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# Antimicrobial and Antihelmintic Effect of Wonderful Kola (*Buchholzia coriacea*)

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# Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

#### Article Information

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**Original Research Article** 

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# ABSTRACT

In the present study, the antimicrobial and antihelminthic effect of wonderful kola (*Buchholzia coriacea*) was examined. The kola nut was diced and dried in an air-draught oven. It was then pulverized using hammer mill. The resulting powder was soaked in ethanol and aqueous solutions to obtain the extracts. Wonderful kola was screened for phytochemical properties. Disc diffusion assay method was used to screen for antimicrobial activities of *B. coriacea*. The test organisms used were obtained from the University of Port Harcourt Teaching Hospitals, Nigeria. It was observed that saponin, flavonoid, carbohydrate, alkaloid were present in wonderful kola examined while other phytochemicals (Oxalate, Diterpenes, Terpenoid, Tanins, Protein, Steroids, Phenols, phlobatannins, Glycoside, Anthraquinones) tested for were absent. Wonderful kola exhibited antifungal and antimicrobial activities on the tested microorganisms. The antihelminthic effect of wonderful kola was also pronounced on the eggs of *Ascaris lumbricoides*. *B. coriacea* was found to be more active on the test pathogens than the ethanol and aqueous extracts. It was concluded that over exposure to air, sunlight, too much artificial heat and rapid drying causes a loss of essential

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oils and *B. coriacea* possesses an invaluable but yet to be tapped potentials which therefore justifies the traditional usefulness and clinical potentials of *Buchholzia coriacea*, a medicinal plant commonly used in different parts of the world.

Keywords: Antimicrobial; antihelminthic; wonderful kola; pathogens and phytochemicals.

#### **1. INTRODUCTION**

The plant Buchholzia coriacea is a shrub or medium-sized tree, evergreen, with a dense crown, large glossy leathery leaves arranged spirally and clustered at the ends of the branches, and conspicuous cream-white flowers in racemes at the end of the branch [1]. The bark of the plant Buchholzia coriacea is smooth, blackish-brown or dark-green. Slashes are deep red turning dark brown [2,3]. Buchholzia coriacea is commonly known as wonderful kola, musk tree, Kola pime, Elephant kola, Ndo, Doe-fiah, Eson-bese, Banda, Esson bossi, Kola Pimente, Okpokolo, Uwuro and Aponmu. Buchholzia coriacea has multiple medicinal values and was named wonderful kola because of its usage in traditional medicine. The plant parts commonly eaten are the seeds which are either cooked or eaten raw [4]. In Africa, it is useful in the treatment of hypertension and also prevents premature aging and has the ability to stop migraine headache when applied on the forehead [5,6]. The stem bark extract is applied as an enema to treat back pain. Non specified bark preparations are also applied externally against pleurisy, rheumatism, conjunctivitis, smallpox, scabies and other skin complaints. Leaf decoctions are used to treat sterility in women and seed oil is taken against menstruation problems and gastro-intestinal complaints. The seeds which have a peppery taste are used as a substitute of capsicum pepper (a hot red pepper fruit) [7,8]. Researchers have reported its traditional relevance in the treatment of illnesses and conditions caused by a variety of microorganisms. Such conditions include fever, headache and gonorrhea [9]. The spread of resistance to existing antibiotics has led to a diminished effectiveness of these useful agents, thereby highlighting the need for novel antibacterial agents. Plants have been sources of medicines for many generations. More than 80% of the populations in developing countries depend on plants for their medical needs [10]. It has been reported that about two-third of all plant species are found in the tropics. Some have been investigated while so many are yet to be studied. Less than 10 % of biodiversity has been tested for biological activity [11]. Substances that can either inhibit the growth of pathogens or kill

them and have little or no toxicity to host cells are considered good agents for developing new antimicrobial drugs [12]. Recent works have revealed the potential of several herbs as sources of drugs [13,14,15]. The screening of plant extracts and products for antimicrobial activity has shown that higher plants are potential sources of novel antibiotic prototypes [16]. This study is therefore aimed at assessing the antimicrobial and antihelmintic effect of wonderful kola (*Buchholzia coriacea*).

#### 2. MATERIALS AND METHODS

#### 2.1 Sample Collection

Fresh *B. coriacea* (wonderful kola) was obtained from Abuja Federal Capital Territory and Rumuola, Port Harcourt Rivers State and were identified at the Botany Department, University of Port Harcourt, Rivers State, Nigeria.

