ANTIMICROBIAL AND ANTIHELMINTIC EFFECT OF WONDERFUL KOLA (*Buchholzia coriacea*)

ABSTRACT

In the present study, the antimicrobial and antihelminthic effect of wonderful kola (*Buchholzia coricea*) 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 antimicromial activities of *B. coricea*. 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 eggs on *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 causeds a loss of essential 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

1.0 INTRODUCTION~~ntroduction:~~

The plant *Buchholzia coricea* 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 branches (Akpayung *et al.*1995). The bark of the plant *Buchholzia coricea* is smooth, blackish-brown or dark-green. Slashes are deep red turning dark brown (Akpayung *et al.*1995; Awouters *et al.* 1995). *Buchholzia coricea* is commonly known as wonderful cola, musk tree, Cola pime, Elephant cola, Ndo, Doe-fiah, Eson-bese, Banda, Esson bossi, Kola Pimente, Okpokolo, Uwuro and Aponmu. *Buchholzia coricea* 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 (Lemmens, 2013). 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 (Anowi *et al*., 2012; Nwachukwu *et al*., 2014). The stem bark extract is applied as an enema to treat back pain. Non specified bark preparations are also applied

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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) (Anowi *et al*., 2012; Nwachukwu *et al*., 2014). Researchers have reported its traditional relevance in the treatment of illnesses and conditions caused by a variety of microorganisms. Such conditions include fevers, headaches and gonorrhea (Nweze *et al.*, 2009; Keay *et al*., 1989). 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 (Farnsworth, 1988). 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. lLess than 10 % of biodiversity has been tested for biological activity (Nwafor *et al.*, 2001). 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 (Masoko *et al.*, 2005). Recent works have revealed the potential of several herbs as sources of drugs (Ajaiyeoba *et al.*, 2001; Nweze and Asuzu, 2006: Ezekiel and Onyeoziri, 2009; Mbata *et al*., 2009). The screening of plant extracts and products for antimicrobial activity has shown that higher plants are potential sources of novel antibiotic prototypes (Afolayan, 2003). This study is therefore aimed at assessing the antimicrobial and antihelmintic effect of wonderful kola (*Buchholzia coricea*).

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, Nigeria and where identified at the Bbotany Ddepartment, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

2.2: Preparation of the Seed Extract

The fresh wonderful kolanuts 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 ($NaClO_4$) to reduce surface contamination. This was followed by rinsing with distilled water. The kolanuts were diced to facilitate drying in an air-draught oven at 60 ºC for 72 h. The dried kolanuts were pulverized using a hammer mill. The powder was stored in desiccators to prevent moisture absorption and contamination.

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

$$\text{Total yield (\%)} = \frac{\text{Weight of extracts}}{\text{Original weight of sample}} \times 100$$

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2.3: Ethanol Extract Preparation

Two hundred grams (200 g) of the pulverized kolanut 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 N~~a~~o.1 filter paper (Azoro, 2002) into a clean beaker and the ethanol was recovered using a S~~s~~oxhlet apparatus and was evaporated to dryness using a steam bath at 100 ºC.

2,4: Aqueous Extract Preparation

Two hundred grams (200 g) of the pulverized kolanut 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 (Azoro, 2002) into a clean beaker, and the filterate was concentrated to dryness by evaporation using the steam bath at 100 ºC.

2.5: Preparation of control Sample

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

2.6: Phytochemical Analysis

Phytochemical tests were carried out using standard procedures to identify the constituents as described by…….(ref…).

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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. Two (2) drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration (Ref….).

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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 steriods: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue, indicating the presence of steroids.

2.6.5: Test for terpenoids (Salkowski test): 5ml 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.

2.6.5: Test for cardiac glycosides (Keller-Killani test): 5ml 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.

2.6.6: Test for Anthraquinones: 0.5 g of the extract was boiled with 10 ml H_2S0_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.

2.6.7: Test for Alkaloids: 0.5 g of the powdered extracts was stirred in 5 ml of 1% HCl solution ~~aq~~ on a steam bath for 5 mins. The mixture was then filtered using Whatman's N~~n~~o. 1 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 ºC. 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 (Bauer *et al*., 1966) was used for sensitivity test on ethanoic extract and the organism tested were *Escherichia* spp., *Staphylococcus* spp, *Pseudomonas* spp. *Candicas albicans, Klebiella* spp. and *Streptococcus* spp.

2.7.6: 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 (Laurens, 2004). Inoculums size of 1x10^8 ml of the organism was pre-inoculated into the media (Baris *et al*., 2006), 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 forcept, each disc was gently pressed to the agar to ensure that the disc is attached to the agar. Plates were incubated for 24 h at an incubation temperature of 37 ºC and 48 h for fungi and Zones of inhibition were measured.

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2.8: Screening for antihelminthic activities

Eggs of *Ascaris lumbricoides* and *Trichuris trichuria* were used for the helminthes identification of *B. coricea*. Eggs of *Ascaris lumbricoides* and *Trichuris trichuria* 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 bijou bottles were prepared for each concentration. 1ml of saline-stool mixture was inoculated into three bijou bottles representing three concentration (10^{-1}, 10^{-2} and 10^{-3}), the control was inoculated with 1 ml of the saline-stool 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. The survivors were recorded and multiplied by 100 eggs/ml.

