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EVALUATION OF ANTIMICROBIAL PROPERTIES OF DIETHYLETHER EXTRACT OF THE ROOT-BARK OF *BUCHHOLZIA CORIACEA FAMILY CAPPARACEAE*

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ABSTRACT

Aim - Buchholzia coriacea had been claimed to have medicinal properties. It has been used by the locals, in Idemili area of Anambra State, Nigeria to manage "wounds and running stomach". This study is therefore aimed at determining this claim which will also serve as criteria to recommend the ethanopharmacological use of the plant. The rootbark of Buchholzia coriacea was dried, powdered and extracted by cold maceration with diethylether for 48hrs, it was concentrated using rotary evaporator. The antimicrobial activity was investigated using agar diffusion method. Phytochemical evaluation revealed the presence of tannins, flavonoids, alkaloids, glycosides, and saponins. The phytochemical analysis of the rootbark of Buchholzia coriacea shows the presence of tannins, flavonoids, glycosides, alkaloids and saponins. The diethylether extract of the rootbark of Buchholzia coriacea exhibit antimicrobial activity. The claim of the locals in Idemili area of Anambra State, Nigeria in traditional medical management of infection, could be supported by the results of this investigation

Key words – Buchholzia Coriacea, Agar Diffusion, Rotary Evaporator, Root Bark

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INTRODUCTION

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population (Sofowura A 2008). This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations (Srinivas *et al,* 2007). Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some case) and the cultural and spiritual point of view of the people of the countries (Srinivas *et al).* In Western developed

countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited (Satyejji and lutfun*, 2007)*. Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over -prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care (Appidi et al 2008). All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine become necessary. (Fagbohun *et al,* 2010).

Before the era of Louis Pasteur (1822-1895), world renowned chemist and biologist who proved the germ theory of disease, the notion that tiny organisms could kill vastly larger ones (including human) seemed ridiculous to many people. Nowadays, it has been accepted that infectious diseases are the number one causes of death worldwide, accounting for approximately one half of all deaths in tropical countries (Iwu *et al*., 1999). In fact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to availableagents(KeysJohnD1988).

The use of plant parts as a source of medicine to treat infectious diseases predates history. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines (Erdemeier et al. 1996; Lino and Deogracious, 2006) to cure infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control. Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria. Even the World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs (WHO Traditional Medicine Strategy, 2002). However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonized by majority of allopathic medical practitioners as it is considered to have no scientific basis. This work is therefore a preliminary work to prove that there is scientific evidence to the use of the root of *Buchholzia coriacea* in the treatment of diseases.

One major problem of herbal medicine practice is that there is no official standard and / or local monograph. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine boards to license and regulate the practice of herbal practitioners under the supervision of ministries of health (Carvalho et al 1996). Many medicines including reserpine, ergotamine, vincristine, and vinblastine are of herbal origin. About one quarter of the present prescription drugs dispensed by community pharmacies in the United States contain at least one active principle originally derived from plant materials (Farms Worth and Moris, 1976). *Buchholzia coriacea* was named after R.W Buchholz who collected the plants in Cameroon in the late 19th century (keay et al 1989). It belongs to the family Capparidaceae. The seed of *buchholzia coriacea* has medicinal values. These seeds gave the plant a common name wonderful kolanut because of its' usage in traditional medicine. The seeds are covered in purple aril which are chewed in Ivory coast and has a pungent taste. It is used to treat a variety of illnesses. Bucholzia coriacea also known as musk tree is a member of the family Capparidaceae. It is an evergreen under-storey tree of lowland rain forest, up to 20 metres high occurring in West Africa, from Guinea to west and east Cameroon and in Gabon. The tree is found

in the southern part of Nigeria, Ghana and Liberia. The bark can be made into a pulp for inhalation or into a snuff to relieve headache, sinusitis, and nasal congestion in Ivory Coast; smallpox or skin itching in Gabon. The pulped bark is applied to the chest to treat chest pains and also boils. In Liberia, the seeds are used on skin eruption and internally for worms. In Ivory Coast, the crushed up seeds, are pasted over the stomach for difficult childbirth. It is also considered anthelmintic (worm expeller). It is used as cough medicine, and in the treatment of ulcer. It is also used in the treatment of hypertension by drinking the fluid squeezed out of the leaves with pea leaves and small salt. Plants that belong to the botanical family Capparidaceae have been used for the treatment of syphilis, dressing of wounds, chronic ulcers and for the treatment of snake bites. Certain plants of the family Capparidaceae have been used for the treatment of gonorrhoea, convulsion in children, as aphrodisiacs and as anthelmintics. In the Ivory coast the twig bark decoction of the plant *Buchholzia coriacea* is used for the treatment of rheumatism and kidney pain, it is also used for the treatment of infections of the eye (bark gruel poured into the flat of the hand and inhaled) and for the treatment of pain in the back (fruit pulp massaged in). For the treatment of earache, seeds are pounded in a little bit of water and the resulting liquid is dropped into the ear. The Ebri tribes bathe smallpox victims with the bark decoction of the plant *Buchholzia coriacea*. Young leaves of the plant *Buchholzia coriacea* are used in a gruel poultice for ulcers and boils. In Gabon pounded bark of the plant *Buchholzia coriacea* is used as a lotion against scabies, the fruit of the plant *Buchholzia coriacea* as an anthelmintic. In former times young warriors were given fresh roots of the plant *Buchholzia coriacea* to stimulate them before battle.

