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Evaluation of the phytochemicals and microbial inhibitory properties of *Piper guineensii* and *Buchhlozia coriacea* seeds of Nigerian Origin

Ojelere O. Olusola*and Adoghe O. Eromosele

Department of Chemistry, Faculty of Sciences, University of Ibadan, 23402, Nigeria.

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ABSTRACT

To solve the problem of microbial outbreak which is a major challenge to human health in developing countries like Nigeria, herbal drugs are being looked as an imperative source for discovery of new agents for treating various ailments related to microbial infections. In this present study, the seeds of *Piper guineensii* and *Buchhlozia coriacea* were evaluated for their phytochemical constituents and antimicrobial properties. The phytochemical analysis revealed the presence of alkaloids, saponins and reducing sugar in the two plants while others like tannin, flavonoids and steroids were also observed interchangeably. The broad spectrum of the antimicrobial activities of the two seeds proved them to be active against various pathogens tested. The outcome of this work is a good evidence to support ethno-medicinal uses of these plants in the treatment of various diseases.

Introduction

According to the World Health Organization, W.H.O. (1999) a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. In Nigeria many spices are used as food and medicine, however, a great number of these plants are traditionally used due to the medicinal properties and availability.

A good proportion of the world population particularly those living in developing countries like Nigeria depend mostly on herbal medicines for their health needs. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. In the battle for survival, plants have evolved with many chemical defenses as means of survival to ward off attackers such as bacteria, insects, fungi, severe weather and, in some cases, mammals that may threaten their existence. Secondary metabolites, while not essential for growth and development, do promote the spread and dominance of plant species in an ecological setting. As a result of this and the reported therapeutic activities associated with different phytochemicals, they are therefore worth the effort in research into the discovery of new drugs or as a viable alternative to the existing drugs.

Nowourdays, the challenge of drug resistance is leading researchers in the direction of Antimicrobial medicinal plant research. According to the World Health Organization (WHO), 80 % of the world's population uses medicinal plants in the treatment of diseases. In African countries this rate is much higher (Ajose, 2007). In recent years, medicinal plants have represented a primary health source for the pharmaceutical industry (Phillipson, 1991). No less than 400 compounds derived from plants are currently used in the preparation of drugs, such as vincristine and vinblastine used in the treatment of cancer (Ajose, 2007) and quinine and artemisinin used as antimalarial (Phillipson, 1991). The utilization of the medicinal plants is often based on ancestral experience, limited scientific evidence regarding safety and efficacy to support the continued therapeutic application of some of these herbal remedies exists compared to such evidence for synthetically formulated drugs.

*Piper guineense*is a spice plant from the family, *piperaceae* and from the genus piper. It is a West African spice plant and is commonly called Ashanti pepper. The seeds, leaves and sometimes the stems are used in preparing soup. It imparts "heat" and a spicy pungent aroma to food. The medicinal properties of *Piper guineese* exert bacteriostatic and bacteriocidal effects on some bacteria. The leaves are considered aperitive, carminative and eupeptic. They are also used for the treatment of cough, bronchitis, intestinal disease and rheumatism. The leaves are also used for female infertility while the fruits are used as an aphrodisiac. It is a climbing plant that can grow up to 20m in length. The seeds are smooth and are prolate-elliptically shaped.

*Buchhlozia coriacea (*Wonderful Kola), as it is commonly called, is known in the world as memory nut because it enhances the memory. It act as cleaner of the blood, facilitates learning ability and strengthens the nervous system, and also effective in the treatment of menstrual problems. It's a brain food which promote memory. It's also useful in treatment of hypertension and also prevents premature aging; it has also been proved in Africa that Wonderful Kola has the ability to stop migraine/headache when on the forehead for about ten minute. Its normally use for the treatment of so many diseases, like cough, chest, pain, waist pain, irregular menstruation, internal pile, malarial, quick ejaculation, headache, hypertension, dysentery, premature aging, memory improvement, blood cleansing, chronic venous, insufficiency, mental function, minor burns, scars, scieroderma, skin ulcers, varicose veins, wound healing, rheumatism, blood disease, congestive heart failure, urinary tract infections, venereal disease, hepatitis and high

Tele:+2348030865335
E-mail addresses: solarry206@yahoo.com

blood pressure phlebitis, leg cramps and abnormal fingling of the extremities, improves circulation, strengthens veins and capillaries. It is also use as adrenal purifier, AIDS, blood purifier, elzema, epilepsy, insanity, hypochondria, hair loss, intermittent fevers, immune system boost (cleansing and nourishing) Psoriasis, skin conditioner, bowel disorder tetnus, convulsions, elephantiasis. It strengthens the adrenal glands and cleans the blood to treat skin impurities. It is said to combats stress and depression, increase libido and improve reflexes. It is a wonderful herb with incredible healing powers and is a rich source of vitamin K.

With the upsurge in the use of herbal remedies, there is a need for a thorough scientific evaluation to validate or disprove the supposedly therapeutic effects of some of these medicinal plants. However, a number of compounds extracted from traditional plants have not been thoroughly studied for phytochemical and efficacy. Therefore, this study was initiated at validating the pharmacological significant of *Piper guineensii* and *Buchhlozia coriacea* seeds.

Materials and methods

Collection of plant material

Fresh seeds of *Piper guineensii* and *Buchhlozia coriacea* were purchased from Bode market in Ibadan, Oyo State, Nigeria in month of April, 2013. The plant seeds were identified and authenticated at the Herbarium Unit of Forest research Institute of Nigeria (FRIN), Jericho Ibadan, Oyo state, Nigeria. Dirt's and other extraneous materials were removed from the seeds. They were air dried and pulverized with a ceramic mortar and pestle to powder and the powder used for subsequent analysis.

