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Antibacterial and Antifungal Activities of Ethanolic and Methanolic Extract of Dried Seeds of *Buchhlozia coriacea*.

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Research Article

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ABSTRACT

The antibacterial activities of the ethanol and methanol extracts of the dried seeds of *Buchlozia 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 been 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* sp was the least susceptible.

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 [1]. 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 without any adverse side effect especially when compared with synthetic drugs and because of low income of the majority of the populace [1]. 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, it's importance cut across all aspects of life and economy of man which include health care delivery and supply of drugs [2] .The search for newer sources of antibiotics is a global challenge preoccupying research institution, pharmaceutical companies and academic since many infectious agents are becoming resistance to synthetic drugs [3]. Plants have the major advantage of still been the most effective and cheaper alternative sources of drugs. The local use of material plants as primary health remedies due to their pharmacological properties is quits common in Asia, Latin America and Africa [4] .The development of medicinal chemistry, as a major note for the discovery of novel and more active therapeutic agents is further investigation into the chemical and biological activities of the plants needed to be carried out [5] .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 analgesic, anti inflammatory antioxidant, hypoglycemic, antibacterial and antifungal agents [6]. Plant generally produce, many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs [7] .Plant products still remain the principal source of pharmaceutical agents used in traditional medicine . The effects of plant extracts on bacterial have been studies 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 [1]. Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associate with synthetic antimicrobials [3] .

Buchholzia coriacea was named after R.W. Buchholzia who collected plants in Cameroon in the late 19th century [8]. 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*.

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MATERIALS AND METHODS

Collection of *B. Coriacea* Seeds

Seeds of *B. coriacea* were brought from Oba Market in Ado-Ekiti, Ekiti State and were identified at the Herbarium Section of the Department of Plant Sciences, Ekiti State University , Ado-Ekiti, Nigeria.

Processing of the Seeds

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

Extraction of Plant Material

Ethanol and methanol were used for extraction of the active components of the plant's seed .The method of [9] 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^{o}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, Escherichia coli* uses for the work were collected from the Medical Laboratory Department of Federal Medical Centre, Owo, Ondo State while laboratory fungal cultures of *Aspergillus nigers Trichoderma spp* and *Rhisopus* sp were collected from the Microbiology Department, Ekiti State University , Ado Ekiti, Ekiti State, the bacterial species were maintained on nutrient agar start while the fungal isolates were maintained on Potato dextrose agar (PDA).

Antibacterial Activity

The antibacterial activity of the extract of *B. coriacea* was done with agar well diffusion method of [9] .Broth culture of the test bacteria (0.1ml) containing 10^6 cells/ml (MacFarland standard) of the organisms were asceptically 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 various concentrations (50,100,150 and 200mg/ml) of the crude extracts were added and allowed to diffuse for about 2 hr. The plates were incubated at 370C for 18-24 hr. Antibacterial activities of the extracts were determined by measured the diameter of inhibition zones (mm) test bacteria.

Antifungal Activity

Radial mycelia growth assay method of [10] was used to test for the antifungal activity of the extracts. About 5ml of different concentrations (50,100,150 and 200mg/ml) of the extracts 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^{o}c for 5days.The radical mycelia growth was measured every 24 hrs 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 zone (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. *B. subtilis* was the most resistant of the isolates tested followed by *E. coli* while *S. 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*. *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 plants origin drugs have been reported with biological properties like antibacterial, antifungal, antioxidants, anti inflammatory and hypoglycemic [6]. According to WHO report 80% of the world population are taking interest in indigenous herbal medicines usually been seed inform of fruits, vegetables, drugs or their extracts for the treatment of the diseases and for maintenance health [11] . The results of this work

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showed that the seeds extract of *B. coriacea* inhibited the growth of all the tested isolates at varying concentration of 50, 100, 150 and 200 mg/ml. The antimicrobial activity of extracts of medicinal materials has been attributed to the phytochemicals constituents present in them [12] and the extracts of *B. coriacea* won't 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 [13]. The active components usually interfere with growth and metabolism of micro organisms in a negative manner [12]. The antibacterial activity of ethanolic and methanolic extract of *B. coriacea* seeds at varying concentrations (50,100, 150, 200 mg/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 it's extraction power [14]. 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 produced 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±0.01 and 27.00± 0.02. The antibacterial activity of the extracts increased with the increase in the concentration. *S. aureus* was the most susceptible with inhibition range between 9.00 ± 0.03 and 27.00 ± 0.02 for ethanolic extract at 50mg/ml and methanolic extract at 200mg/ml respectively.

