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PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF *GYNANDROPSIS GYNANDRA* AND *BUCHHOLZIA CORIACEAE* EXTRACTS

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Extracts of leaves and stems of Gynandropsis gynandra L. (Briq.) and Buchholzia coriaceae Engl. (A. Chev.) were screened phytochemically for the presence of secondary metabolites and for in vitro antibacterial and antifungal properties respectively. The main secondary metabolites indicated in both plants were alkaloids, cyanogenetic glycosides and steroidal nuclues. Anthraquinones were slightly indicated. Hexane and methanolic extracts of each of the plant materials of the two plants were screened for antimicrobial properties using eleven clinical strains of human pathogenic microorganisms. At a concentration of 200mg/ml, the extracts displayed various degrees of activity in both bioassays. Of the eight extracts investigated, B. coriaceae stem hexane extract displayed the highest activities in both assays in the agar cup diffussion technique. The microorganisms that were used included six bacteria and five fungi. Ampicillin and tioconazole were used as standard reference drugs while methanol was incuded as a solubilising agent as well as a negative control in the study. Diameters of zones of inhibition were in the range of 10-24mm for the extracts and drugs.

Key words : Buchholzia coriaceae, Gynandropsis gynandra, Phytochemical, Antibacterial, Antifungal.

Gynandropsis gynandra and *Buchholzia coriaceae* belong to the plant family Capparidaceae. *Gynandropsis gynandra* L. (Briq.) syn *Cleome gynandra* L. (Briq) and *Gynandropsis pentaphylla* DC.). Common names include spider flower and cat whiskers. It is a herb indigenous to the tropical and pan tropical regions. The herb is edible and grows up to about 60cm high (Dalziel, 1937; Burkhill, 1985; Irvine, 1961; Adjanohun and Ake Assi, 1972). *Buchholzia coriaceae* Engl. (A. Chev) is commonly known as the Musk tree. It is an evergreen understorey of the lowland rain forest usually ataining a height of about 20m.

Both plants have been used for several years in African traditional medical practices. The bark of *B. coriaceae* is inhaled or snuffed to relieve the symptoms of headache, sinusitis and nasal congestion. The bark sap is also applied for chest pain, bronchitis, and kidney pains. Fresh bark is used in some regions for earache and a decoction of the bark is used for washing of small pox (Kerharo and Bouquet, 1950; Bouquet and Debray, 1974; Irvine, 1961). The leaves have been used for treatment of boils; fruits, for fever; oil of fruits for fish poison; fruits as anthelmintic (Dalziel, 1937; Walker, 1953). *G. gynandra* leaves with a high percentage of Vitamin C is taken as a pot herb in soups, fresh or dried (Ainsle, 1937; Watt and Breyer - Brandigk, 1962). The leaves are used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for earache and eye wash (Dalziel, 1937; Oliver, 1960). Seeds have been reputed to have anthelmintic properties and oil is used as fish poison (Walker and Sillians, 1961; Walker, 1953).

Glucosinolates, also known as mustard oil glycosides are characteristic chemical components of Capparidaceae plants. On enzymatic hydrolysis by myrosinase (present in the plants), isothiocynates are produced from the glucosinolates. An example is Cleomin which has been isolated from *G. gynandra* (Ahmed et al, 1972). Sterols are also found in in these plants; with lupeol, campesterol and epi-lupeol having been isolated from *B. coriaceae* (Kondagbo et al, 1972; Lakshimi and Chanhan, 1977).

In previous studies, the anthelmintic and antimicrobial properties of Capparidaceae plants have been reported ((Ajaiyeoba and Okogun,1996; Ajaiyeoba et al, 1998; Ajaiyeoba et al, 2000) and in continuation of these objectives on this plant family, the phytochemical, antibacterial and antifungal properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* are presented.

MATERIALS AND METHODS

Plants collection and authentication

Gynandropsis gynandra leaves (405g) and stem (500g) were obtained from Olodo area of Ibadan, Nigeria, in December 1997. While leaves (748g) and stem (420g) of *Buchholzia coriaceae* were collected from the Nifor forest reserve, in the outskirks of Benin-City, Nigeria in January 1998. *B. coriaceae* was authenticated under FHI 32885 and *G. gynandra*; FHI 18486 at the Forest Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens were deposited.

Plant extraction

Plant materials were successively extracted in redistilled hexane and methanol by marceration at room temperature (29°C) for 72 hours respectively. Percentage yields were calculated after removal of solvents and the resulting plant extracts were stored in the refrigerator till needed for analysis.

Phytochemical screening

Preliminary phytochemical screening of the four plant materials for various secondary metabolites were done as stated below:

