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Research Article

Phyto-composition and antimicrobial activities of the ethanol seed extracts of *Buchholzia coriacea*

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Background: There is a growing awareness in correlating phytochemical compounds with their biological activities. Folk medicines suggest that *Bulccholzia coriacea* is effective in the management of bacterial infections.

Objectives: This research was aimed at investigating the phyto-composition and antimicrobial activities of *Bulccholzia coriacea* ethanol seed extract.

Methods: Phyto-composition of *Bulccholzia coriacea* seeds were determined using gas chromatography mass spectroscopy (GC-MS) technique. Clinical isolates; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Streptococcus faecalis*, and *Candida albicans* were used to test for the antimicrobial activities of *Bulccholzia coriacea* seeds following disc diffusion and broth dilution methods.

Results: Gas chromatogram reveal six peaks depicting different phyto-constituents with Oleic acid (75.57%) highest peaked. *Candida albican*, *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *Staphylococcus aureus* had the same minimum inhibitory concentration (MIC) value of 6.25mg/ml and minimum bactericidal concentration (MBC) value of 12.5mg/ml.

Conclusion: Findings from this study supports the claims of local people who use *B. coriacea* seeds for the management of infectious diseases.

Keywords: Phyto-composition; antimicrobial; *Buchholzia coriacea*

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1. Introduction

Infectious diseases account for one half of all deaths in tropical countries irrespective of effort made in controlling incidence of the epidemic (Iwu, 1993; Okigbo and Ajalie, 2005). Clinical efficacy of many existing synthetic antibiotics today is being threatened. This is as a result of the emergence of multidrug-resistant pathogens such as some bacteria, fungi and viruses (Bandow et al, 2003). Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industries. Countless efforts

have been made to reverse this trend. One of them is the widespread screening of medicinal plants from the traditional system of medicine with the hope of getting newer, safer, and more effective agents to fight infectious diseases (Colombo and Bosisio, 1996; Natarajan et al, 2003). Phyto-medicines have shown great promise in the treatment of intractable infectious diseases (Idu et al, 2007).

The several medicinal uses of *Buchholzia coriacea* seeds amongst most West African countries coin its common name "wonderful kolanut". In Ivory Coast, the crushed

up seeds of *Bucchozia*, are pasted over the stomach for difficult childbirth and also to treat a variety of illnesses (Irvine, 1961; Burkill, 1985). In Liberia, wonderful kolanuts are used on skin eruption and also considered anthelmintic (worm expeller). In Western Nigeria, wonderful kola is used as a cough medicine, and in the treatment of ulcer (Ajaiyeoba et al, 2001; Ajaiyeoba et al, 2003; Ezekiel and Onyeoziri, 2009). The antibacterial efficacy of hot water and methanol extracts of dried seeds of *Bucchozia coriacea* against gastrointestinal pathogens have been tested effective (Mbata et al, 2009). The numerous medicinal assertions of *Bucchozia coriacea* seeds suggest that it contains bioactive phyto-constituents (Ajaiyeoba et al, 2003; Oluseyi and Francisca, 2009; Onyekaba et al, 2011; Umeokoli et al, 2016). With an increasing awareness in the correlation between plant phytochemicals and their biological usefulness (Fernie et al, 2004; Robertson, 2005), this study was aimed at investigating the phyto-composition and antimicrobial activities of *Bucchozia coriacea* ethanol seed extract on clinical pathogens.

2. Methods

2.1 Collection, identification and authentication of plant material

Fresh seeds of *B. coriacea* were purchased from Edo State, Nigeria. The seeds were authenticated at the herbarium, Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State (Voucher specimen No.: FHI 109920).

2.2 Preparation and extraction of plant material

Fresh seeds of *B. coriacea* were washed, chopped into pieces, sun-dried and pulverized with a mechanical grinder. The powdered seed was collected and stored in closed vessels. Extraction was done following procedures adopted by Omoregie et al, 2015 using absolute ethanol as the extraction solvent.

2.3 Experimental procedures for phyto-composition

GC-MS analysis was done on the ethanol extracts of *Bucchozia coriacea* following methods previously reported by Omoregie et al, 2015.

2.4 Preparation of stock solutions for antimicrobial screening

Fresh stock concentration of 300 mg/ml of extract was prepared by dissolving 3 g of extract in 10 ml of 50 % dimethyl sulfoxide (DMSO) in a test tube.

