

THE PHYTOCHEMICAL, ANTISPASMODIC AND ANTIDIARRHOEA PROPERTIES OF THE METHANOL EXTRACT OF THE LEAVES OF *BUCHHOLZIA CORIACEA* FAMILY CAPPARACEAE

CHINEDU FRED ANOWI¹, CHIBEZE IKE², EMMA EZEOKAFOR³, CHUKWUENWEIWE EBERE⁴

¹Department of Pharmacognosy and Traditional Medicine, ²Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, ³Department of Physiology, Faculty of Medical Sciences, ⁴Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmacy, Madonna University, Elele.

Received: 19 March 2012, Revised and Accepted: 30 April 2012

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 frequent 'stooling'. This study is therefore aimed at determining this claim which will also serve as a criteria to recommend the ethanopharmacological use of the plant.

Methodology: The leaves of *Buchholzia coriacea* were dried, powdered and extracted by cold maceration with methanol for 48hrs, it was concentrated using rotary evaporator. The anti-diarrhoeal activity of the leaves extract was investigated using castor oil induced diarrhoea in rats. Whereas antispasmodic study was investigated using charcoal meal in tragacanth mucilage. Phytochemical evaluation revealed the presence of tannins, flavonoids, alkaloids, glycosides, and saponins .

Result: *Buchholzia coriacea* leaves extract (100 -500 mg/kg) caused a significant and dose-related inhibition of the frequency of diarrhoeic drops in rats ($p < 0.05$) and prolonged the time for diarrhoea induction. It also exhibited antispasmodic effect.

Conclusion: The claimed benefits of *Buchholzia coriacea* in traditional medical management of diarrhea as well as "stomach bite", could be supported by the results of this investigation.

Keywords: *Buchholzia coriacea*, Castor oil, Loperamide tablet, Atropine, Charcoal meal.

INTRODUCTION

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 . *Buchholzia 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 coriacea* are a component of a traditional and valued aphrodisiac or stimulant that is sold on local markets in Africa (Cameroon). The African plant *Buchholzia coriacea* is used as stimulant, tonic, aphrodisiac.

Taxonomy Profile

Family: capparaceae Juss

Super order: Rosanae Takht

Order: brassicales Bromhead

Genus: *Buchholzia* engl

Class: Equissetopsida 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

Loperamide
Castor oil
Atropine
Methanol
Charcoal meal

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 Handkerchief (as porcelain 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)

Animal

Albino rats (57 – 220g) and albino mice (18 – 29g) of both sexes.

Plant Material

Collection and Identification

Young fresh leaves of *Buchholzia coriacea* were collected from Ogidi, Idemili North local government area of Anambra State in July, 2011, during the rainy season and was identified by Dr. Ezugwu, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Extraction – Methanol Extraction

Fresh leaves *Buchholzia coriacea* were dried at ambient temperature until their weight which was measured at intervals was about the same. The dried leaves were pulverized using laboratory miller, 200g of the powder was macerated in 500ml of methanol and were placed on a mechanical shaker for 48 hours. The extract was filtered using clean white handkerchief, 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 Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for tannins: About 0.5 g of the dried powdered samples 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 colouration.

Test for saponin: About 2 g of the powdered sample 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, then observed for the formation of emulsion.

Test for flavonoids: A portion of the powdered plant sample 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 steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H₂SO₄. 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 H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): 5ml of the extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid 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 H₂SO₄ and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette 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 extracts was stirred in 5ml of 1% HCl on a steam bath for 5mins. The mixture was then filtered using Whatman's no1 filter paper. To the filtrate, 2-4 drops of Dragendoff's reagent was added to 1ml of the filtrate. An orange colour was observed indicating the presence of alkaloids.

