



## Research Article

# Antibacterial and Antifungal Activity of Ethanolic and Methanolic Extract of Dried Seeds of *Buchhlozia Coriacea*

<sup>1</sup>Ibrahim T.A.\* and <sup>2</sup>Fagbohun E.D.

<sup>1</sup>Department of Food Science & Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.

<sup>2</sup>Department of Microbiology, Ekiti State University, Ado Ekiti, Nigeria

## ARTICLE INFO

## ABSTRACT

Article No.: 100912103

DOI: 10.15580/GJAS.2013.6.100912103

Submitted: 10/09/2012

Accepted: 22/06/2013

Published: 29/06/2013

\*Corresponding Author

Ibrahim T.A.

E-mail: [tessieuptown@yahoo.ca](mailto:tessieuptown@yahoo.ca)

Phone: +2348035774200

The antibacterial activities of the ethanol and methanol extract of the dried seeds of *B. coriacea* against clinically significant bacterial isolates were determined using the agar well diffusion method. It showed *Staphylococcus aureus* was the most susceptible while *Bacillus cereus* was the least susceptible to the extracts with methanol extract being more effective against the clinical bacterial isolates. The extracts inhibited the growth of the bacterial isolates in a concentration dependant manner. The antifungal activity of the extracts on radial mycelical growth of the test fungi showed that *Aspergillus niger* was the most susceptible while *Rhizopus spp* was the least susceptible.

**Keywords:**

Antibacterial, Antifungal, Extract, Seeds, *Buchhlozia coriacea*

## INTRODUCTION

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 2008a). In recent years, there has been a gradual renewal of interest in the use of medicinal plants in developing countries because herbal medicines have been reported to be safe and work out any adverse side effect especially when compared with synthetic drugs and because of the low income of majority of the populace (Sofowora, 2008a). Thus a search for new drugs with better and cheaper substitute of plant origin is a natural choice. The importance of higher plants to human existence cannot be over-emphasized, its importance cut across all aspects of life and economy of man which include health care delivery and supply of drugs (Bringman *et al*; 1999). The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academics since many infection agents are becoming resistance to synthetic drugs (Latha and Kanabiran, 2006). Plants have the major advantage of still being the most effective and cheaper alternative source of drugs. The local use of material plants as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha *et al*, 2002). The development of medicinal chemistry, as a major note for the discovery of novel and more active therapeutic agents is further invitation that investigations into the chemical and biological activities of the plants needs to be carried out (Rao and Raja, 2007). Plants are the best sources of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported to contain biological properties like analgesic, anti inflammatory antioxidant, hypoglycemic, antibacterial and antifungal agents (Sindhu, 2009). Plants generally produce secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Doughari *et al*, 2008). Plant products still remain the principal source of pharmaceutical agents used in traditional medicine. The effects of plant extracts on bacterial have been studied by a very large number of researchers in different parts of the world. The potential for developing antimicrobials from higher plants appear rewarding as it will lead to the development of a phytomedicine to act against microbes (Sofowora, 2008). Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associate with synthetic antimicrobials (Rao and Raja, 2007).

*Buchholzia coriacea* was named after R.W. Buchholzia who collected plants in Cameroon in the late 19<sup>th</sup> century (Keay *et al*; 1989). It belongs to the

Capparaceae family. The seed of *B. Coriacea* has medicinal values.

These seeds gave the plants its common name of "wonderful kola" because of its usage in traditional medicine. The seeds are covered in a purple aril which is chewed in Ivory Coast and has a sharp pungent taste. As a result of its supported broad-spectrum affinity, there is need to conduct studies on potential utilization of wonderful kola in foods. This work was aimed at evaluating the antimicrobial potentials of ethanol and methanol extracts of *B. coriacea*.

## MATERIALS AND METHODS

**Collection of *B. Coriacea* Seeds:** Seeds of *B. coriacea* were brought from Oba market, Post Office in Ado-Ekiti, Ekiti State and were identified by the herbarium section of the Department of Plant Sciences, University of Ado-Ekiti, Ekiti State.