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.

RESULTS

3.1: Results of Phytochemical screening

~~Table 1. presents t~~The preliminary phytochemical screening of the test plant *B. coricea* ~~.~~ 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, P~~p~~hlobatannins, Glycoside, Anthraquinones.

3.2: Antibacterial and Antifungal Activity of Wonderful cola

The antibacterial and antifungal activity of a *Buchholzia coricea* 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. coricea*.

3.3: Antihelminthic Activity of *B. coricea*

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

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Table. 1.Chemical screening of the non-nutrient phytochemicals From *B. coricea*

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

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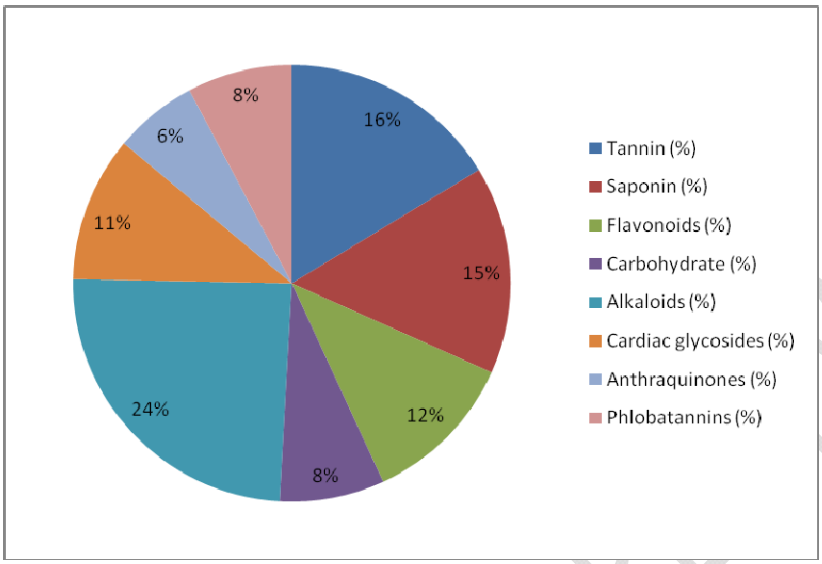

Fig.1. % Availability of Non-nutrient Phytochemicals from *B. coricea*

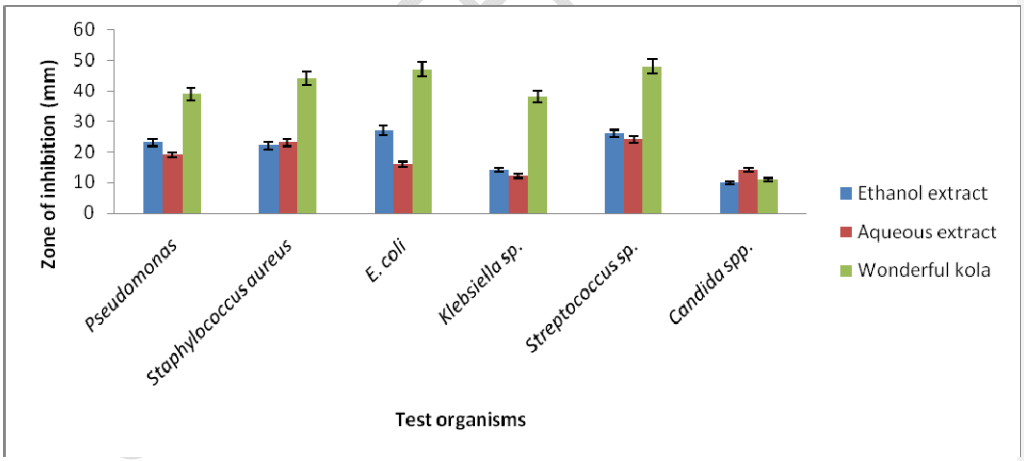

Fig.2. Mean diameter of zones of inhibition of extracts obtained from various extraction techniques

Table.2. Antihelminthic activity of *B. coricea*

Concentrations (w/v)	0 hours (eggs/ml)	24 hours (eggs/ml)
Neat	200	0
10^{-1}	200	0
10^{-2}	200	0
10^{-3}	200	0
Control (Normal saline)	200	100

DISCUSION ~~iscussions~~

The ethanol extracts of *B. coriacea* showed inhibitory zones ranging from 14–27 mm with all test organisms (*Pseudomonas* spp., *E. coli*., *S. aureus*., *Klesiella* 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 Chika *et al*.(2012) 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. Zaika (1988) 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* (Ajayeoba *et al*., 2003). The fresh kolanut 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 and Onyeoziri (2009) observed a similar result when they carried out a study on the effect of the fresh kola, hexane and methanol extracts of *B. coricea* 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 (Savitri *et al*., 1986). 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 (Desrosier, 1977). 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

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antibacterial activity (Uchechi and Oghenerobo, 2010). 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. coricea* was absolute. *Ascaris lumbricoides* and *Trichuris trichuria* used were observed to be completely eliminated by *B. coricea* in all concentrations tested of 10^{-1} to 10^{-4}. The data obtained from the study implies that *B. coricea* has ~~is~~ more antiheliminthic activity than antibacterial effect. Ajaiyeoba *et al*. reported similar findings when the anthelmintic properties of *Buchholzia coriaceae* was tested against *Fasciola gigantica, Taenia solium and Pheritima pasthuma*.

