Original Research Article

JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY

Antibacterial and synergistic efficacy of acetone extracts of *Garcinia kola* (Bitter kola) and *Buchholzia coriacea* (Wonderful kola)

Lovet T. Kigigha, Remita E. Selekere, Sylvester C. Izah*

Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria

Abstract

This study investigated the antibacterial and synergistic efficacy of acetone extracts of seed of *Buchholzia coriacea* (wonderful kola) and *Garcinia kola* (bitter kola). Extraction was carried out using acetone, and Agar well diffusion sensitivity testing method was adopted. Results showed that the highest zone of inhibition was achieved when both kola was mixed at ratio of 1:1. The zone of inhibition of synergy extract of *Garcinia kola* nut and *Buchholzia coriacea* seed for *E. coli, Pseudomonas sp, Staphylococcus aureus* and *Bacillus* sp were 14.00 mm, 12.33 mm, 13.0 mm and 12.00 mm respectively. Furthermore, individual zones of inhibition for *E. coli, Pseudomonas* sp., *Staphylococcus aureus* and *Bacillus* sp. were 11.33 mm, 9.67 mm, 13.67 mm and 11.00 mm respectively for *Buchholzia coriacea* and 13.00 mm, 11.00 mm, 13.0 mm and 12.67 mm respectively for *Garcinia kola.* Analysis of variance showed that there was no significant difference ($p > 0.05$) among the different isolate for the different extracts except for that of *Buchholzia coriacea.* This study showed that synergistic efficacy of bitter kola and wonderful kola produced superior antimicrobial effects. Again, the positive result for both gram negative bacteria (*E. coli, Pseudomonas* sp.) and gram positive bacteria (*Staphylococcus aureus* and *Bacillus* sp.) suggest that they could be used as broad spectrum antibiotics.

Keywords: *Garcinia kola*, *Buchholzia coriacea*, Antimicrobial activity, Bitter Kola, Wonderful kola

1. Introduction

Drug resistance, incidence of emerging and reemerging disease is a major challenge in the field of pharmaceutical microbiology despite its advancements [1-3]. According to Ezeigbo *et al*. [4], Ejikeugwu *et al*. [5], drug resistant or multi drug resistant strain is a major factor leading to search of new antimicrobial agents. Several plants have demonstrated antimicrobial properties [2, 3, 6, 7]. Basically, medicinal plants are plants whose tissues including roots, leaves, bark possess healing properties [2, 3, 6 – 8]. The use of plant for food/spices and herbal medicine against some disease is a long term practice [9]. Hence the field of herbal science is as old as human history.

According to world health organization, over 80% of global population rely on herbs for cure of several diseases especially individuals residing in developing countries [8, 10-14]. Under rural areas, Amole and Ilori [15] attributed the use of herbal remedies to inaccessibility of modern drugs and economic factor. Poverty and educational level are also contributing factor on the use of medicinal plants.

Plants have proven to be a credible source of new drugs [10, 16]. Globally, plants are used for treatment of specific disease condition [17]. Several plant species are abound in the world. Some medicinal plants are important to a particular locality and not to another. Several cola, sometimes spelled as kola, are also abound in the world.

Buchholzia coriacea (Wonderful kola), belonging to the family of Capparidaceae is an evergreen shrub, which is distributed in Cameroon, Central African Republic, Gabon, Congo, Angola, Nigeria, Ghana etc [9, 18, 19]. *Buchholzia coriacea* can grow up to 20 m tall with large, glossy, leathery leaves and conspicous creamy white flower [20].

Garcinia kola often called bitter cola/ kola is another kola family that is highly distributed in West Africa including Sierra Leone and Nigeria [4]. *Garcinia kola* belongs to the Guttiferae family and thrives in forest. The plant can attain up 12 m height and produces fruit mostly between July to October of each year.

Garcinia kola have been proven to have several medicinal properties including antioxidant, antimicrobial, anti-inflammatory, hypocholesterolemic, antiviral, anti-diarrhoeal, antiproliferative, antiandrogenic and anticoronary disease activities [10, 21]. On the specific parts, the seed can be used as a stimulant and for many other illnesses such as respiratory tract infection, gastritis, bronchitis, antiseptic, hepatitis and throat problems [4]. Authors have also reported that it has antibacterial potentials [4, 22, 23].