**Processing of the Seeds:** The seeds were washed, chopped into pieces and air dried for 21 days. After drying, the seeds were grounded into powder using a mortar and pestle and stored in well labeled air tight containers for the antibacterial and antifungal activity of the ethanolic and methanolic extracts of the seed.

**Extraction of Plant Materials:** Ethanol and methanol solvents were used for extraction of the active components of the plant's seed. The method of Alanis *et al* (2005) was used for both ethanolic and methanolic extraction of the seed active ingredients. Exactly 150g each of the powdered seeds were separately extracted in cold using 60% methanol and 95% ethanol and shaken at 150rpm for 4 days at ambient temperature. The mixture was then filtered. The filtrate was evaporated using vacuum rotary evaporator (BUCHL Rolavapour R200/205 model R205V800) and stored at 4°C in dark sample bottles prior to use.

**Test microorganisms:** Clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis*, *Salmonella typhimurium*, *E. coli* used for the work were collated from the medical Laboratory Department of Federal Medical Centre, Owo Ondo State while laboratory fungal cultures of *Aspergillus nigers* *Trichoderma spp* and *Rhizopus Spp* were collected from the Microbiology Department, University of Ado Ekiti, Ekiti State, the bacterial species were maintained on nutrient agar start while the fungal isolates were maintained on Potato dextrose agar (PDA) and kept at 4°C prior to use.

**Table 1: Antibacterial activity of methanolic extract of *B. coriacea* seeds**

|                               | Concentration of extract (mg/ml) Diameter of inhibition zone (min) |          |          |         |          |         |          |         |
|-------------------------------|--|----------|----------|---------|----------|---------|----------|---------|
|                               | 50mg/ml  |          | 100mg/ml |         | 150mg/ml |         | 200mg/ml |         |
|                               | METH   | ETH      | METH     | ETH     | METH     | ETH     | METH     | ETH     |
| <i>Staphylococcus aureus</i>  | 10+0.04  | 9+0.33   | 17+0.03  | 14+0.16 | 22+0.06  | 20+0.16 | 27+0.02  | 24+0.16 |
| <i>Klebsiella pneumonia</i>   | 8+0.02   | 6+0.04   | 12+0.10  | 10+0.23 | 16+0.07  | 15+0.01 | 18+0.05  | 16+0.17 |
| <i>Pseudomonas aeruginosa</i> | 2 + 0.01   | 1+0.03   | 6+0.11   | 5+0.14  | 9+0.16   | 7+0.06  | 12+0.02  | 10+0.16 |
| <i>Proteus vulgaris</i>       | 5 + 0.02   | 3+0.01   | 7+0.12   | 5+0.06  | 10+0.14  | 8+0.10  | 14+0.01  | 12+0.01 |
| <i>Bacillus subtilis</i>      | 1 +0.01  | 1.0+0.01 | 2+0.14   | 1+0.05  | 4+0.06   | 3+0.04  | 7+0.06   | 6+0.06  |
| <i>E. coli</i>                | 2 +0.02  | 1.0+0.32 | 3+0.13   | 3+0.12  | 6+0.11   | 4+0.02  | 9+0.21   | 8+0.16  |
| <i>Salmonella typhimurium</i> | 7 + 0.04   | 5.0±0.03 | 14+0.17  | 12+0.04 | 18+0.21  | 16+0.10 | 22+0.06  | 19+0.21 |

Values are means of duplicate results  $\pm$  S. D

METH=METHANOLIC EXTRACT, ETH =Ethanollic extract

**Antibacterial Activity:** The antibacterial activity of the extract of *B. coriacea* was done with agar well diffusion method of Osadebe and Ukwæze (2004). Both culture of the test bacterial isolate (0.1ml) containing  $10^6$  cells/ml (macfarland standard) of organism was aseptically inoculated by spreading evenly onto the surface of nutrient agar plates using a sterile spreader. Five wells (6.0 mm diameter) were made in the plate using a sterile cork borer and 0.5ml of the crude extracts were added and allowed to diffuse for about 2h. The plates were incubated at 37°C for 18-24hr. Antibacterial activities of the extracts were determined by measuring the diameter of inhibitions zone (mm) against each test bacteria.

