

ANTIMICROBIAL AND ANTIHELMINTIC EFFECT OF WONDERFUL KOLA

(*Buchholzia coriacea*)

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 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 caused 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

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 branches (Akpaiyung *et al.* 1995). The bark of the plant *Buchholzia coriacea* is smooth, blackish-brown or dark-green. Slashes are deep red turning dark brown (Akpaiyung *et al.* 1995; Awouters *et al.* 1995). *Buchholzia coriacea* is commonly known as wonderful kola, musk tree, Cola pime, Elephant cola, 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 (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. Less 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*).

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62 **MATERIALS AND METHODS**

63 **2.1: Sample Collection**

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

67 **2.2: Preparation of the Seed Extract**

68 The fresh wonderful kolanuts were cleaned by the double disinfection method. They were
69 washed thoroughly with distilled water to remove adhering particles after which they
70 were soaked in 80% ethanol for 30 min. They were rinsed with distilled water and then
71 washed with aqueous sodium hypochlorite (NaClO₄) to reduce surface contamination.
72 This was followed by rinsing with distilled water. The kolanuts were diced to facilitate
73 drying in an air-draught oven at 60 °C for 72 h. The dried kolanuts were pulverized using
74 a hammer mill. The powder was stored in desiccators to prevent moisture absorption and
75 contamination.

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

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

Comment [AO4]: Kindly insert unit of weight used.

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 No.1 filter paper (Azoro, 2002) 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 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 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 ofloxacin. Ofloxacin (280 mg) was prepared by diluting 1ml of ofloxacin in 19_mls of distilled water that is, 1:20 dilution (1+19 ml) giving 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

110 filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was
111 observed, indicating a positive test for flavonoids.

112 | **2.6.4: Test for steroids:** Two ml of acetic anhydride was added to 0.5 g ethanolic extract
113 of each sample with 2 ml H₂SO₄. The colour changed from violet to blue, indicating the
114 presence of steroids.

115 | **2.6.5: Test for terpenoids (Salkowski test):** 5ml of the extract was mixed in 2 ml of
116 chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A
117 reddish brown colouration of the inter face was formed to show positive results for the
118 presence of terpenoids.

119 | **2.6.5: Test for cardiac glycosides (Keller-Killani test):** 5ml of the extracts was treated
120 with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was
121 underlayered with 1 ml of concentrated sulphuric acid. A brown ring at the interface
122 indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the
123 brown ring, while in the acetic acid layer, a greenish ring may form just gradually
124 throughout thin layer.

125 | **2.6.6: Test for Anthraquinones:** 0.5 g of the extract was boiled with 10 ml H₂SO₄ and
126 filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer
127 was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting
128 solution was observed for colour change.

129 | **2.6.7: Test for Alkaloids:** 0.5 g of the powdered extracts was stirred in 5 ml of 1% HCl
130 solution on a steam bath for 5 mins. The mixture was then filtered using Whatman's
131 No. 1 filter paper. To the filtrate, 2-4 drops of Dragendoff's reagent was added to 1 ml
132 of the filtrate. An orange colour was observed indicating the presence of alkaloids.

133 | **2.7: Determination and characterization of antimicrobial effects**

134 | **2.7.1: Disc Diffusion method**

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

137 | **2.7.2: Preparation of disc**

138 | 0.1 ml of extract was dropped into sterile disc and allowed to dry. A sterile container was used
139 to store the dry disc in a sterile laminar flow cabinet and store containers at frozen temperature in
140 darkness until used.

141 | **2.7.3: Preparation of Plates**

142 Sterile Petri dishes were used and Mueller Hinton agar cooled below 50°C was poured 4 mm
143 deep into the sterile petri dish (70 ml in 150 mm Petri dish, 25 ml in 90 mm diameter Petri dish)
144 and the agar allowed to set. The prepared plate was stored in a sealed plastic at a temperature of

145 4 - 80 °C. The surface of the agar was dried before plates were used to avoid any form of wetness
146 on the agar plate.

147

148 **2.7.4: Determination of resistance/susceptibility of Clinical Isolates to the Seed extract**

149 The Kirby Bauer method (Bauer *et al.*, 1966) was used for sensitivity test on ethanoic extract and
150 the organism tested were *Escherichia* spp., *Staphylococcus* spp, *Pseudomonas* spp. *Candida*
151 *albicans*, *Klebsiella* spp. and *Streptococcus* spp.