Buchholzia coriacea also has antimicrobial, anti-inflammatory, anthelmintic, anti-trypanosomal, hypoglycaemic and analgesic activities [9, 24]. It is also used against headache, sinusitis, bronchitis, opthalmias, pleurisy, kidney pain and nasal congestion [9]. Specifically the seed is used to make decoctions against hyperthesion, diabetes, cold, cough, rheumatism, headache, catarrh and

*Corresponding author; E-mail: chivestizah@gmail.com

improve memory [9]. The seed also have antimicrobial properties [19, 20, 25]. In a review study, Erhirhie *et al*. [26] reported that wonderful kola is a potential therapy for several diseases including diarrhoea, malaria, rheumatism, ulcers, worm infection, asthma and cough, diabetes, hypertension, psychiatric disorders and impotence among others.

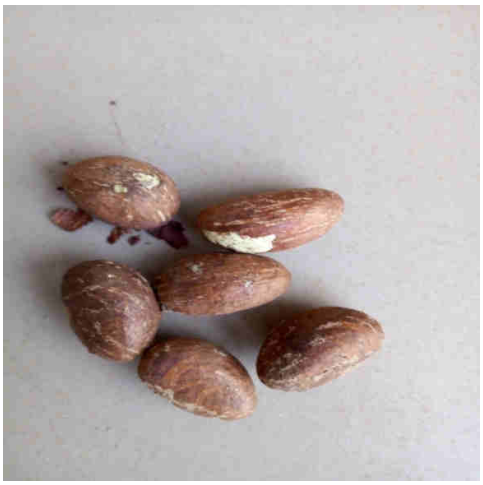
Most of the study on the antimicrobial activities of both kola focused against both gram positive and gram negative bacteria. But informaton on the synergistic efficacy of both plant is deficient in literature. Hence, this study aimed at assessing the antibacterial and synergistic efficacy of acetone extract of wonderful kola and bitter kola.

2. Materials and methods

2.1. Sample procurement

Triplicate samples of the Wonder kola and Bitter kola were purchased from Swali market in Yenagoa, Bayelsa state, Nigeria and were transported to the laboratory of the Department of Biological Sciences, Faculty of Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State where the experimental set up was conducted.

Wonder kola



Bitter kola

Figure 1. Pictures of Wonder kola and Bitter kola

2.2. Preparation of plant extracts

Both kola were first grounded using mortar and pestle. The samples were sun dried and blended to powder. About 40 g of dried kola were weighed and soaked in 100 mL of acetone. After 72 hr of soaking, it was filtered using muslin cloth and re-filtered using Whatman No.1 filter paper.

2.3. Source of the microorganisms for the experiment

The microorganisms used in this study were obtained from the stock culture in the Medical Microbiology unit, Federal Medical Centre, Yenagoa, Bayelsa state. The purity of the bacteria was checked using the scheme of Benson [27] and Cheesbrough [28]. Other confirmatory test for special microbes such as *Staphylococcus aureus* using Mannitol salt Agar; *E. coli* using Levine's eosin Methylene Blue (EMB) Agar were carried out.

2.4. Experimental design

The Completely Randomized Design (CRD) experimental method was adopted for this study. In the sensitivity studies, each isolate was analysed in triplicate for the individual extracts. Furthermore, synergy of both type of kola was carried out at a ratio of 1:1.

2.5. Antibacterial susceptibility test

Agar well diffusion method previously described by Opoku and Akoto [29] with slight modification using the guide of Kigigha *et al*. [6, 7], Epidi *et al*. [2, 3] were adopted in this study. The test organisms were inoculated into prepared peptone water and incubated. After 24 hr of incubation, 0.2 mL of bacteria was aseptically inoculated into gelled sterile nutrient agar and then swabbed uniformly on the entire surface. Three wells of 6.0 mm diameter were made with sterile cock borer. Thereafter 0.3 mL of the concentrated extracted was dispensed into the wells. The plates were masked with tape to avoid shifting and contamination. All the plates were incubated at 37 ºC for 24 hr and then, the resultant zone of inhibition was measured.

