabs from ear, eye and wound. The organisms which were isolated and characterized using stan-dard laboratory methods as contained in the standard laboratory manual and standard operating procedure (SOP) of the laboratory, include both sensitive and multi-resistant bacterial isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

The fungal agent studied were 17 different isolates of *Candida* species, these included 6 *Candida albicans,* and 10 *Candida* species (based on "germ-tube" test) (Cheesbrough, 1987).

The 10 *Candida* species included 1 isolate of *Candida tropicalis,* 2 *Candida krusei* and 1 *Candida glabrata* based on isolation on CHROMagar (CHROMagar Paris, France) and 6 other Candida whose species could not be determined within the scope of the current experiment.

The species of *Candida* from blood were initially isolated from blood stream of patient with fungaemia using BACTEC Pêds Plus/F (Becton, Dickinson and company) before further characterization was carried out. Other sources of *Candida* were the Vagina, throat and ear of patients. The subculture was done on Sabouraud dextrose agar (BIOTEC laboratories,UK) and Sheep blood agar.

Following isolation of the *Candida*, further characterization and identification was carried out on CHROMagar which gives different

Table 1. showing the range of susceptibility of the various isolates.

Isolate types	Diameter of Inhibition With extract 0.1 mls (mm)	Ceftriaxone 30 µg (mm)	Gentamicin 10 µg (mm)	Ceftazidime 30 µg (mm)
P. aeruginosa	12-18	12-22	20-28	22-24
K. pneumoniae	10-16	12-16	10-16	0-10
E. coli	10-20	12-16	0-12	12-24
P. mirabilis	12-16	22-30	0-12	Not tested
S. aureus	18-24	25-30	19-24	Not tested
C. albicans	18-25			
C. krusei	20			
C. tropicalis	22		Not applicable	
C. glabrata	24			
Candida species(others)	14-18			

pigmentation with different species of *Candida*. The culture plates were examined within 24 - 48 h.

Extract sensitivity testing

A pure culture of the different isolates (bacterial and fungal) was prepared and an inoculum size of 0.5 McFarland turbidity standards was made from the broth of the overnight culture of each isolate. This procedure is used for standardization of quantity of organism per preparation.

Mueller Hilton agar (Oxoid CM409) for bacterial sensitivity testing and SDA agar (Oxoid CM41) for fungal were flooded with the different isolates depending on what is being tested. The agar surface was allowed to dry and a punched-out-hole (ditch) was made in each plate with a steel borer of 5 mm diameter and 0.1 mls (9 drops) of the extract was added to the ditches with the aid of sterile 2 ml syringe and 21G needle.

For bacterial isolates, 3 or 4 standard antibiotic discs (Ceftazidime 30 µg, ceftriaxone 30 µg, Cefuroxime 30 µg, gentamicin 10 µg) (Oxoid) depending on isolates being tested were included for comparison. The charged plates were then incubated aerobically at 35 - 37°C overnight (16 - 18 h) for bacteria and 48 h for *Candida*, in Gallenkamp incubator.

The susceptibility was read by measuring the zone of inhibition (confluent area surrounding the discs and ditches, where there was absence of growth or clearing of organism) for each species and each preparation. The diameter is equal to the addition of the two annular radii and width of disc (6 mm) and ditch (6 mm). The measurement was read and recorded in millimetres (mm). For illustration, an organism with 2 mm annular radius will have a diameter of 10 mm.

RESULTS

Bacterial isolates tested showed varying degree of susceptibility ranging from annular radius of 2 - 7 mm (diameter 10 - 20 mm) in Gram negative bacteria and 2 - 9 mm (diameter 10 - 24 mm) in Gram positive bacteria.

Candida species also varied in their susceptibility with a diameter ranging from 18 - 25 mm in *Candida albicans* to 14 mm in some other *Candida* species.