152 **2.7.6: Kirby Bauer Antimicrobial susceptibility testing**

153 The kola seed extract was dissolved with distilled water and solutions were applied to the sterile
154 filter paper discs (Whatman grade) the sterile filter paper disc was allowed to soak in the kola
155 seed extract for 2 h and placed on the surface of the assay plates. Mueller Hinton was used
156 (Laurens, 2004). Inoculums size of 1×10^8 ml of the organism was pre-inoculated into the media
157 (Baris *et al.*, 2006), the plates were seeded with disc containing the extract and labeled
158 appropriately. Twenty milligrams ofloxacin disc and fluconazole (for candida) were used as
159 control. Using flame sterilized forceps, each disc was gently pressed to the agar to ensure that the
160 disc is attached to the agar. Plates were incubated for 24 h at an incubation temperature of 37 °C
161 and 48 h for fungi and Zones of inhibition were measured.

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162 **2.8: Screening for antihelminthic activities**

163 Eggs of *Ascaris lumbricoides* and *Trichuris trichuria* were used for the helminthes
164 identification of *B. coricea*. Eggs of *Ascaris lumbricoides* and *Trichuris trichuria* used
165 were obtained from Parasitology laboratory in the University of Port Harcourt Teaching
166 Hospital, [Nigeria](#). The extract was tested at 3 concentrations of 10^{-1} , 10^{-2} , and 10^{-3} g/ml.
167 Three bijou bottles were prepared for each concentration. 1ml of saline-stool mixture was
168 inoculated into three bijou bottles representing three concentration (10^{-1} , 10^{-2} and 10^{-3}),
169 the control was inoculated with 1 ml of the saline-stool mixture. The test substance was
170 mix in the bijou bottle and incubated for 24 h at room temperature in the dark. After 24 h
171 0.15 ml from the bijou bottle smeared on a glass slide and a drop of iodine was added. The
172 slide was examined under oil immersion microscope for the presence of eggs. The
173 survivors were recorded and multiplied by 100 eggs/ml.

174 **2.8.1: Screening for antimicrobial activities**

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

176 **2.9: Determination of antifungal activity of the extracts:**

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

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184 RESULTS

185 **3.1: Results of Phytochemical screening**

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

190 **3.2: Antibacterial and Antifungal Activity of Wonderful cola**

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

195 **3.3: Anthelmintic Activity of *B. coricea***

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

199

200
201

**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

202
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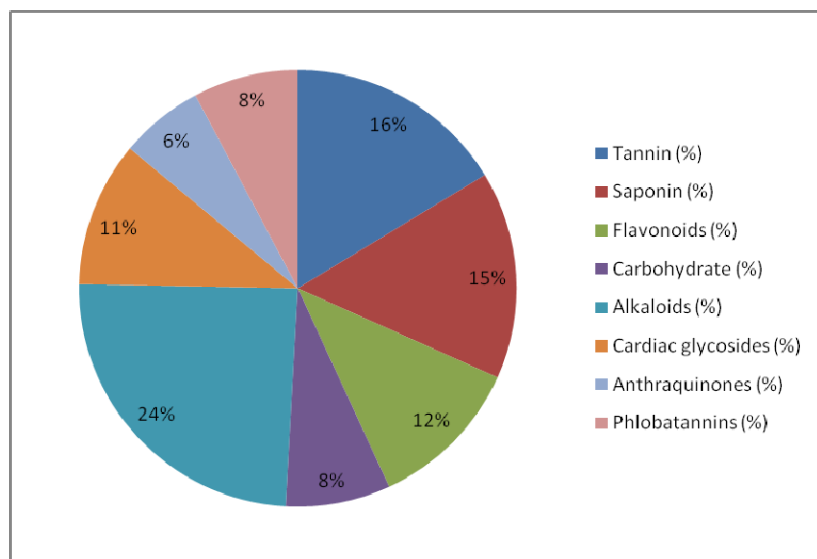