#### 2.2 Preparation of the Seed Extract

The fresh wonderful kola nuts were cleaned by the double disinfection method. They were washed thoroughly with distilled water to remove adhering particles after which they were soaked in 80% ethanol for 30 min. They were rinsed with distilled water and then washed with aqueous sodium hypochlorite (NaClO<sub>4</sub>) to reduce surface contamination. This was followed by rinsing with distilled water. The kola nuts were diced to facilitate drying in an air-draught oven at 60 °C for 72 h. The dried kola nuts were pulverized using a hammer mill. The powder was stored in desiccators to prevent moisture absorption and contamination.

Ethanol and aqueous extracts from *B. coriacea* powder were obtained and the percentage yield of the extracts was calculated as:

Total yield (%)

 $= \frac{Weight of extracts (g)}{Original weight of sample(g)} x 100$ 

#### 2.3 Ethanol Extract Preparation

Two hundred grams (200 g) of the pulverized kola nut was weighed using Satoric AG

Gottingen Electronic weighing balance. The weighed sample was soaked in 500 ml of ethanol contained in a conical flask mixture, swirled and allowed to stand for 24 h with interval stirring. The mixture was filtered using Whatman No.1 filter paper [17] into a clean beaker and the ethanol was recovered using a Soxhlet apparatus and was evaporated to dryness using a steam bath at 100°C.

# 2.4 Aqueous Extract Preparation

Two hundred grams (200 g) of the pulverized kola nut was weighed and macerated in 500 ml of distilled water. The mixtures were vigorously swirled. After the elaption of 24 h with interval stirring, the mixture was filtered using Whatman No.1 filter paper [18] into a clean beaker, and the filtrate was concentrated to dryness by evaporation using the steam bath at 100°C.

# 2.5 Preparation of Control Sample

Standardized antibiotics (ofloxacin and fluconazole) was aseptically used as the control in order to compare the diameter of zone of clearance from the extracts and 1ofloxacin. Ofloxacin (280 mg) was prepared by diluting 1 ml of oflaxacin in 19 mls of distilled water that is, 1:20 dilution (1+19 ml) given a final concentration of 2 mg/ml.

# 2.6 Phytochemical Analysis

Phytochemical tests were carried out using standard procedures to identify the constituents according to the methods of [19,20].

#### 2.6.1 Test for tannins

0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. 2 drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

#### 2.6.2 Test for saponin

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and swirled vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

#### 2.6.3 Test for flavonoids

A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed, indicating a positive test for flavonoids.

#### 2.6.4 Test for steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml  $H_2SO_4$ . The colour changed from violet to blue, indicating the presence of steroids.

#### 2.6.5 Test for terpenoids (Salkowski test)

5 ml of the extract was mixed in 2 ml of chloroform, and concentrated  $H_2SO_4$  (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

# 2.6.6 Test for cardiac glycosides (Keller – Killiani test)

5 ml of the extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

#### 2.6.7 Test for anthraquinones

0.5 g of the extract was boiled with 10 ml  $H_2SO_4$ and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour change.

#### 2.6.8 Test for alkaloids

0.5 g of the powdered extracts was stirred in 5 ml of 1% HCl solution on a steam bath for 5 mins. The mixture was then filtered using Whatman's No1 filter paper. To the filtrate, 2-4 drops of Dragendoff's reagent was added to 1 ml of the filtrate. An orange colour was observed indicating the presence of alkaloids.

# 2.7 Determination and Characterization of Antimicrobial Effects

#### 2.7.1 Disc diffusion method

Muller Hinton agar was used and sterile disc of 6 mm in diameter was impregnated with extract per disc.

#### 2.7.2 Preparation of disc

0. 1 ml of extract was dropped into sterile disc and allowed to dry .A sterile container was used to store the dry disc in a sterile laminar flow cabinet and store containers at frozen temperature in darkness until used.

### 2.7.3 Preparation of plates

Sterile Petri dishes were used and Mueller Hinton agar cooled below 500°C was poured 4 mm deep into the sterile petri dish (70 ml in 150 mm Petri dish, 25 ml in 90 mm diameter Petri dish) and the agar allowed to set. The prepared plate was stored in a sealed plastic at a temperature of 4 - 80°C. The surface of the agar was dried before plates were used to avoid any form of wetness on the agar plate.

# 2.7.4 Determination of resistance / susceptibility of Clinical Isolates to the Seed extract

The Kirby Bauer method [21] was used for sensitivity test on ethanoic extract and the organism tested were Escherichia spp., Staphylococcus Pseudomonas spp, spp. Candica albicans, Klebiella spp. and Streptococcus spp.