The seeds or kernels of the plant *Buchholzia coriacea* are edible and that they have a spicy taste and that they can be used as a condiment (spice). The ground seeds or kernels of the plant *Buchholzia coriaceae* are a component of a traditional and valued aphrodisiac or stimulant that is sold on local markets in Africa (Cameroon). The African plant Buchholzia coriaceae is used as stimulant, tonic, and aphrodisiac.

TAXONOMY PROFILE

Family: capparaceae Juss , Super order: Rosanae Takht , Order: brassicales Bromhead

Genus: Buchholzia engl

Class: Eqissetopsida c. Agardh

Sub class: magnoliidae nov'ak ex takht

Specie: Coriaceae

DISCRIPTION

The plant *Buchholzia coriacea* is a shrub or medium-sized tree, evergreen, with a dense crown, large glossy leathery leaves arranged spirally and clustered at the ends of the branches, and conspicuous cream-white flowers in racemes at the end of the branches. The bark of the plant *Buchholzia coriacea* is smooth, blackish-brown or dark-green. Slashes are deep red turning dark brown; Akpayunget al (1995) and Awouters et al (1995). The leaves of the plant *Buchholzia coriacea* can be described as follows: large, obovate, oblanceolate to elliptic, shortly acuminate or acute

at apex, cuneate at base, 15-30×5-11 cm, thinly coriaceous, glabrous, midrib very prominent below, about 10 lateral nerves, each running directly into the one above and forming distinct loops close to the margin, prominent below, stalk 10-15 cm long, swollen for about 1 cm at both ends, pale green.

The flowers of the plant *Buchholzia coriacea* can be described as follows: in simple or lightly-branched lax racemes among the leaves at the ends of the shoots, up to 24 cm long, individual flowers with a stalk less than 1,3 cm. 4 small rounded sepals bent right back exposing the thick saucer-shaped purplish receptacle, without petals, 40 to 45 stamens with cream-yellow filaments and small purplish-black anthers and a narrow elongated ovary projecting beyond the stamens at the end of a thin stalk. The fruits of the plant *Buchholzia coriacea* can be described as follows: large, long-stalked, ellipsoid, resembling avocado pears, 12×5-8 cm, endocarp up to 1.3 cm thick and woody, yellowish when ripe, flesh yellow, edible, containing a few large blackish seeds, about 2.5 cm long; Culpeper(1995), Grieve Maud(1984) and Ketende AB et al (1995). The plant *Buchholzia coriacea* is a tree of the lowland rain forest in the region Guinea to Cameroon, and in Gabon. In Gabon the plant *Buchholzia coriacea* is sometimes cultivated as a medicinal and fetish plant. Gbile et al (1993). Vernacular names of the plant *Buchholzia coriacea* are Cola pimento, elephant cola, oignon de Gorille and Okpokolo in Igbo; Palombo EA (2006), Andrews(1982) and Arber Agnes(1986)

MATERIALS AND METHOD

DRUGS AND CHEMICALS

Diethylether

Nutrient agar

Nutrient broth

MATERIALS

Miller (Thomas Laboratory Mill,U.K)

Mechanical Weighing Balance (Ohaus,Poland)

Electronic Weighing Balance (Gulfes Mediqal and Scientific,England)

Filter Paper (No. 1 wattman)

White Clean Handkarchief (as porcelin cloth)

Rotary Evaporator (Fulton,china)

Oven (Harris,England)

Mechanical shaker (Surgifrend,England)

Incubator (Genlab,U.K)

Culture Plates

Autoclave (health team instrument,England)

Beakers (10ml,25ml,50ml,500ml capacities)

Cotton wool

Hand gloves

Syringes and Needle (1ml,2ml,5ml)

PLANT MATERIAL

COLLECTION AND IDENTIFICATION

The plant was identified by Mr Ozioko, a Taxonomist of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

EXTRACTION

The rootbark of *Buchholzia coriacea* was dried at ambient temperature until their weight which was measured at intervals was about the same. The dried root was pulverized using laboratory miller; 250g of the powder was macerated in 500ml of diethylether which was placed on a mechanical shaker for 48 hours. The content was filtered using clean white handkerchief, and then the filtrate was further filtered using No.1 wattman filter paper. The filtrate was concentrated using rotary evaporator. The extract was stored in the refrigerator for future use.

PHYTOCHEMICAL ANALYSIS

Phytochemical tests were carried out using standard procedures to identify the constituents as described by Sofowura (1993),Trease and Evans (1989) and Harborne (1973).