2.6. Statistical analysis

SPSS software version 20 was used to carry out the statistical analysis. The data were expressed as mean ± standard error. A one-way analysis of variance was used to show significant variation at $p = 0.05$. Multiple comparisons were carried out using Tukey Honestly Significant Difference to compare the means.

3. Results and Discussion

The zone of inhibition at varying concentrations for the acetone extracts of *Buchholzia coriacea* nut is presented in Table 1. The zone of inhibition for *E. coli, Pseudomonas sp, Staphylococcus aureus* and *Bacillus* spp was 11.33 mm, 9.67 mm, 13.67 mm and 11.00 mm respectively (100%), 8.00 mm, 7.67 mm, 10.33 mm and 10.33 mm respectively (95%) and 7.33 mm, 0.00 mm, 8.33 mm and 0.00 mm respectively (90%). At 85%, the extract was not effective against the selected pathogens under study. Basically, there was significant difference ($p < 0.05$) among the different microbes for each of the concentrations. Furthermore, there was no significant variation between the means of *Pseudomonas* and *Bacillus* sp, and between *Staphylococcus aureus* and *E. coli.*

Table 1. Zones of Inhibition (mm) of acetone extracts of *Buchholzia coriacea* nut

Isolates	100%	95%	90%	85%
E. coli	11.33±0.33ab	8.00±0.58b	7.33±0.33b	0.00±0.00
Pseudomonas sp	9.67±0.33a	7.67±0.67a	0.00±0.00a	0.00±0.00
Staphylococcus aureus	13.67±0.88b	10.33±033b	8.33±0.33b	0.00±0.00
Bacillus sp	11.00±0.58a	7.67±0.33a	0.00±0.00a	0.00±0.00

Data is expressed as mean ± SE; a and b represent significant difference ($p < 0.05$) according to Tukey HSD statistics

Table 2. Zones of Inhibition (mm) of acetone extracts of *Garcinia kola* nut

Isolates	100%	95%	90%	85%
E.coli	13.00±0.58a	9.67±0.33b	7.67±0.33b	0.00±0.00
Pseudomonas sp	11.00±0.58a	7.67±0.33a	0.00±0.00a	0.00±0.00
Staphylococcus aureus	13.00±0.58a	10.00±0.58b	7.33±0.33b	0.00±0.00
Bacillus sp	12.67±0.33a	8.67±0.33ab	0.00±0.00a	0.00±0.00

Data is expressed as mean ± SE; a and b represent significantly difference ($p < 0.05$) according to Tukey HSD statistics

Table 3. Zones of Inhibition (mm) of acetone extracts of mixture of *Garcinia kola* nut and *Buchholzia coriacea* nut

Isolates	100%	95%	90%	85%
E. coli	14.00±0.58a	11.00±0.58a	8.33±0.33a	0.00±0.00
Pseudomonas sp	12.33±0.33a	9.33±0.33a	7.33±0.33a	0.00±0.00
Staphylococcus aureus	13.00±0.58a	10.67±0.33a	8.33±0.33a	0.00±0.00
Bacillus sp	12.00±0.58a	9.00±0.58a	7.33±0.33a	0.00±0.00

Data is expressed as mean ± SE; a and b represent significantly difference ($p < 0.05$) according to Tukey HSD statistics

Table 2 presents the zone of inhibition at varying concentrations for the acetone extracts of *Garcinia kola* nut. The zone of inhibition for *E. coli, Pseudomonas sp, Staphylococcus aureus* and *Bacillus* spp was 13.00 mm, 11.00 mm, 13.0 mm and 12.67 mm respectively (100%), 9.76 mm, 7.67 mm, 10.00 mm and 8.67 mm respectively (95%), and 7.67 mm, 0.00 mm, 7.33 mm and 0.00 mm respectively (90%). At 85%, the extract was not effective against the selected pathogens under study. Basically, there was a significant difference ($p < 0.05$) among the different microbes for each concentration apart from 100% concentration. In 95% concentration, *Pseudomonas* species were the source of the observed difference. Furthermore, there was no significant variation between the means of *Pseudomonas* and *Bacillus* sp (not effective at 90% of the extract), and between *Staphylococcus aureus* and *E. coli.*