**Antifungal Activity:** Radial mycelia growth assay method of Okafor (1995) was used to test for the antifungal activity of the extracts. Different concentrations of the extracts (5ml) were aseptically added to sterile potato dextrose agar medium (15ml) in Petri dishes. The plates were gently swirled and allowed to solidify. The extract-amended medium in the Petri dishes were inoculated, each alone at the centre with mycelia discs (6mm) of the test fungus and incubated at

28°C for 5days. The radical mycelia growth was measured every 24hrs for 5days.

## RESULTS AND DISCUSSION

Table 1 showed the antibacterial activity of methanolic and ethanolic extract of air dried seeds of *B. coriacea* against selected clinical bacterial isolates. The diameter of inhibition zones (mm) of four different concentrations (50,100,150 and 200mg/ml) were presented. The higher the concentration the better the anti bacterial activity of the extracts while methanolic extract showed better antibacterial than the ethanolic extract from the overall results. *Bacillus subtilis* was the most resistant of the isolates tested followed by *E. coli* while *Staphylococcus aureus* was the most susceptible.

The antifungal activity of the extracts of air dried seeds of *B. coriacea* at four different concentrations (50, 100, 150 and 200mg/ml) was given in table 2 against laboratory fungal isolates of *Aspergillus niger*, *Trichoderma viride* and *Rhizopus spp.*

**Table 2: Antifungal activity of methanolic extract of B. Coriacea seeds**

| Test fungi               | Concentration of extract (mg/ml) /Radial mycelia growth (mm) |        |          |         |          |         |          |         |
|--------------------------|--|--------|----------|---------|----------|---------|----------|---------|
|                          | 50mg/ml  |        | 100mg/ml |         | 150mg/ml |         | 200mg/ml |         |
|                          | METH   | ETH    | METH     | ETH     | METH     | ETH     | METH     | ETH     |
| <i>Aspergillus niger</i> | 8+0.2  | 7+0.11 | 14+0.16  | 10+0.01 | 22+0.15  | 22+0.07 | 34+0.02  | 28+0.01 |
| <i>Trichoderma</i>       | 4+0.01   | 4+0.02 | 8+0.06   | 7+0.06  | 18+0.23  | 15+0.16 | 26+0.05  | 22+0.16 |
| <i>SPP</i>               | 4+0.16   | 3+0.45 | 7+0.23   | 6+0.18  | 14+0.01  | 13+0.02 | 22+0.06  | 18+0.16 |
| <i>Rhizopus spp</i>      |  |        |          |         |          |         |          |         |

Values are means of duplicate results  $\pm$  S.D

METH=METHANOLIC EXTRACT, ETH =Ethanolic extract

*Aspergillus niger* was the most susceptible followed by *Rhizopus spp* while *T. viride* was least susceptible to the extracts. Plants are the best sources of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties like antibacterial, antifungal, antioxidants, anti inflammatory and hypoglycemic (Sindhu, 2009). According to WHO report 80% of the world population are taking interest in indigenous herbal medicines usually being seed in form of fruits, vegetables, drugs or their extracts for the treatment of the diseases and for maintenance of health (Sahito *et al*, 2003). The results of this work showed that the seeds extract of *B. coriacea* inhibited the growth of all the tested isolates at varying concentrations of 50, 100, 150 and 200mg/ml. The antimicrobial activity of extracts of medicinal materials has been attributed to the phytochemicals constituents present in them (Aboaba *et al*, 2006) and the extracts of *B. coriacea* wont be an exception. Antimicrobial properties of substances are desirable tools in the control of undesirable micro organisms especially in the treatment of infectious diseases and in food spoilage (Mohanta *et al*, 2007). The active components usually interfere with growth and metabolism of micro organisms in a negative manner (Aboaba *et al*, 2006). The antibacterial activity of ethanolic and methanolic extract of *B. coriacea* seeds at varying concentrations (50,100, 150, 200mg/ml) are given in table 1. The results showed that the higher the concentration the higher the diameter of inhibition zone, the better the bacterial activity of the extract while methanolic extract exhibited better activity than the ethanolic extract. This has been observed that the more polar the solvent, the better its extraction power (Chang *et al*, 1997). Methanol, although not a recommended