Isolates from vaginal showed better response of 22 - 25 mm, than those from throat and blood stream (Candidaemia) with diameter of zone of inhibition of 18 mm. The isolate from the ear showed a response of 14 - 18 mm.

Table 1 showed the range of response from all isolates tested and Plates 1 - 3 also showed some of the plates with susceptibility tests.

DISCUSSIONS

This experiment showed that crude extract obtained by boiling a fresh ripe stem-bark of *B. ferruginea* has a wide range of anti-infective activity which includes both antifungal and anti-bacterial.

Phytochemical screening of methanolic extract of the bark revealed the presence of alkaloids, tannins, saponins, steroids and cardiac glycosides (Adebayo and Ishola, 2009), while other constituent such as gallocatechin-(4-O-7)-epigallocatechin, isoflavone, bridelone and bridelonine etc have been found from the various species and also from the different parts such as fruits, root, and leaves of the tree- *Bridelia.* However, a further study to determine the detail constituents of crude extract by boiling of the ripe fresh stem-bark is in progress, since most of the previous authors got extracts from dried bark or powdered form, some of which were bought from herb seller, there is a probability that some of its constituents could have been lost in the process.

The emphasis on ripeness is because the stem bark, depending on the age of the tree, grows in stages and some are more ripe than the other and ripeness is determined by the scaliness of the outer portion and redness of the fleshy portion of the bark. The ripe bark gives a better result from experience.

This study showed that the efficacy attributed to consumption of crude extract obtained by boiling is true, however, there is a need to determine the best and most cost effective form of extraction that will bring out all the various component in adequate quantity.

In the current study, the crude extract showed comparative activity with standard antimicrobials against

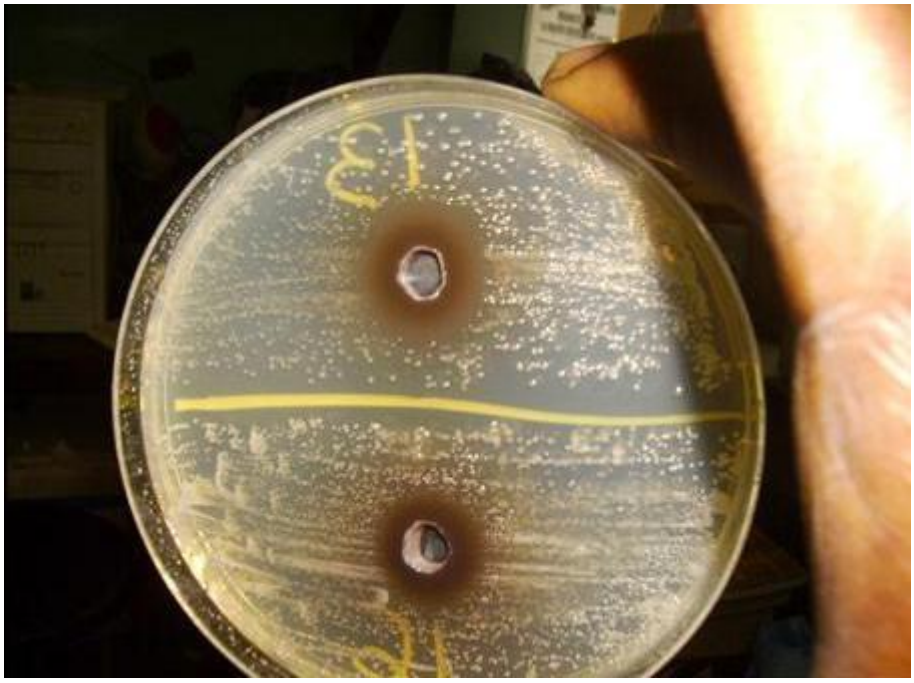

Plate 1. Showing zone of inhibition of 2 *Candida* isolates around the ditches with extract.

Plate 2. Zone of inhibition of one of the *P. aeruginosa* isolates around the ditches of extract and antibiotic discs.