Table 3 presents the zone of inhibition at varying concentrations for the acetone extracts of *Garcinia kola* nut and *Buchholzia coriacea* nut mixture (ratio 1:1). The zone of inhibition for *E. coli, Pseudomonas sp, Staphylococcus aureus* and *Bacillus* spp was 14.00 mm, 12.33 mm, 13.0 mm and 12.00 mm respectively (100%), 11.00 mm, 9.33 mm, 10.67 mm and 9.00 respectively (95%), and 8.33 mm, 7.33 mm, 8.33 mm and 7.33 mm respectively (90%). At 85%, the extract was not effective against the selected pathogens under study. Typically, there was no significant difference ($p > 0.05$) among the different microbes for each of the extract concentration.

Garcinia kola nut and *Buchholzia coriacea* nut have antimocribal potentials. This is in consonance with the report of Okigbo and Mmeka [23], Osadebe *et al*. [30], Ejikengwu *et al.* [5], Ghamba *et al*. [22], Ibrahim and Fagbohun [18], Ezekiel and Onyeoziri [19], Nwachukwu *et al*. [25], Umeokoli *et al*. [9]. The antimicrobial and other therapeutic potential could be due to the presence of phytochemicals or bioactive constituents [2, 3, 6, 7, 31, 32]. Authors have variously reported that both *Garcinia kola* nut and *Buchholzia coriacea* contain phytochemical

constituents such as alkaloids, phlobatannins, saponin, flavonoids, tannins, cardiac glycoside, steroid, terpenoids for *Buchholzia coriacea* [5, 9, 30], and flavonoids, saponins, tannins, sterols and terpenes for *Garcinia kola* [22]. For instance, alkaloids have defense mechanisms through which plants wade off pests such as microorganisms, insects and herbivores [2, 3, 6, 7, 33]. Flavonoids can also be used as antioxidant, anticarcinogens, antimicrobial and antitumor agents [2, 3, 6, 7, 34].

The significant variation ($p < 0.05$) among the different bacteria investigated could be due to difference in the metabolism and physiology of the different organisms under study [2, 3, 7]. The zone of inhibition decreased with dilution of the extracts. Furthermore, the variation in antibacterial activity among both type of kola under study could be due to differences in their bioactive constituents. For instance, the concentration of tannin could contribute to the antimicrobial properties of both nuts.

The synergy of both nuts had superior efficacy against the microbes under study. These trends have been reported by Epidi [35]. Several solvents have been used to extract plant materials for antimicrobial activities. Some of the common solvents include methanol, ethanol, water, acetone, chloroform. Slight variation exists among the antimicrobial potential of microbes extracted with different solvents [2, 3, 7, 36]. The variation could be due to the polarity of the solvents. Furthermore, Kigigha *et al*. [7], Okigbo and Mmeka [23] is with the opinion that moisture content, plant parts, environmental conditions including habitats, temperature, pH etc. and the concentration of the plant extracts affects the therapeutic potential.

The zone of inhibition of this study is lower than the values previously reported by Mbata *et al*. [20], Ezekiel and Onyeoziri [19], Nwachukwu *et al*. [25], but had some similarity with the study of Ibrahim and Fagbohun [18], Kigigha *et al*. [6, 7], Epidi *et al*. [2, 3], Epidi [35], Osadebe *et al*. [30], Ejikeuwu *et al*. [5], Ezeigbo *et al*. [4] on some plants. The variation that exists could be due to the choice of microbes, bioactive contents of the plants, concentration used for the sensitivity testing, physical constituents of the plants among others factors.

4. Conclusion

Garcinia kola and *Buchholzia coriacea* nuts are widely consumed, and there have been several claims by traditional health practitioners about their medicinal potentials for the cure of different ailments. This study investiated the efficiacy of acetone extracts of seeds/nut of *Garcinia kola* and *Buchholzia coriacea* against some common pathogens including *E.coli, Pseudomonas* sp, *Bacillus* sp and *Staphyloccus aureus*. The study found that both extracts have antibacterial potential against the tested isolates, but the synergistic effects have superior effects. The zone of inhibition for all the microbial isolates suggests that the extracts can be used as broad spectrum antibiotics i.e. against gram positive and gram negative organisms.