2.5 Test organisms

Clinical isolates including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus faecalis*, and *Candida albicans* were used in this study. Isolates were obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. All the organisms were laboratory identified and maintained at 4 °C in slants of nutrient agar and Sabouraud dextrose agar (SDA) for bacteria and fungi respectively.

2.6 Assay for antimicrobial activity:

Bucchozia coriacea ethanol seed extract was assayed for its antimicrobial activity of using the disc diffusion technique. The discs were prepared as described by Isu and Onyeagba (1998). Sterile paper discs (Whatman No. 1 filter paper) of 5 mm diameter were impregnated with extract graded with different concentration; 300, 250, 200, 150, 100, 50 mg/ml and dried in the oven at 55°C for 15 min. Agar plates were seeded with 0.1 ml broth culture of test organisms and the prepared discs were placed on the plates. They were incubated at 37 °C for 24 hrs and observed for clear zone diameters of inhibition against the organisms. The zone diameters were measured with a transparent rule and the result recorded in millimeters (mm). The assay was done in triplicates, Sterilized distilled water as negative and 50 mg/ml ciprofloxacin as positive control, were used.

2.7 Minimum Inhibitory Concentration (MIC) - Broth Dilution Method

MIC of extract was carried out using broth dilution method as described by Ibekwe et al, (2001). The nutrient broth and Sabouraud dextrose liquid were prepared according to the manufacturer's instruction (10 ml of each broth was dispensed into separate test-tube and was sterilized at 121 °C for 15 mins and then allowed to cool). Dilutions of the extracts in the broth were made from the stock concentration of the extract to obtain 50, 25, 12.5, 6.25, 3.125 mg/ml extract. Standardized inoculums (0.1 ml) of the microbes were inoculated into the different concentrations of the extracts in broth. The test tubes of the broth were incubated at 37 °C for 24 hrs and 30 °C for 1-7 days for bacteria and fungi respectively and observed for turbidity. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

2.8 Minimum Bactericidal/Fungicidal Concentration - Broth Dilution Method

Fresh Nutrient agar and Sabouraud media were prepared, sterilized at 121 °C for 15 mins and poured into sterile petri-dishes to cool and solidify. All contents of the MIC in the serial dilution were sub-cultured onto the media and incubated at 37 °C for 24 hrs and 30 °C for 1-7 days for bacteria and fungi respectively then, observed for colony growth. The MBC/MFC was the plate with the lowest concentration of extract and without colony growth.

3. Results

GC-MS analyzed results of *Bucchozia coriacea* seeds show six distinct peaks representing possible biologically relevant compounds. **Figure 1** presents a gas chromatogram showing the retention time of peaked compounds present in the ethanol extracts of *B. coriacea* seeds.

Table 1 shows the compounds represented by the peaks on **Figure 1** and their respective names, retention time, molecular formula, molecular weight, and nature of the compound.