Pharmacological tests

Acute Toxicity Test

The acute toxicity study of *Buchholzia coriacea* was assessed by oral administration in albino mice using the method of Lorke (1983) also Carvalho et al (1978). Briefly, the tests involved two phases. The first phase involved the determination of the toxic range. The mice were placed in three groups (n = 3) and the extract (10, 100 and 1000 mg/kg) suspended in distilled water was administered orally. The treated mice were constantly observed for the next 4hrs, then intermittently for the next 6hrs, then over a period of 24hrs. Then the number of deaths in each group was recorded. The death pattern in the first phase determined the doses used for the second phase. In this phase, four groups (n = 1) of mice were used for each dose. Each group received different doses of the extract (p. o.) 1500 mg/kg, 2500 mg/kg, 3500 mg/kg and 5000 mg/kg respectively. The animals were observed for lethality or signs of acute intoxication for the next 24hrs. The LD₅₀ was calculated using the relation

$\sqrt{2a \times b}$. Where 'a' is the lowest dose that brought death and 'b' is the highest dose that did not bring death.

Antispasmodic Activity

Groups of three rats each were treated as outlined:

Group 1-0.5ml of distilled water

Group 2-1mg of atropine intraperitoneally

Group 3-50mg/kg of extract

Group 4-100mg/kg of extract

Group 5-200mg/kg of extract

The animals were starved for 18hrs prior to this experiment. All the animals were given orally 1ml of 3% deactivated charcoal in the tragacanth mucilage. Immediately after the administration, the drugs were administered orally. 30mins after administration, all the animals in each group were sacrificed, dissected and the intestine were cut out and the distance the charcoal travelled from the stomach to the caecum were measured using meter rule and were expressed as percentage to the total length.

Castor oil-induced diarrhoea in rats

A modified method of castor-oil induced diarrhoea previously described (Sunil et al., 2001) and Appidi et al (2008) was used to simulate diarrhoea condition in rats. Rats were fasted for 24hour prior to the experiment and were randomly divided into five groups (n=5). The rats in the first group served as the control treatment and were given 0.5ml of distilled water orally using orogastric cannula. The second group received a standard anti-diarrhoea agent, loperamide (2 mg/kg). The last three groups are the test group and were treated with the extract (100, 250, and 500 mg/kg; p. o). After 1hr of the different treatments, castor oil 1.0 mL/rat was administered orally to each rat and thereafter the rats were separated into single cages for observation. The time of diarrhoeal onset was recorded for each animal in the group, which is the time interval between castor oil administration and the appearance of the

first diarrhoeal drop. The mean number of defecation and the number of wet drops were also recorded for the test group (Dt) and for the control (Dc). Observation for defecation continued up to 6 hrs on pre-weighed (W_0) filter paper placed beneath the individual rat cages; Awouters et al (1978) and Havigiray et al (2004). The filter paper was replaced at hourly intervals and reweighed (W_1) with the wet faeces. The fresh weight of the faecal droppings was determined, ($W_1 - W_0$) g. The fluid content of the faeces was also determined by drying the filter paper to a constant weight and then re-weighed (W_2). The water content was estimated as ($W_1 - W_2$) g. Inhibition of diarrhoeic drops was calculated by the relation

$$\text{Inhibition of diarrhoeic drop (\%)} = 100 - (\text{Dt}/\text{Dc} \times 100)$$

Where, 'Dt' is the test group and 'Dc' is the control group.

Statistical analysis

The procedure was repeated three times and results expressed as mean \pm standard error of mean (SEM). Differences in observation were determined by Analysis of Variance (ANOVA) using Dunnett comparison method and regarded as slightly significant at $p \leq 0.05$ and extremely significant at $p \leq 0.01$.

RESULT**Phytochemical constituents of Buchholzia coriacea**

Phytochemical analysis	Results
Alkaloid	+
Anthraquinones	+
Cardiac glycosides	+
Flavonoids	+
Saponins	+
Tannins	+

The plant extract revealed the presence of the following secondary metabolites alkaloids, anthraquinone, cardiac glycosides, flavonoids, glycosides, saponins, tannins.

Anti Spasmodic Activity

Group	Dose & agent	Distance travelled by charcoal	Total length of the intestine	% Distance travelled by charcoal
1	0.5ml distilled water	95 \pm 1.27	110 \pm 2.34	86.36%
2	1mg/kg atropine	59.5 \pm 4.27	104 \pm 5.11	56.73%
3	50mg/kg extract	84.5 \pm 1.12	98.5 \pm 1.00	85.75%
4	100mg/kg extract	61.5 \pm 1.27	100.5 \pm 2.42	61.00%
5	200mg/kg extract	89.5 \pm 3.11	105.8 \pm 1.21	84.7%

Mean \pm SEM

The extract was effective as an antispasmodic agent as it reduces the movement of charcoal in the intestine.