References

1. Masih H, Paul S, Yadav J, Pandey S, Peter JK. Antibacterial properties of selected medicinal plants against pathogenic bacteria. Intern J Scientific Res Manage. 2014;2(5):915-924.
2. Epidi JO, Izah SC, Ohimain EI, Epidi TT. Antibacterial and synergistic potency of tissues of *Vitex grandifolia.* Biotechnol Res. 2016;2(2):69-76.
3. Epidi JO, Izah SC, Ohimain EI. Antibacterial and Synergistic Efficacy of Extracts of *Alstonia boonei* Tissues. Bri J Appl Res. 2016;1(1):0021-0026.
4. Ezeigbo OR, Ejike EN, Nwachukwu I, Ejike BU. Comparatie antibacterial activity of methanolic, ethanolic and aqueous extract of Garcinia kola (bitter kola) and Cola nitida (Kola nut). Intern J Plant Res. 2016;6(3):53–56.
5. Ejikeugwu C, Umeokoli B, Iroha I, Ugwu M, Esimone C. Phytochemical and antibacterial screening of crude extracts from leaves and wonderful kola. Amer J Life Sci. 2014;2(6):9-12.
6. Kigigha LT, Izah SC, Ehizibue M. Activities of *Aframomum melegueta* Seed Against *Escherichia coli*, *S. aureus* and *Bacillus* species. Point J Bot Microbiol Res. 2015;1(2):23–29.
7. Kigigha LT, Biye SE, Izah SC. Phytochemical and antibacterial activities of *Musanga cecropioides* tissues against *Escherichia coli*, *Pseudomonas aeruginosa Staphylococcus aureus, Proteus* and *Bacillus* species. Intern J Appl Res Technol. 2016;5(1):100–107.
8. Minocheherhomji FP, Vyas BM. Presence of alkaloids in medicinal plants and their importance in antimicrobial activities of some pathogenic microbial strains. J Environ Res Develop. 2014;9(1):144–150.
9. Umeokoli BO, Abba C, Ezeh P, Ajaghaku D. Antimicrobial, anti-inflammatory, and chemical evaluation of Buchholzia coriacea seed (Wonderful kola). Amer J Life Sci. 2016;4(5):106–112.
10. Buba CI, Okhale SE, Muazzam I. *Garcinia kola*: the phytochemistry, pharmacology and therapeutic applications. Intern J Pharma. 2016;3(2):67-81.
11. Akinmoladun A, Ibukun E, Emmanuel A, Akinrinola B, Akinboboye A, Obuofor E, Farombi E. Chemical Constituents and Antioxidant Activity of *Alstonia boonei.* J Biotechnol. 2007;6:1197–1201.
12. Momoh J, Longe AO, Campbell CA. In vivo anti-plasmodial and in vitro antioxidant activity of ethanolic leaf extract of *Alstonia boonie* (Ewe ahun) and its effect on some biochemical parameters in Swiss albino mice infected with Plasmodium berghei NK 65. Euro Scientific J. 2014;10(8):68–82.
13. Fatima N, Ahmad T, Khan SJ, Deeba F, Zaidi N. Assessment of antibacterial activity of in vitro and in vivo grown garlic (*Allium sativum* L.). Pak J Bot. 2011;43(6):3029-3033.
14. Gahlaut A, Chhillar AK. Evaluation of Antibacterial Potential of Plant Extracts using Resazurin based Microtiter Dilution Assay. Intern J pharm Sci. 2013;5(2):372-376.
15. Amole OO, Ilori OO. Antimicrobial Activity of the Aqueous and Ethanolic Extracts of the Stem Bark of *Alstonia boonei.* Intern J Phytopharmacol. 2010;1(2):119-123.