# 2.7.5 Kirby Bauer antimicrobial susceptibility testing

The kola seed extract was dissolved with distilled water and solutions were applied to the sterile filter paper discs (Whatman grade) the sterile filter paper disc was allowed to soak in the kola seed extract for 2 h and placed on the surface of the assay plates. Mueller Hinton was used [22]. Inoculums size of  $1 \times 10^8$  ml of the organism was pre-inoculated into the media [23], the plates were seeded with disc containing the extract and labeled appropriately. Twenty milligrams ofloxacin disc and fluconazole (for candida) were used as control. Using flame sterilized forceps; each disc was gently pressed to the agar.

Plates were incubated for 24 h at an incubation temperature of 37°C and 48 h for fungi and Zones of inhibition were measured.

# 2.8 Screening for Antihelminthic Activities

Eggs of Ascaris lumbricoides and Trichuris trichuria were used for the helminthes identification of B. coriacea. Eggs of Ascaris lumbricoides and Trichuris trichiuria used were obtained from Parasitology laboratory in the University of Port Harcourt Teaching Hospital, Nigeria. The extract was tested at 3 concentrations of  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  g/ml. Three bottles were prepared for biiou each concentration. 1ml of saline-stool mixture was inoculated into three bijou bottles representing three concentration  $(10^{-1}, 10^{-2} \text{ and } 10^{-3})$ , the control was inoculated with 1 ml of the salinestool mixture. The test substance was mix in the bijou bottle and incubated for 24 h at room temperature in the dark. After 24 h 0.15 ml from the bijou bottle smeared on a glass slid and a drop of iodine was added. The slide was examined under oil immersion microscope for the presence of eggs.

#### 2.8.1 Screening for antimicrobial activities

The zone of inhibition of extracts and control experiments was measured.

# 2.9 Determination of Antifungal Activity of the Extracts

Nutrient agar was poured into Petri dishes, allowed to set and bored with a Durham tube. Fungal culture was used to inoculate each of the agar plates after which about 0.01 ml of the extract was added. Incubation was done at 28°C for 120 h after which the plates were inspected for zones of inhibition.

# 3. RESULTS

# 3.1 Results of Phytochemical Screening

The preliminary phytochemical screening of the test plant *B. coriacea* are shown in Table 1. It showed the presence of saponin, flavonoid, carbohydrate, alkaloid and the absence of Oxalate, Diterpenes, Terpenoid, Tanins, Protein, Steroids, Phenols, Phlobatannins, Glycoside, Anthraquinones.

# Table 1. Chemical screening of the nonnutrient phytochemicals From *B. coriacea*

Compound	Test
Saponin	+ve
Alkaloid	+ve
Flavonoids	+ve
Oxalate	-ve
Diterpenes	-ve
Terpenoid	-ve
Tanins	+ve
Carbohydrates	+ve
Protein	-ve
Steroids	-ve
Phenols	-ve
phlobatannins	+ve
Glycoside	-ve
Anthraquinones	-ve

# 3.2 Antibacterial and Antifungal Activity of Wonderful Kola

The antibacterial and antifungal activity of a *Buchholzia coriacea* extract was assayed *in vitro* by agar disc diffusion against three bacterial species and a fungal species. Fig. 2 summarizes the microbial growth inhibition of both aqueous and ethanol extracts of *B. coriacea*.

# 3.3 Antihelminthic Activity of B. coriacea

The antihelminthic effect of *B. coriacea* after 24 h exposure of the eggs of *Ascaris lumbricoides* and *Trichuris trichuria* indicates that *B. coriacea* completely eliminated helminthic lives at all concentrations.

### Table 2. Antihelminthic activity of B. coriacea

Concentrations (w/v)	0 hours (eggs/ml)	24 hours (eggs/ml)
Neat	200	0
10 <sup>-1</sup> 10 <sup>-2</sup>	200	0
10 <sup>-2</sup>	200	0
10 <sup>-3</sup>	200	0
Control (Normal saline)	200	100