food solvent because of its toxicity, gave a higher quantitative phytochemical. The results showed that the values obtained are quite higher for the methanolic extract than the ethanolic extract suggesting that extraction with methanol produces better active antimicrobial phytochemicals which are contained in the seed. The presence of phytochemicals in the seed extract showed that the extract possess antibacterial properties which are seen in results of the antimicrobial activities of the extracts. The antibacterial activity of crude extracts of the seeds at varying concentrations showed that the inhibition zones ranged between  $1.00 \pm 0.01$  and  $27.00 \pm 0.02$ . The antibacterial activity of the extracts increases with the increase in the concentration. *Staphylococcus aureus* was the most susceptible with inhibition range between  $9.00 \pm 0.03$  and  $27.00 \pm 0.02$  for ethanolic extract at 50mg/ml and methanolic extract at 200mg/ml respectively. This was followed by *Salmonella typhimurium* with inhibition zone ranging between  $2.0 \pm 0.04$  and  $22.0 \pm 0.06$ , *Proteus vulgaris* has inhibition zone ranging between  $3.00 \pm 0.01$  and  $14.0 \pm 0.01$ . The most resistant to the extracts was *Bacillus subtilis* with inhibition zone range between  $1.00 \pm 0.01$  and  $7.00 \pm 0.06$  for ethanolic extract at 50 mg/ml and methanolic extract at 200mg/ml. This was followed by *E. coli* with inhibition range between  $1.00 \pm 0.32$  and  $9.00 \pm 0.21$ .

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their anti-microbial activity may provide new anti-microbial substances hence the present investigation clearly reveals, the antibacterial nature of these seeds and suggests that these seeds could be exploited in the management of diseases caused by these bacteria in human systems. The results of the

antibacterial activity showed that the plant seeds are rich reservoirs of antimicrobial as observed by other works such as Mbata *et al*; (2009), Ezekiel and Onyeoziri, (2009). The active components usually interfere with growth and metabolism of microorganisms in a negative manner (Aboaba *et al*; 2006). The varying degree of sensitivity of the bacterial isolates may be due to the intrinsic tolerance of the microbes and the nature and combination of phytochemicals present in the extracts. The test organisms used in this study are associated with various forms of human infections from a clinical point of view *Klebsiella pneumoniae* is the most important member of the *Klebsiella* genus and it is emerging as an important cause of neonatal nosocomial infections (Gupta *et al*; 1993). *E. coli* causes Septicemias and can infect the gall bladder, surgical wounds, skin lesions and the lungs especially in debilitate and immuno deficient patients (Black, 1996), *proteus vulgaris* is capable of causing inflammation of the bladder (Nester *et al*, 2004). *Pseudomonas aeruginosa* which is an opportunity pathogen that is wide spread in the environment, a major cause of nosocomial infections and an occasional cause of community acquired infections (Nester *et al*; 2004). *Staphylococcus aureus* and *Salmonella typhimurium* had been associated with food poisoning and typhoid fever respectively (Nester *et al*; 2004) while *Bacillus subtilis* is capable of causing eye infection, food spoilage and food borne intoxication (Nester *et al*; 2004).