16. Izah SC, Zige DV, Alagoa KJ, Uhunmwangho EJ, Iyamu AO. Antibacterial Efficacy of Aqueous Extract of Myristica fragrans (Common Nutmeg). EC pharmacol Toxicol. 2018; In press.
17. Ikpeazu OV, Otuokere IE, Igwe KK. Preliminary Studies on the Secondary Metabolites of *Buchholzia coriacea* (Wonderful Kola) Seed Ethanol Extract by GC-MS Analysis. Intern J Res Eng Appl. 2017;7(3):17-26.
18. Ibrahim TA, Fagbohun ED. Antibacterial and antifungi activities of ethanolic and methanolic extract of dried seeds of buchhlozia coriacea. Res Rev: J Microbiol Biotechnol. 2014;3(2):5–9.
19. Ezekiel OO, Onyeoziri N. Preliminary studies on the antmicrobial properties of Buchholzia coriacea (wonderful Kola). Afr J Biotechnol. 2009;8(3):472–474.
20. Mbata TI, Duru CM, Onwumelu HA. Antibacterial activity of crude seed extracts of Buchholzia coriacea E. on some patogenic bacteria. J Develop Biol Tissue Eng. 2009;1(1):1–5.
21. Fapohunda S, Arinola A, Otuneme O, Adaramola B, Jegede D, Adewumi A, Aleshinloye A, Onigbinde A. Evaluation of the nutraceutical potential of *Garcinia kola* Seed Oil. J Pharm Phytochem. 2017;6(5):1894-1901.
22. Ghamba PE, Agbo EB, Umar AF, Bukbuk DN. The effects of diethyl ether and aqqueous Garcinia kola seeds extracts on some bacteria isolates. Academia Arean, 2011;3(2):87–94.
23. Okigbo RN, Mmeka EC. Antimicrobial effects of three tropical plant extracts on Staphylococcus aureus, Esherichia coli and Candida albicans. Afr J Tradit. Complem Altern Med. 2008;5(3):226–229.
24. Eze JI, Ekelozie CF, Nweze NE. Immunomodulatory Activity of the Methanolic Extract of *Buchholzia coriacea* Seeds on *Trypanosoma brucei Brucei* Infected Mice. *Global Veterinaria* 2015;14(4):582-588.
25. Nwachukwu MI, Duru MKC, Amadi BA, Nwachukwu IO. 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. Intern J Pharm Inven. 2014;3(6):41–49.
26. Erhirhie EO, Ben-Azu B, Moke GE, Chinwuba P, Omonjiahio IA. Ethno-pharmacological Review of *Buchhoizia coriacea* (Wonderful Kola). Intern J Adv Pharm Biol Chem. 2015;4(1):149–155.
27. Benson HJ. *Microbiological Applications:* Laboratory Manual in General Microbiology. Complete version, 5th edition. McGraw-Hill, New York, 2002.
28. Cheesbrough M. *District Laboratory Practice in Tropical Countries.* Low price Edition part 2. Cambridge press, England, 2004.
29. Opoku F, Akoto O. Antimicrobial and Phytochemical Properties of Alstonia Boonei Extracts. Org Chem Curr Res. 2015;4(1): http://dx.doi.org/10.4172/2161-0401.1000137.
30. Osadebe PO, Awemu GA, Ezealisiji KM, Agbo MO. Phytochemical and antimicrobial studies of the leaf extracts of Bucholzia coriaceae. Intern J Curr Res Rev. 2011;03(12):17-22.
31. Kigigha LT, Kalunta CG. Antimicrobial efficacy of leaf extracts of Piper nigrum against Escherichia coli, Staphylococcus aureus and Candida albicans. J Basic Pharmacol Toxicol. 2017;1(2):32–36.
32. Kalunta CG. Antimicrobial effect of different seed extracts of Piper nigrum against Escherichia coli, Staphylococcus aureus and Candida albican. Biotechnol Res. 2017;3(3):71-76.
33. Agu GC, Thomas BT. Antibacterial Activities of Ethanol and Aqueous Extracts of Five Nigerian Medicinal Plants on Some Wound Pathogens. Nat. Sci. 2012;10(2):78-84.
34. Osuntokun OT, Oluwafoise BO. Phytochemical screening of ten Nigerian medicinal plants. Intern J Multidispl Res Develop. 2015;2(4):390-396.
35. Epidi JO. Antibacterial and synergistic studies of crude extracts of parts of *Alstonia boonei* De Wild and *Vitex grandifolia* Gürke" A M.Sc thesis submitted to Post graduate school, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, 2016.
36. Kigigha LT, Atuzie MN. Assessment of Traditional Medicinal Application of *Alchornea cordifolia*. Afr J Biotechnol. 2012;11(8):2083–2086.