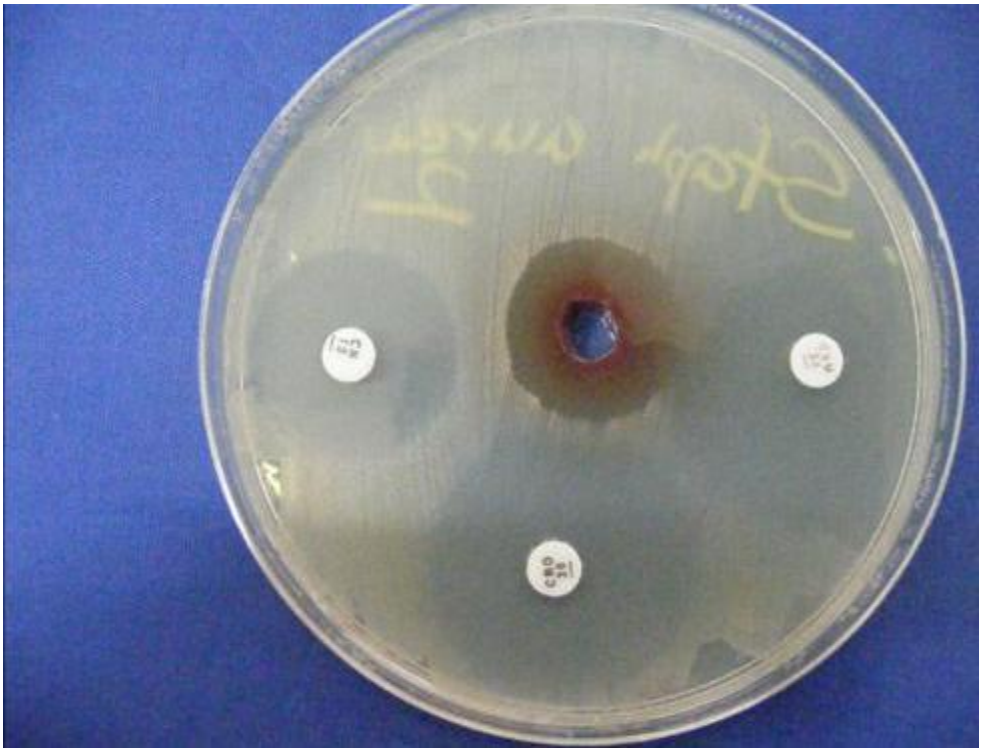

Plate 3. One of the *S. aureus* isolate and susceptibility profile.

both Gram positive and Gram negative bacterial isolates, (Ceftriaxone 12 - 30 mm, Gentamicin 0 - 28 mm, *B. ferruginea* extract 10 - 24 mm (Table 1 and Plates 1 - 3) however, further study will be carried out on larger number of microorganisms to establish this finding.

Irobi (1994) and colleaques reported that the Gram negative bacteria appeared more susceptible to the anti-microbial effects of the extracts than the Gram positive. This fact is in contrast to our findings, as all the *S. aureus,* a Gram positive bacteria tested showed better response than majority of the Gram negative bacteria tested (Plate 3 and Table 1).

This study has confirmed that the clinical response observed by the local people has scientific basis. Previous studies by Irobi et al. (1994), Muanza et al. (1994) and Jose and Kayode (2009) have also confirmed these results, although their mode of extraction was different. Therefore, further studies will therefore be necessary to determine the components of the crude extract obtained by boiling freshly collected stem bark as compared to other methods of extraction, and also to identify the components of extract that are responsible for the various anti-infective activities observed including the mode of action.

The experiences have shown that applications both topically and orally for 5 days to 1 week have produced cure in the treated individuals. Further studies are also necessary to determine optimal dosage, efficacy and safety of the extract.

In conclusion, this study has established that the crude extract obtained by boiling of the stem bark of *B. ferruginea* is effective for both fungal and bacterial agents and is effective in the treatment of Candidiasis.

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