The antifungal activity of the *B. coriacea* seeds extracts as shown in table 2 indicates a significant antifungal activity on the fungi used. Many scientists, Anjum and Khan (2003), Adedokun *et al* (2002), Bajwa *et al*, (2006), Theo and Abro (2000), Pizada *et al* (2007) Sanjary and Ashok, (2006), Fardos, (2009) just to mention few have worked on the antifungal activities of medicinal plants from different regions of the world. *Aspergillus niger* was the most susceptible with inhibition zone between  $7.00 \pm 0.11$  and  $34.00 \pm 0.02$  (mm) followed by *Trichoderma* sp between  $4.00 \pm 0.01$  and  $26.00 \pm 0.05$  while *Rhizopus* sp showed the lowest susceptibility with inhibition zone between  $3.00 \pm 0.15$  to  $22.00 \pm 0.06$  (mm). Many investigations were carried out to discover plant products that inhibit the fungi like *Aspergillus sp*, *Trichophyton sp* (rubrum), *Rhizopus sp*. These fungi species causes infections in human which are difficult to control effectively and the pharmaceutical arsenal currently available organize than is rather limited (Gupta *et al*, 1991). Hence, plant products that inhibit their growth without harming the host represents potential therapeutic agent. The antifungal activities of the seed extracts were obtained using methanol and ethanol.

The trend in the activity followed the antibacterial activity earlier mentioned in which the methanolic extract showed better inhibition zones than the ethanolic extract in terms of radial mycelia growth of the fungal species used. Ezekiel and Onyeoziri (2009) also found out the hexane and methanolic extract of *B. coriacea* seeds inhibit the growth of *Trichoderma viride* *Aspergillus niger*.

## CONCLUSION

The plant extracts have great potentials as antimicrobial compounds against microorganism, thus they can be used in the treatment of infections diseases caused by the tested isolates. The seeds could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

## REFERENCES

- Aboaba O.O, Smith S.I, Olide F.O (2006). Antimicrobial effect of Edible plant extract on *Escherichia coli* 0157:H7, Pak. J. Nutr. 5(4):325-327.
- Adedokun, A.A., and Okoli, S.O. (2002). Antifungal activity of Crude extract of *Alfia barteri* oliver (Apocynaceae) and *Chasmanthera dependens* Hochst (Menispermaceae). *Hamdard Medicus*, 45:52-56.
- Alani S. A.D. Glazada, F; Gerrantes J.A. Tarres J. and Ceballas, G.M., (2005). Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastro intestine disorders *J. Ethnopharmacot*, 100 (1-2): 153-157. (doi:10.1016/s.jep.2005.02.022.).
- Anjum N and Khan Z. (2003). Antimicrobial activity of the Crude extract of *Cuscuta reflexa* Roxb. *Pakistan Journal of Botany*, 35:999-1007.
- Bajwa, R, Anjum, T, and Shafique, S. (2006). Evaluation of antifungal activity of *cicerarietinum* L. *Pakistan Journal of Botany* 38:175-184.
- Bibitha b, Jisha V.K, Salitha C.V, Mohan S and Valsa A.K (2002). Antibacterial activity of different plants extracts. Short Communication, *Indian Journal of Microbiology*, 42:361-363.
- Black JG (1996). *Microbiology: Principles and Application* Prentice Hall, New York. P. 260.
- Brimman G. Oehse M, Wolf K, Krans J, Peter S.K., Peter E.M., Herderieh M; Akaeasi L and Tayman F.K (1999). 4-oxonicotinamide 1-1 (B-d-ribofuranoside) from *Erothmannsa Loengithora salish* (Rubiaceae) phytochemistry..
- Chang S.S, Ostrich-Mates J.B, Hsieh O.A, Hurg C.L (1977). Natural antioxidants from rosemary and sage. *J. Food Sci.* 42:1102-1106.
- Cimaga K, Kamba K, Tona L, Apes S, DC-Bruyne T, Hermans N, Totte J, Pieters L, and Vlietinck A.J. (2002). Correlation between chemical composition and antibacterial activity of essential oil of some aromatic medicinal plants growing in the Democratic Republic of Conco *J. Ethnopharmacol* 2(79)213-225.
- Doughari J.H, EL- Mahmood A.M and Tyoyina I. (2008). Antimicrobial activity of leaf extracts of *Senna obtuse folia* (C) African *J. of pharmacy and pharmacology*, vol 2(1) pp 7-13.
- Ezekiel O.O, Onyeoziri NF (2009). Preliminary studies on the antimicrobial properties of *Buchholzia coriacea* (wonderful kola). *Afr.J. Biotechnol.* 8 (3).472-474.