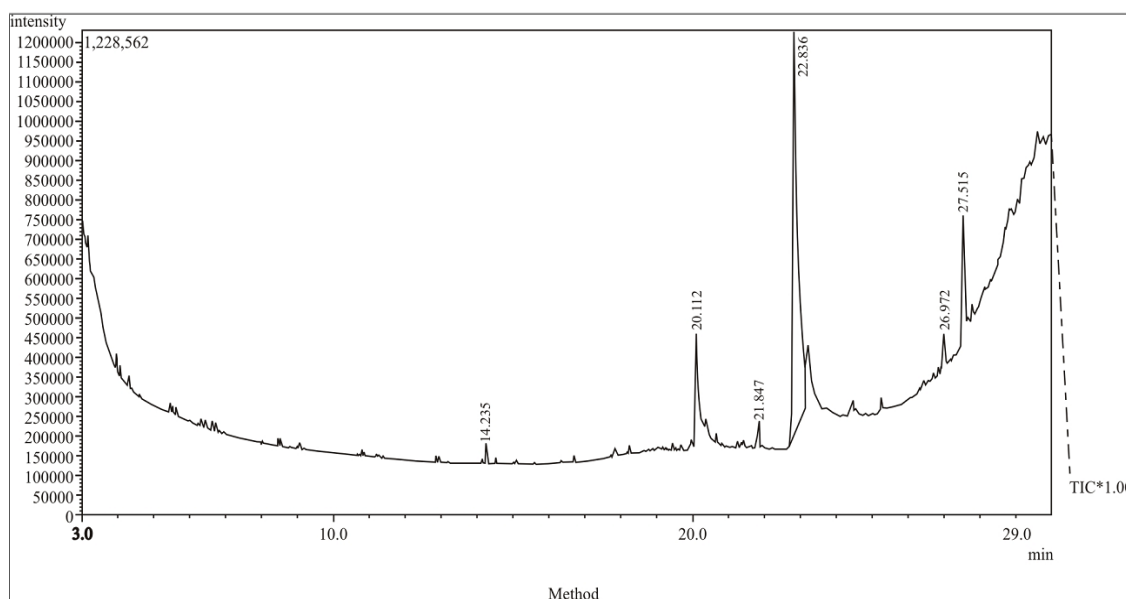


Figure 1: Gas chromatogram of *Buchholzia coriacea* ethanol seed extract.

Table 1: GC-MS analysis of *B. coriacea* ethanol seeds extracts.

No	RT (min)	Name of compound	Molecular Formula	Molecular Weight	Peak Area %	Compound Nature
1.	14.233	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	C ₁₉ H ₃₀ O ₃	306	0.56	Carboxylic acid
2.	20.108	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	10.88	Palmitic acid
3.	21.850	Thiirane, octyl-	C ₁₀ H ₂₀ S	172	1.28	Organosulphur
4.	22.833	Oleic acid	C ₁₈ H ₃₄ O ₂	282	75.57	Oleic acid
5.	26.975	9-Octadecene, 1,1-dimethoxy-, (Z)-	C ₂₀ H ₄₀ O ₂	312	2.46	Oleic acid
6.	27.517	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298	9.25	Linoleic acid

Table 2: Mean zones diameter of inhibition (mm) of different concentrations of *B. coriacea* ethanol seeds extract

Test isolates	Mean zones diameter of inhibition (mm) of different extract concentrations						+ve Ctrl	-ve Ctrl
	300 (mg/ml)	250 (mg/ml)	200 (mg/ml)	150 (mg/ml)	100 (mg/ml)	50 (mg/ml)		
<i>C.albicans</i>	28±0.02	22±0.05	18±0.01	13±0.00	8±0.05	6±0.03	22	0
<i>S.epidermidis</i>	33±0.01	27±0.02	19±0.02	15±0.03	13±0.00	10±0.00	25	0
<i>S.faecalis</i>	30±0.00	25±0.01	19±0.00	13±0.05	10±0.03	5±0.01	20	0
<i>P.aeruginosa</i>	25±0.01	20±0.03	12±0.05	8±0.05	5±0.01	2±0.02	28	0
<i>K.pneumonia</i>	34±0.03	30±0.00	22±0.00	17±0.02	10±0.04	4±0.05	18	0
<i>S.aureus</i>	40±0.01	32±0.00	26±0.01	20±0.01	13±0.03	10±0.00	19	0

Key: +ve control (Ciprofloxacin, 50 mg/ml); -ve control (Distilled water); n=3.

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *B. coriacea* seeds extract.

Test Isolates	Concentrations (mg/ml)	
	MIC	MBC
<i>Candida albicans</i>	6.25	12.5
<i>Staphylococcus epidermidis</i>	6.25	12.5
<i>Streptococcus faecalis</i>	12.5	50.0
<i>Pseudomonas aeruginosa</i>	3.12	6.5
<i>Klebsiella pneumonia</i>	6.25	12.5
<i>Staphylococcus aureus</i>	6.25	12.5

n=3

The antimicrobial activities, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of *B. coriacea* seed extract at different concentrations against the test organisms are presented on **Table 2** and **Table 3**. It was observed that the higher the concentration, the higher the mean zone of inhibition.

4.0 Discussion

The presence of bioactive components in plants, produce some biological activity in man and animals and are responsible for their usefulness as herbal remedies. These compounds also protect plants against infection by microorganisms, predation by insects and herbivores (El-Mahmood et al, 2010). The valuable pharmaceutical properties of *B. coriacea* seeds may be attributed to the presence of bioactive compounds (Ibrahim and Fagbohun, 2012). Results from the mean diameter zone of inhibition, MIC and MBC of *Bucchozia coriacea* seeds in the present study further lays credence to the fact that *Bucchozia coriacea* seeds have antimicrobial properties.