This was followed by *S. typhimurium* with inhibition zone range between 2.0± 0.04 and 22.0± 0.06, *P. vulgaris* has inhibition zone ranging between 3.00± 0.01 and 14.0± 0.01. The most resistant to the extracts was *B. subtilis* with inhibition zone range between 1.00 ± 0.01 and 7.00± 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± 0.32 and 9.00± 0.21.

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

Bacterial isolates	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
Staphylococcus aureus	10 ± 0.04	9± 0.33	17± 0.03	14± 0.16	22± 0.06	20± 0.16	27± 0.02	24± 0.16
Klebsiella pneumonia	8±0.02	6± 0.04	12± 0.10	10± 0.23	16± 0.07	15± 0.01	18± 0.05	160±.17
Pseudomonas aeruginosa	2± 0.01	1± 0.03	6± 0.11	5± 0.14	9± 0.16	7± 0.06	12± 0.02	10± 0.16
Proteus vulgaris	5± 0.02	3± 0.01	7± 0.12	5± 0.06	10± 0.14	8± 0.10	14± 0.01	12± 0.01
Bacillus subtilis	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
E. coli	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
Salmonella typhimurium	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 ± S. D. METH=Methanolic extract, ETH=Ethanolic extract

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
Aspergillus niger	8± 0.2	7± 0.11	14± 0.16	10± 0.01	22± 0.15	22± 0.07	34± 0.02	28± 0.01
Trichoderma spp	4± 0.01	4± 0.02	8± 0.06	7± 0.06	18± 0.23	15± 0.16	26± 0.05	22± 0.16
Rhizopus spp	4± 0.16	3± 0.45	7± 0.23	6± 0.18	14± 0.01	13± 0.02	22± 0.06	18± 0.16

Values are means of duplicate results ± S. D. METH=Methanolic extract, ETH=Ethanolic extract

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 revealed the antibacterial nature of these seeds and suggests that these seeds could be exploited in the management of disease caused by these bacteria in human systems. The results of the antibacterial activity showed that the plant seeds are rich reservoir of antimicrobial as observed by other workers such as [15,16] .The active components usually interfere with growth and metabolism of microorganisms in a negative manner [12] . 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 genus Klebsiella and it is emerging as an important cause of neonatal nosocomial infections [17] .*E. coli* causes Septicemias and can infect the gall bladder, surgical wounds, skin lesions and the lungs especially in debilitate and immuno deficient [18] . *Proteus vulgaris* is capable of causing inflammation of the

bladder [19]. *Pseudomonas aeruginosa* which is an opportunistic pathogen that is wide spread in the environment, a major cause of nosocomial infections and an occasional cause of community acquired infections [19]. *Staphylococcus aureus* and *Salmonella typhimurium* had been associated with food poisoning and typhoid fever respectively while *Bacillus subtilis* is capable of causing eye infection, food spoilage and food borne intoxication [19]. The antifungal activity of the *B.coriacea* seeds extracts are shown in table 2 indicated a significant antifungal activity on the fungi used. Many scientists [20,21,22,23,24,25,26] just to mention few had work on the antifungal activities of medicinal plants from different regions of the world. *Aspergillus niger* was the most susceptible with inhibition zone ranged between 7.00± 0.11 and 34.00± 0.02 (mm) followed by *Trichoderma* sp between 4.00± 0.01 and 26.00± 0.05 while *Rhizopus* sp showed the lowest susceptibility with inhibition zone between 3.00 ± 0.15 to 22.00± 0.06 (mm). Many investigations were carried out to discover plant products that inhibit the fungi like *Aspergillus* sp*, Trichophytan* sp (rubrum), *Rhizopus* sp*.* These fungi species causes infections in human which are difficult to control effectively and the pharmaceutical arsenal currently available [27]. Hence, plant products that inhibit their growth without harming the host represent 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 that the methanolic extract showed higher inhibition zones than the ethanolic extract in terms of radial mycelia growth of the fungal species used. [16] also found out the hexane and methanolic extract of *B. coriacea* seeds inhibit the growth of *Trichoderma viride* and *Aspergillus niger*.

CONCLUSION

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

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