Figure 1: *Piper guineensii* and *Buchhlozia coriacea* seeds

Preparation of plant extract

The air dried finely ground seeds (40 g) were taken separately in air tight bottles and 200 ml of ethanol was added and kept under dark. After 3 days, the contents were stirred well and filtered using Whatmann no: 1 filter paper. Thereafter, the filtrate was evaporated to dryness by means of a rotary evaporator attached to a vacuum pump. The extracts were stored in refrigerator until needed for further analysis.

Collection of Microorganisms

All the microorganisms used for this study were pure isolates obtained from the Laboratory stock culture unit of the Department of Microbiology, Faculty of science, University of Ibadan, Oyo State, Nigeria.

Phytochemical Screening of Crude Extracts

Phytochemical screening of the crude extracts for alkaloid, saponin, glycoside, tannin, steroids, flavonoids and cardiac glycoside were carried out by the methods described by Evans (2002) and Sofowora (2001).

Determination Antimicrobial Activity

The antimicrobial activity of the two seeds against clinical pathogens was determined by using agar well diffusion method based on the guidelines of the National Committee for Clinical Laboratory Standards NCCLS (2002). Sterile nutrient agar plates were prepared for bacterial strains and Sterile Sabouraud's dextrose agar (SDA) were prepared for fungal strains inoculated by a spread plate method under aseptic conditions. 20 ml of sterilized nutrient agar was poured into Petri dishes and allowed for solidification. After solidification, 24 hours nutrient broth grown pathogenic cultures were swabbed on the respective agar plates using sterilized cotton swabs. Wells of 6 mm diameter were punched over the agar plates using a sterile gel puncher. About 100 µl of different concentrations of plant solvent extracts were added using sterile syringe into the wells and allowed to diffuse at room temperature for 1hour and the plates were incubated at 37° C for 18-24 hours for bacterial pathogens and 28° C for 48 hours fungal pathogens respectively. After incubation, the diameter of inhibition zones formed around each wells were measured and expressed in millimeter (mm) and recorded against the corresponding concentrations to evaluate the antimicrobial activity. Positive control was set using standard antibiotics drugs while a negative control was set using extraction solvents.

Statistical analysis

All data generated were analyzed using descriptive statistic (Olawuyi, 1996). Statistical values were calculated using mean. All experiments were performed in triplicate.

Results and discussion

Phytochemical screening

The phytochemical screening conducted on the crude extracts of *Piper guineensii* and *Buchhlozia coriacea* seeds as shown in the table 1 below revealed that both of them contain alkaloids, saponins and reducing sugar while other secondary metabolites like tannin, flavonoids and steroids were also observed interchangeably. Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones; Okwu, D.E., (2001). Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombic and vasodilatory activity; Alan and Miller (1996). Various studies have shown that saponins although non-toxic can generate adverse physiological responses in animals that consume them. These compounds have been shown to be active against potentially significant pathogens including those that are responsible for enteric infections Owolabi, *et al*. (2007). Apart from their potential antibacterial activity, compounds present in this study such as alkaloids are known as antimalarial agents, analgesics and can act as stimulants. Glycoside moieties such as saponins, anthraquinones, cardiac glycosides and flavonoids can inhibit tumor growth, act as an antiparasitic agent, and can be used as an antidepressant.

Antimicrobial activity

Phyto-constituents employed by plants to protect them against pathogenic insects, bacteria, fungi or protozoa have found applications in human medicine (Nascimento*et al*., 2000). Some phytochemicals such as phenolic acids act essentially by helping in the reduction of particular adherence of organisms to the cells lining the bladder, and the teeth, which ultimately lowers the incidence of urinary-tract infections (UTI) and the usual dental caries. Plants can also exert either bacteriostatic or bactericidal activity on microbes.

Table 1: Phytochemical constituents of *Piper guineensii* & *Buchhlozia coriacea* seeds

Phytochemicals	*Piper guineensii*	*Buchhlozia coriacea*
Alkaloids	+	+
Saponin	+	+
Tannins	+	-
Flavonoids	-	+
Steroids	-	+
Cardiac glycoside	+	-
Reducing sugar	+	+

Table 2: Antimicrobial activities of *Piper guineensii* & *Buchhlozia coriacea* seeds extract on isolated pathogens by zone of inhibition in (mm)

Extract (mg/ml) *P. guineensii*	***Aspergillus niger***	***Bacillus subtilis***	***Candida albicans***	***Escherichia coli***	***Penicillium notatum***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
160	18	15	21	20	22	13	11
80	14	13	18	14	20		10
40	12		16	-	17		
20	-	-	-	-	12		
Extract(mg/ml) *B. coriacea*							
160	16	19	18	18	20	17	21
80	14	14	12	15	12	14	13
40		12		12			
20	-	-	-	-	-	-	-
Gentamycin 10µg/ml	22	24	22	21	23	20	24

The ethanolic extract from the seeds of *Piper guineensii* showed higher effect in diameter of zone of inhibition than *Buchhlozia coriacea* ethanolic extract against *Escherichia coli, Aspergillus niger, Penicillium notatum, and Candida albican* while *Buchhlozia coriacea* showed higher effect than *Piper guineensii* against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. These results are comparable to the commercially available broad spectrum antibiotics (Gentamycin).

Conclusion

The result of this research work revealed that the two plant seeds investigated showed reasonable degrees of antimicrobial properties against the clinical pathogens tested as revealed by the broad spectrum of the zone of inhibition, which suggest that the seeds could probably be good use as antimicrobial agents in new drug formulation.

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