The GC-MS chromatogram on **Figure 1** shows that *Bucchozia coriacea* seeds are rich in six basic phyto-compounds represented by peaks. Oleic acid (75.57%) which was the highest peaked compound has been implicated to possess other activities other than antimicrobial activities (Table 2) (Igor, 2014). Others are n-Hexadecanoic (10.88%), 9,12-Octadecadienoyl chloride (Z,Z)- (9.25%), 9-Octadecene, 1,1-dimethoxy-, (Z)- (2.46%), Thiirane, octyl- (1.28%) and Pentanoic acid, 5-hydroxy-,2,4-di-t-butylphenyl esters (0.56%). In contrast to previously reported studies on the GC-MS analysis of *Bucchozia coriacea* aqueous seed extracts, two phyto-constituents (n-Hexadecanoic and 9,12-Octadecadienoyl chloride (Z,Z)-) were found present in the aqueous extract of the plant (Omoregie et al, 2015). This phenomenon suggests that the nature of compounds isolated or elucidated from GC-MS analysis of *Bucchozia coriacea* seed depends on the solvent used. The previously reported biological uses of the compounds of the compounds presented on **Table 1** are summarized in **Table 4**.

Table 4: Bioactive compounds detected in the seeds ethanol extract of *B.coricea* by GC-MS (Brahmachari, 2010; Rajeswari et al, 2013; Bais and Kakkar, 2013; Raval et al, 2016; Mustapha et al, 2016)

Compound Name	Molecular Formula	Bio-activity
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	C ₁₉ H ₃₀ O ₃	Food additives, flavouring, immune boosters, aids lipid and carbohydrate metabolism, reduces stress, anti-depressant, hypocholesterolemic.
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Antioxidant, larvicidal effect, hypocholesterolemic, nematocide, pesticide, lubricant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor
Thiirane, octyl-	C ₁₀ H ₂₀ S	Polyamide stabilizers, Antioxidant, nucleating agent, UV absorbers and light stabilizers, metal deactivators, base co-stabilizers.
Oleic acid	C ₁₈ H ₃₄ O ₂	Hypo-cholesterolemic, 5-Alpha reductase inhibitor, Flavor, anti-inflammatory, immune booster, support respiratory and cardiovascular health, anti-cancer, anti-oxidant and useful in weight reduction.
9-Octadecene, 1,1-dimethoxy-, (Z)-	C ₂₀ H ₄₀ O ₂	Anti-inflammatory, hypo-cholesterolemic, cancer preventive, hepatoprotective, nematocide insectifuge, anti-histaminic, anti-eczemic, anti-acne, 5-alpha reductase inhibitor anti-androgenic, anti-arthritis, anti-coronary, insecticide.
9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	Anti-inflammatory, hypocholesterolemic, cancer preventive, antihistaminic, anti-eczemic, anti-acne, emulsifying agent, anti-arthritis, anti-coronary disease agent.

The antimicrobial activities of *Buchholzia coriacea* seeds on the test organisms showed clear zones of inhibition (mm) on the tested isolates. At 200mg/ml, the zones of inhibition for *Staphylococcus aureus* (26 mm), *Klebsiella pneumonia* (22 mm) and *Pseudomonas aeruginosa* (12 mm) in this study (**Table 2**) were comparable with findings of Ibrahim and Fagbohun, 2013. This implies that the factors responsible for the antimicrobial activities of *B. coriacea* are perhaps the secondary metabolites as enumerated in that study. *Candida albican*, *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *Staphylococcus aureus* had the same minimum inhibitory concentration (MIC) value of 6.25mg/ml and minimum bactericidal concentration (MBC) value of 12.5mg/ml. This may suggest that the seed of *B. coriacea* has similar potency on those test isolates.

5.0 Conclusion

In conclusion, *B. coriacea* seeds can be used as an antibacterial agent against infections. This supports the claimed use of crude plant for the management of infectious diseases by local people across Africa. The phyto-constituents in *B. coriacea* can be useful in synthesizing new antimicrobial drugs. However, further study is recommended to ascertain fully the plant toxicity.

Conflict of Interest declaration

The authors declare no conflict of interest.

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