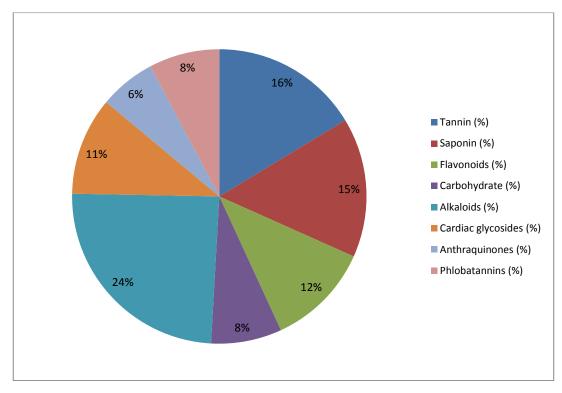


Fig. 1. % availability of non-nutrient phytochemicals from B. coriacea

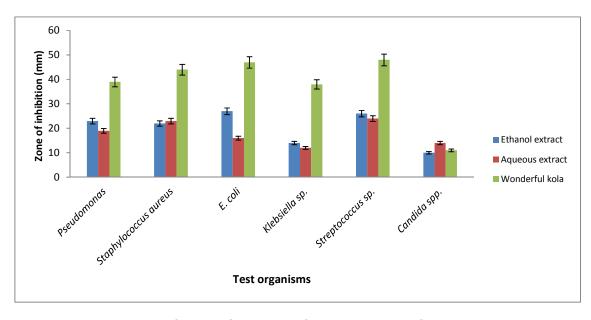


Fig. 2. Mean diameter of zones of inhibition of extracts obtained from various extraction techniques

# 4. DISCUSSION

The ethanol extracts of B. coriacea showed inhibitory zones ranging from 14-27 mm with all test organisms (Pseudomonas spp., E. coli., S. aureus., Klebsiella sp., Streptococcus sp. and Candida albicans. The aqueous extract of B. coriacea showed inhibitory zones of 12-23 mm with the test bacteria. In a related work by [24] the isolates were treated with n-hexane, methanol and chloroform extracts of B. coriacea leaf elicited modest antibacterial activities against the test isolates with E. coli, Staphylococcus aureus, Shigella species, Klebsiella pneumoniae and Bacillus subtilis susceptible. [24] noted that extracting solvents could bring about variation in spice extractive components, which may influence their antimicrobial activities. C. albicans resisted the ethanol extract of B. coriacea but could not resist the aqueous extract. Stem bark fractions of B. coriacea have been found to inhibit S. aureus, E. coli, S. typhii, P. aeruginosa, Candida albicans and A. flavus [25]. The fresh kola nut exhibited greater inhibitory effect on the test organisms than the ethanol and aqueous extracts, it showed inhibitory zones ranging from 39-48 mm with the three test bacteria (Pseudomonas, E. coli, and S. aureus) it was exposed to and it completely inhibited the growth of C. albicans. Ezekiel OO and Onyeoziri NF [26] observed a similar result when they carried out a study on the effect of the fresh kola, hexane and methanol extracts of B. coriacea on some food

borne pathogens (Esherichia coli, Enterococcus faecalis, Staphylococcus aureus, Trichoderma viride and Aspergillus niger). The relatively poor inhibitory effect of the extracts of B. coriacea compared with the fresh wonderful kola could be attributed to the heat applied during drying. The unit operations during the production of powder from the kola might have influenced their activity as some of the active ingredients may be volatile in nature. Likewise the low level of activity at a low extract concentration may suggest that the concentrations of the active constituent in the extracts are too low for any appreciable antibacterial activity. The phytochemical analysis revealed the presence of alkaloids, tannins, saponins, and flavonoids. It is also possible that the plant showed low antibacterial potential because all the aforementioned secondary metabolites were present in low concentration and the concentration of plant extract used was also low.

Then antihelminthic effect of *B. coriacea* was absolute. Ascaris lumbricoides and Trichuris trichuria used were observed to be completely eliminated by *B. coriacea* in all concentrations tested of  $10^{-1}$  to  $10^{-4}$ . The data obtained from the study implies that *B. coriacea* has more antiheliminthic activity than antibacterial. Ajaiyeoba et al. reported similar findings when the anthelmintic properties of Buchholzia coriaceae was tested against *Fasciola gigantica*, *Taenia solium* and *Pheritima pasthuma*.

# 5. CONCLUSION

The fresh kola was found to be more active on the test organism than the ethanol and aqueous extracts. The lower inhibitory properties of the extracts suggests that over exposure to air, sunlight, too much artificial heat and rapid drying can cause a loss of essential oils. This study indicates clearly that *B. coriacea* possesses an invaluable but yet to be tapped potentials which therefore justify the traditional usefulness and clinical potentials of *Buchholzia coriacea*, a medicinal plant commonly used in different parts of the world.

# 6. RECOMMENDATIONS

It is therefore recommended that:

- The attention of the general public should be drawn to the use of natural products in the management of diseases
- 2. More work should be done to ascertain the active principles of the plant.
- 3. The development of plant products into standardized, quality-controlled phytopharmaceuticals should be encouraged
- The characterization of its bioactive component, which can be used in the development of more reliable and safer drugs.

# COMPETING INTERESTS

Authors have declared that no competing interests exist.

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