- Farsos M.B (2009) .Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia, Mycopath, 7(1):51-57.
- Gupta M, Mazunder U.K, Pal D.K, Bhatta and Charyas (2003). Onset of puberty and Ovarian Steroidogenesis following administration of methanolic extract of *Cuscuta reflexa* Roxb. Stem and *Corchorus olitorius* Linn seed in Mice Journal of Ethnopharmacology 89:55-59.
- Gupta P, Murali P. Murali MV, Faridi MMA, Kaul PB, Ramachandran VC, and Talwar V (1993). Clinical profile of Klebsiella Spticaemia in neonates Ind.J. Paediatr. 60:565-572.
- Latha S.P and Kannabiran K (2006). Antimicrobial activity and phytochemicals of *Solanum trinobatum* linn. Afri. J. Biotechnol. 5(3) 2402-2012.
- Mbata T.I, Dura C.M AND Onwumelu H.A (2009). Antibacterial activity of crude seed extracts of *Bucholzia coriacea* on some pathogenic bacteria. J. of Dev. Biol. And Tiss.Eng. Vol 1:001-005.
- Mohanta T.K, Patra J.K, Ralf S.K, Pal D.K and Thata H.N (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semi carpus anacardium* L.F. Scientific Research and Essay vol.2 (II) pp486-490.
- Nester E.W, Anderson O.G, Robert C, Pearlsadl N and Nester M.T (2004). Microbiology: a human perspective, 4<sup>th</sup> edition, MacGraw-Hill Companies, N.Y U.S.A pp.635-637.
- Okafor J.J (1995). Preliminary studies of the antifungal activities of some medicinal plants against *Bsidiobulus* and some pathogenic fungi mycoses 38:191-195.
- Osadebe P.O and Ukwueze S.E (2004). Comparative Study of the phytochemical and antimicrobial properties of the Eastern Nigerian species of African Mistletoe (*Loranthus micranthus*) sourced from different host trees. J. Biol. Res. Biotech. 2(1):18-23.
- Pirzada A.J, Shaikh W, Ghani K.V and Laghari K.A (2009). Study of antifungal activity and some basic elements of medicinal plant *Cress cretica* linn against fungal causing skin disease. Sindh univ. Res. Jour (Sci. Ser.). Vol. 41 (2) 15-20.
- Roja G AND Rao P.S (2002). Anticancer Compounds from tissue culture of medicinal plants. J. Herbs, Spices and Medicinal plants. Vol 1, pp. 71-79.
- Sahito S.R, M.A Memon, T.G Kazi and G.H Kazi (2003). Evaluation of Mineral content in medicinal plant *Azadirachta indica* (neem). J. chem. Soc. Pak 25(2): 139-143.
- Sanjay Gulena and Ashok Kumar (2006). Antifungal activity of some Himalayan Medicinal plants using direct bioautography. J. of cell and molecular biology 5:95-98.
- Sindhu G (2009). Antibacterial and antifungal studies of *Abutilon indicum* leaf extract. Pharmacology 2:567-571.
- Sofowora E.A (2008). Medicinal Plants and Traditional medicine in Africa. John wiley and Sons Ltd; pp1-10
- Thebo, N.K and H.Abro (2000). Antifungal activity of *Azadirachta indica* (neem) against human pathogenic fungi. Sindh Univ. Res. Jour. (Sci. Ser.) 32 (2):35-42.

**Cite this Article:** Ibrahim TA and Fagbohun ED (2013). Antibacterial and Antifungal Activity of Ethanolic and Methanolic Extract of Dried Seeds of *Buchholzia Coriacea*. Greener Journal of Agricultural Sciences, 3(6): 458-463, <http://doi.org/10.15580/GJAS.2013.6.100912103>.