Anti Diarrhoea Activity

Grou p	Dose & agent	No of solid faeces				No of wet faeces				Weight of solid faeces	Weight of wet faeces	Total weight of faeces	% Inhibition
		1hr	2hrs	3hrs	4hrs	1hr	2hrs	3hrs	4hrs				
1	0.5ml distilled water	7 \pm 0.4 0	7 \pm 0.40	7 \pm 0.4 0	7 \pm 0.4 0	3 \pm 0.1 2	4 \pm 0.2 5	5 \pm 0.0 7	7 \pm 0.1 3	1.98 \pm 0.1 6	3.95 \pm 0.5 0	5.93	0
2	2mg/kg loperamide	** 0 \pm 0.0 0	** 0 \pm 0.00	** 0 \pm 0.0 0	* 1 \pm 0.5 0	** 0 \pm 0.0 0	** 0 \pm 0.0 0	** 0 \pm 0.0 0	** 2 \pm 0.2 5	** 0.07 \pm 0.0 2	** 0.12 \pm 0.0 2	0.19	71.45
3	50mg/kg	** 0 \pm 0.0 0	** 0 \pm 0.00	** 2 \pm 0.4 6	** 2 \pm 0.4 6	Ns 2 \pm 0.2 5	*2 \pm 0.2 5	*2 \pm 0.2 0.25	** 3 \pm 0.5 0	** 0.30 \pm 0.0 5	** 0.19 \pm 0.0 2	0.49	57.14
4	100mg/kg extract	** 1 \pm 0.0 7	** 1 \pm 0.00	** 1 \pm 0.0 7	** 1 \pm 0.0 7	Ns 2 \pm 0.2 5	*2 \pm 0.2 5	*3 \pm 0.4 6	** 2 \pm 0.2 8	** 0.08 \pm 0.0 2	** 0.18 \pm 0.0 2	0.26	71.43
5	200mg/kg extract	** 1 \pm 0.0 7	** 1 \pm 0.07	** 2 \pm 0.2 8	** 2 \pm 0.2 8	** 0 \pm 0.0 0	** 0.07	** 2 \pm 0.28 2 \pm 0.28	*2 \pm 0.28 2	** 0.29 \pm 0.0 2	** 0.16 \pm 0.0 5	0.45	71.43

Mean \pm Sem

*=slightly significant; **=extremely significant; Ns=not significant

The number of wet faeces produced by the animals that received the extract at the doses of 100mg/kg and 200mg/kg has no significant difference ($p>0.05$) with the group that received loperamide at the dose of 2mg/kg. But the weight of the wet faeces is less in the loperamide group when compared with other groups.

Acute Toxicity Test Result

Phase	Dose	Death
1	10mg/kg	0/3
	100mg/kg	0/3
	1000mg/kg	0/3
2	2000mg/kg	0/1
	3000mg/kg	0/1
	4000mg/kg	0/1
	5000mg/kg	0/1

From the result of the LD50, the extract is well tolerated even at dose up to 5000mg/kg. It is therefore safe for acute administration.

DISCUSSION

Administration of *Bucchozia coriacea* prolonged the time for diarrhoeal induction in a dose dependent manner. The extract significantly inhibited the frequency of diarrhoeic drops in rats by as much as (75.0%) at a dose of 100mg/kg. This is comparable to the effect of loperamide used as a standard drug. The fresh weight of and the water content of faecal matter were also reduced in a like manner by the extract administration.

In previous studies, the antidarrhoeal properties of medicinal plants has been associated with the presence of some plant metabolites such as tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids (Havagiray et al., 2004; Mukherjee et al., 1998); Palombo et al (2006) Our preliminary phytochemical investigation showed the presence of some of these phytoconstituents in *Bucchozia coriacea* leaf extract was found to contain abundant plant tannins, flavonoids, glycosides, alka;oids anthraquinoneglycosides, saponins. Tannins in medicinal plants are known to denature proteins to form protein tannates, an effect postulated to improve the resistance of the intestinal mucosa to chemical alteration leading to reduced hyper-secretion in diarrhoea (Tripathi, 1994; Havagiray et al., 2004). These metabolites could act in synergism to produce the antidarrhoeal effects exhibited by plant extracts. These results suggest that there could be scientific merit in the folkloric use of *Bucchozia coriacea* leaf extract in the management of diarrhea Appidi et al (2008). The extract also reduced the movement of charcoal meal in the intestinal tract of rat.