Fig.1. % Availability of Non-nutrient Phytochemicals from *B. coricea*

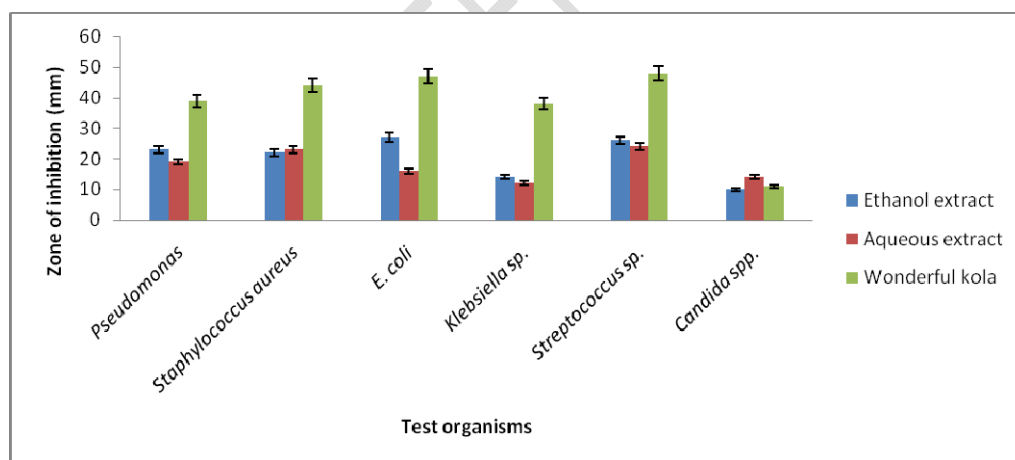


Fig.2. Mean diameter of zones of inhibition of extracts obtained from various extraction techniques

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

217

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

218

219 **DISCUSSION**

220 The ethanol extracts of *B. coriacea* showed inhibitory zones ranging from 14–27 mm
 221 with all test organisms (*Pseudomonas* spp., *E. coli*, *S. aureus*, *Klesiella* sp.,
 222 *Streptococcus* sp. and *Candida albicans*. The aqueous extract of *B. coriacea* showed
 223 inhibitory zones of 12-23 mm with the test bacteria. In a related work by Chika *et*
 224 *al.*(2012) the isolates were treated with n-hexane, methanol and chloroform extracts of *B.*
 225 *coriacea* leaf elicited modest antibacterial activities against the test isolates with *E. coli*,
 226 *Staphylococcus aureus*, *Shigella species*, *Klebsiella pneumoniae* and *Bacillus subtilis*
 227 susceptible. Zaika (1988) noted that extracting solvents could bring about variation in
 228 spice extractive components, which may influence their antimicrobial activities. *C.*
 229 *albicans* resisted the ethanol extract of *B. coriacea* but could not resist the aqueous
 230 extract. Stem bark fractions of *B. coriacea* have been found to inhibit *S. aureus*, *E. coli*,
 231 *S. typhii*, *P. aeruginosa*, *Candida albicans* and *A. flavus* (Ajayeoba *et al.*, 2003). The
 232 fresh kolanut exhibited greater inhibitory effect on the test organisms than the ethanol
 233 and aqueous extracts, it showed inhibitory zones ranging from 39-48 mm with the three
 234 test bacteria (*Pseudomonas*, *E. coli*, and *S. aureus*) it was exposed to and it completely
 235 inhibited the growth of *C. albicans*. Ezekiel and Onyeoziri (2009) observed a similar
 236 result when they carried out a study on the effect of the fresh kola, hexane and methanol
 237 | extracts of *B. coricea* on some food borne pathogens (*Escherichia coli*, *Enterococcus*
 238 *faecalis*, *Staphylococcus aureus*, *Trichoderma viride* and *Aspergillus niger*). The
 239 relatively poor inhibitory effect of the extracts of *B. coriacea* compared with the fresh
 240 wonderful kola could be attributed to the heat applied during drying (Savitri *et al.*, 1986).
 241 The unit operations during the production of powder from the kola might have influenced
 242 their activity as some of the active ingredients may be volatile in nature (Desrosier,
 243 1977). Likewise the low level of activity at a low extract concentration may suggest that
 244 the concentrations of the active constituent in the extracts are too low for any appreciable

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245 antibacterial activity (Uchechi and Oghenerobo, 2010). The phytochemical analysis
246 revealed the presence of alkaloids, tannins, saponins, and flavonoids. It is also possible
247 that the plant showed low antibacterial potential because all the aforementioned
248 secondary metabolites were present in low concentration and the concentration of plant
249 extract used was also low.

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

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256 Conclusion

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

264 Recommendations

265 It is therefore recommended that

- 266 | 1. The attention of the general public should be drawn to the use of natural products in
267 the management of diseases
- 268 | 2. More work should be done to ascertain the active principles of the plant.
- 269 | 3. The development of plant products into standardized, quality-controlled phyto-
270 pharmaceuticals should be encouraged.
- 271 | 4. The characterization of its bioactive component, which can be used in the
272 development of more reliable and safer drugs should be investigated.

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274

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Comment [A08]: Most of the references are too old. Please include new/recent finding about the nut.

Comment [A09]: Should read "Coriacea"

Comment [A010]: "Antispasmodic"

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Comment [AO12]: "coriacea"