Test for tannins: About 0.2 g of the extract was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.
Test for saponin: About 2 g of the extract was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and then the formation of emulsion was observed.
Test for flavonoids: A portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.
Test for steriods: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of sample with 2 ml H_2S0_4. The colour changed from violet to blue indicating the presence of steroids.
Test for terpenoids (Salkowski test): Five ml of the extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddishbrown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): 5ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test **for Anthraquinones :** 0.5g of the extract was boiled with 10ml H_2S0_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour change.

Test for Alkaloids: 0.5g of the powdered extract was stirred in 5ml of 1%HClaq on a steam bath for 5mins. The mixture was then filtered using Whatman's no1 filter paper. To the filtrate, 2-4drops of Dragendoffs' reagent was added to 1ml of the filtrate. An orange colour was observed indicating the presence of alkaloids.

ANTIMICROBIAL ASSAY

Microorganisms: 24hour Cultures of five human pathogenic bacteria made up of both gram positive (*S. aureus,* and *B.subtilis*) and and gram negative (*P. aeruginosa, E. coli* and *S. typhi*) bacteria were used for the *in-vitro* antibacterial assay. All microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka.

Preparation of media: nutrient agar, was used in the assays. Dimethylsulphoxide (DMSO) was used in solubilizing the extracts and drugs and as a negative control in the study. The media was prepared by dispersing the weighed amount in water and then sterilized in autoclave. The plates of nutrient agar were poured and allowed to solidify after the appropriate organisms were seeded. Majorie Murphy Cowan (1999).

Antimicrobial agents: Ampicillin, 20ug/ml (Mecure indusrial ltd Lagos Nigeria.); was used in the study as standard reference drug.

Antimicrobial activity determination: An overnight broth culture used to obtain 0.5 marcfarland standard of bacterium was used to seed sterile molten nutrient agar medium maintained at 45°C. Seven holes (6mm) respectively, were bored in each of the plates (9cm, diameter) with an aseptic cork borer, when seeded plates had solidified; 400mg/ml, 200mg/ml,100mg/ml,50mg/ml,25mg/ml12.5mg/ml and 6.25mg/ml of extract were prepared in dimethylsulphoxide (DMSO) by preparing a stock solution and carrying out double fold dilutions on it. And with the aid of a Syringe, the wells were filled with 0.25 ml (5drops) of different dilutions of the extract while the centre well was filled with 20µg/ml of ampicillin (also dissolved in DMSO). Diameters of zones of inhibition were determined after incubating plates at 37°C for 24h for bacteria. This test was conducted first on the crude extract and the solvent dimethylsulphoxide was used as negative control while ampicillin was used as positive control.

RESULT: Table 1 Phytochemical constituents of Buchholzia coriacea

PHYTOCHEMICAL ANALYSIS	RESULTS
Alkaloid	+
glycosides	+
Flavonoids	+
Saponins	+
Tannins	+

+ indicates presence of secondary metabolite

-Indicates absence of secondary metabolite

Table2 The results of antimicrobial screening

Bacteria	400mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Amp20ug/ml
Staphilococcus aureus	6mm	4mm	2mm	-	-	-	-	6mm
Escherichia coli	-	-	-	-	-	-	-	9mm
Bacillus subtilis	8mm	6mm	4mm	2mm	-	-	-	5mm
Pseudomonas aeruginosa	4mm	2mm	-	-		-	-	6mm
Salmonella typhi	6mm	4mm	2mm	-	-	-	-	6mm

DISCUSSION

The results of phytochemical screening showed presence of simple sugar and flavonoid, essential oil, phenolic group, glycoside, and saponin in the methanol root extract screened for secondary metabolites. Some of these active principles (secondary metabolites) have been reported to have activity against micro-organisms. Flavonoid, phenolics, Alkloids, triterpenes and essential oils have been shown to have activities (Majorie, 1999). The Presence of alkaloids, cyanogenetic glycosides, steroidal nucleus and reducing sugars, phenolic group and essential oil are normal with the plants of this family capparidaceae (Kjaer and Thomson, 1973; Lakshimi and Chanhan, 1977) Ajaiyeoba E. O., 2000). The extracts displayed various activities against bacteria inhibiting it at various concentrations ranging from 400 to 6.25 mg/ml. The inhibition zone of the extract at 400mg/ml, 200mg/ml, and 100mg/ml are 6mm, 4mm and 2mm respectively against Staphiloccocus aureus and Salmonella typhi. At 400mg/ml the activity of the extract is comparable to the standard antibiotic ampicillin with inhibition zone of 6mm. it has no activity against E.coli. But it is effective against Pseudomonas aeruginosa with inhibition zones of, 4mm, and 2mm at concentration of 400mg/ml, 200mg/ml respectively. It is very effective against Bacilus subtilis with the inhibition zone of 8mm, 6mm, 4mm, 2 mm at concentrations of 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml respectively

CONCLUSION

The present study shows that the root of *Buccholzia coriacea* has a lot of potential as an anti microbial agent. These observed activities appear to justify the ethno pharmacological uses of the plant.

RECOMMENDATION

There is need for further study and characterization of the plant to ascertain the active constituent of the drug for easy design and synthesis.

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