CONCLUSION

The extract exhibited antidiarrheal and antispasmodic effect. This work therefore justifies the traditional use of the leaves. However more work should be done to ascertain the active principles of the plant.

REFERENCE

1. Akpanyung, E.O., Udoh, A.P and Akpan, E.J. (1995). Chemical composition of the edible leaves of *Pterocarpus mildbreadii*. Plant Foods Hum Nutr. 43 (3) :209.
2. Appidi JR, Grierson DS, Afolayan AJ (2008). Ethnobotanical study of plants used for the treatment of diarrhoea in the Eastern Cape, South Africa, *Pakistan J Biol Sci* 11(15): 1961-1963
3. Andrews, Theodaora 1982.A Bibliography on Herbs, Herbal Medicine, Natural Foods, and Unconventional Medical treatment. Littleton, Colarado Libraries Unlimited, Inc.
4. Arber, Agnes 1986 herbals, The origin and evolution, a chapter in the History of Botany 1470–1670 . Cambrigde:Cambrigde University press.(fist published in 1912)
5. Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ (1978). Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. *J Pharm Pharmacol* 30: 41–45.
6. Carvalho, V.; Melo, V.M.; Aguiar, A.; Matos, F.S. Toxicity evaluation of medicinal plant extracts by the brine shrimp (Arthenus salina Leah) bioassay. *Ciência e Cultura* 1988; 1109-1111: 1988.
7. Culpeper, Nicholas 1995.Culpeper's Complete Herbal:A Book Of Remedies Of Ancient Ills. (The World's Worth Reference Collection Library) Contemporary Publishing Company.
8. Gbile Z.O, Adesina SK, Odukoya OA, Akinwusi DD (1993). Survey on indogenous useful plants of West Africa with special emphasis on medicinal plants and issues associated with their management.
9. Grieve Maud 1984.A Modern Herbal. Harmonds worth. England:Penguin Books. (first published in 1931).
10. Hong–Yen Hsu 1980.Oriental Materia Medica:A Precise Guide. Long Beach:CA: Orienta Healing Arts Institute.
11. Harbourne JB (1984): Phytochemical methods: A guide to modern technique of plant analysis, 3rd ed. London, Chapman and Hall, p. 1-302. ISBN 0412572605
12. Keys, John D.1976. Chinese Herbs, Their Botany, Chemistry, Pharmacodynamics, Rutland, Vermont:Charles E.Tuttle Company.
13. Havagiray R, Ramesh C, Sadhna K (2004). Study of antidiarrhoeal activity of *Calotropis gigantean* r.b.r. in experimental animals. *J Pharmacol Pharm Sci* 7: 70-75 612 p.
14. Lorke D (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol* 53: 275-289.
15. Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP (1998). Screening of antidiarrheal profile of some plant extract of specific regions of west Bengal. *J. Ethnopharmacol.* 60: 85-89.
16. Odetola AA, Akojenu SM (2000). Anti-diarrhoeal and gastro-intestinal potentials of the aqueous extract of *Phyllanthus amarus* (Euphorbiaceae). *Afr J Med Med Sci.* 29(2):119-22.
17. Palombo EA (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother Res* 20 (9): 717-724
18. Raphael, Sandra 1986.Herbal.In Goode, Partrick & Lancaster, Michael."The Oxford Companion to Gardens" Oxford:Oxford University Press.
19. Rohde, Eleanour Sinclair. The old English Hrbals. London:Minerva Press.
20. Stuart, malcolm (ed.)1979. Herbs & Herbalism. London:Orbis Purblishing Limited.
21. Trease, E.E, Evans, W.C (2002). Pharmacognosy. 15th Edition, W.B. Saunders Compound USA.