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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 suggest ~~confirms~~ 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.

Recommendations

It is therefore recommended that

1. 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 phyto-pharmaceuticals should be encouraged.
4. The characterization of its bioactive component, which can be used in the development of more reliable and safer drugs should be investigated.

REFERENCE

Adisa, R. A., Choudhary, M.I. and Olorunsogo, O. O. (2011). Hypoglycemic activity of *Buchholzia coriacea* (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice. *Experimental Toxicology Pathology*. 7: 619-25.

Ajaiyeoba, E. O., Onocha, P. A. and Olarenwaju, O. T. (2001). *In vitro* Anthelmintic Properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* Extracts. *Pharmaceutical Biology*. 3: 217-220.

Ajaiyeoba, E. O., Onocha, P. A., Nwozo, S. O. and Sama, W. (2003). Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. *Fitoterapia*. 7: 706-9.

Anowi, F. C., Ike, C., Ezeokafor, E. and Ebere, C. (2012). The Phytochemical, Antispamodic and Antidiarrhoea properties of the methanol extract of the leaves of *Buchholzia coriacea family Capparaceae. International Journal of Current Pharmaceutical Research.* 4(3): 52-55.

Chika, E., Ikegbunam, M., Ugwu, C., Araka, O., Iroha, I., Adikwu, M. l. and Esimone, C. (2012). Evaluation of antibacterial activity of the leave extracts of *Buchholzia coriacea*. *Asian Journal of Pharmaceutical and Biological Research. 2*(4): 204-208.

Chinaka, O. N., Okwoche, J. O., Florence, C. N. and Nkeiruka, E. U. (2012). Effects of Methanol Extract of *Buchholzia coriacea* Fruit in Streptozotocin induced Diabetic Rats. *Journal of Pharmacology and Toxicology*. 7 (4): 181-191.

Chinedu, F. A., Chibeze, I., Uchechukwu, A. U. and Chukwuenweiwe, E. (2012). Phytochemical Analysis and Antipyretic Properties Of The Methanol Extract Of The Leaves Of *Buchholzia coriacea* (Family Capparaceae). *Asian Journal of Biochemical and Pharmaceutical Research. 2* (2); 340-345.

Ejikeugwu, C., Umeokoli, B., Iroha, I., Ugwu, M., Esimone, C. (2014). Phytochemical and Antibacterial Screening of Crude Extracts from Leaves of Wonderful Kola. *American Journal of Life Sciences*. 2 (6-3): 9-12.

Ezekiel, O. O. and Onyeoziri, N. F. (2009). Preliminary studies on the antimicrobial properties of *Buchholzia coriacea* (wonderful kola). *African Journal of Biotechnology*. 8 (3): 472-474.

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Lemmens, R. H. M. J. (2013). *Buchholzia coriacea* Engl. In: Schmelzer, G.H. and Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. Accessed 3 Jan. 2016.

Nwachukwu, M. I., Duru, M. K. C., Amadi, B. A. and Nwachukwu, I. O. (2014). Comparative Evaluation of Phytoconstituents, Antibacterial Activities and Proximate Contents of Fresh, Oven Dried Uncooked and Cooked Samples of *Buchholzia coriacea* Seed and Their Effects on Hepatocellular Integrity. *International Journal of Pharmaceutical Science Invention*. 3 (6): 41-49.

Nweze, N. E., Anene, B. M. and Asuzu, I. U. (2011). Investigation of the antitrypanosomal activity of *Buchholzia coriacea* seed extract against a field strain of *Trypanosoma congolense*. *African Journal of Traditional, Complementary, and Alternative Medicines*. 8 (5): 175-180.

Ogunmefun, O. T. and Ajaiyeoba, E. O. (2013). Phytochemical Analysis and Antifungal Activities of *Gynandropsis gynandra* (Spider flower) and *Buchholzia coriacea* (Musk tree) (Fam: Capparidaceae) on Some Common Fungal Isolates. *Journal of Biological Sciences and Bioconservation*. 5 (1): 75-85.

Okoli, B. J., Okere, O. S. and Adeyemo, S. O. (2010). Antiplasmodial activity of *Buchholzia coriancea*. *Journal of Medical and Applied Biosciences*. *2*: 21-29.

Theophine, C. O., Peter. A. A, Chinenye, L. L., Adaobi., C. E., Collins, A. O. (2012). Anti-diabetic Effects of Methanol Extract of the Seeds of *Buchholzia coriacea* and its Synergistic Effects with Metformin. *Asian Journal of Biomedical and Pharmaceutical Sciences*. 2(12): 32-36.

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