i. **Anthraquinones:** Powdered plant material was boiled with 10% HCl for a few minutes, filtered and allowed to cool. This was then partioned against equal volumes of chloroform. Formation of a rose-pink colour in the aqueous layer on addition of 10% ammonia solution indicated the presence of combined anthraquinones.
ii. **Tannins:** Plant material was boiled with water for a few minutes, this was filtered and diluted with more water. Bluish-black colour formation on addition of a few drops of ferric chloride, is indicative of the presence of tannins.
iii. **Cardiac glycosides**: Sample was extracted with 10 mls of 80% methanol for 5 minutes on a steam bath, filtered and diluted with an equal volume of distilled water. A few drops of lead acatate solution were added, shaken and filtered after a while. Filtrate was then extracted with methylene chloride (two times) and was evaporated to dryness on a steam bath. Then, about 1 ml of 2% 3,5-dinitro benzoic acid in ethanol was added to the residue and the solution was made alkaline with 5% NaOH. The formation of a brownish purple colour is indicative of the presence of unsaturated lactones.
iv. **Cyanogenetic glycosides:** About 1 g of powdered sample was bioled with distilled water and moist sodium picrate paper held inside the tube with a cork. A colour change from yellow to brick-red of the picrate paper is positive for cyanogenetic glycosides.
v. **Steroidal nucleus**: A few grams of the powdered material were heated over a Bunsen flame with 10% HCl and FeCl3 for 15 mins, after which it was filtered and cooled. Then extracted with 2 volumes of CHCl3 . The organic phase was concentrated to a small volume and acidified with 5ml acetic anhydride. The formation of a redish-brown ring at the interface on carefully pouring conc. H_2SO_4, is indicative of the presence of a steroidal nucleus. For details of the phytochemical screeing procedures, please see Harborne, 1991.

Antimicrobial Assay

Microorganisms: Cultures of six human pathogenic bacteria made up of three gram positive and three gram negative bacteria were used for the *in-vitro* antibacterial assay. For the antifungal assay, five fungi were utilised for the studies and this was made up of three yeasts and two molds. All microorganisms were obtained from the the laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan.

Media: Nutrient broth, nutrient agar, sabourand dextrose agar (SDA), tryptone soya agar (Oxoid Laboratories, U.K:) were used in the assays. Methanol (Merck) was also used in solublising the extracts / drugs and as a negative control in the studies.

Antimicrobial agents: Ampicillin, 2.5 μg/ml (Lab Oftalmiso, Spain); tioconazole cream, 1mg/ml (Pfizer Inc., New York) were included in the study as standard reference drugs.

Antimicrobial activity determination: An overnight broth culture of 1-2 X 10^7 CFU of each bacterium was used to seed sterile molten agar medium maintained at 45°C. Sterile tryptone soya agar plate was similarly seeded with fungi. Five wells (10mm) respectively, were bored in each plate (9cm, diameter) with an aseptic cork borer, when seeded plates had solidified. 200mg/ml of extract was reconstituted in methanol and by the aid of a Pastuer pippette, each of the well was filled with 80μl of extract. Diameters of zones of inhibition were determined after incubating plates at 37°C for 24h (bacteria) and at 25°C for 72h (fungi). When seeded with bacteria, each plate had wells filled with methanol as well as ampicillin and for fungi, tioconazole was filled in one of the wells also. This method is similar to previuos procedures (Kavanagh, 1977).

Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as means and standard errors on means. Student´s "T" test was used to test probability at $P < 0.05$.

RESULTS

The result of the phytochemical screening of the leaves and stem of *G. gynandra* and *B. coriaceae* and the yield of each extract are presented in Table 1. The antibacterial properties of extracts at a concentration of 200mg/ml are presented in Table 2. The bacteria used were clinical

strains of *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* (gram positive); *Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus faecalis* (gram negative). Order of susceptibility of the bacteria to the extracts is: *B. subtilis* > *S. faecalis* > *B. cereus* > *E. coli* > *S. aureus* > *P. aeruginosa*. (Table 2).

TABLE 1
Phytochemical Screening of *Gynandropsis gynandra* and *Buchholzia coriaceae* and yields of extracts

2° Metabolites	Plants			
	G. gynandra		*B. coriaceae*	
	Leaves	Stem	Leaves	Stem
Alkaloids	+++	+++	+++	+++
Cyanidins	+++	+++	+++	+++
Anthraquinones	-	-	±	±
Tannins	-	-	-	-
Steroids	+++	++	+++	++
Cardiotonics	-	-	-	-
Reducing sugsrs	+++	+++	+++	+++
	Yields of extracts (%)			
Hexane	1.06	0.48	0.94	1.90
Methanol	8.04	4.73	2.88	4.26

-: not detectable; ± : doubtful; + : low concentration; ++: medium concentration; +++ : High concentration.

Table 2
Antibacterial Activities of *G. gynandra* and *B. coriaceae* extracts

Extracts/ Drugs[a,b]	**Mean Diameters of zones of Inhibition of bacteria in mm (+ SEM)**					
	B. cereus	*B. subtilis*	*S.aureus*	*P. aeruginosa*	*E. coli*	*S. faecalis*
BCSH	21.5±0.5	24.0±1.0	17.5±0.5	11.5±0.1	17.5±0.7	21.0±0.9
GGSH	10.0±0.1	17.5±0.5	11.0±0.6	11.5±0.5	15.5±0.6	14.4±0.4
GGLH	14.2±0.8	21.5±1.5	10.5±0.5	12.0±0.8	10.0±0.1	18.5±1.0
BCLH	21.1±1.0	18.5±0.6	12.5±0.4	10.3±0.2	10.0±0.0	21.5±0.4
BCSM	11.5±0.2	26.5±1.5	12.5±0.5	11.2±0.1	16.5±0.4	16.5±0.5
BCLM	10.2±0.5	17.5±0.5	14.5±0.2	12.5±0.8	13.2±0.8	17.4±0.6
GGSM	15.5±0.7	15.5±0.4	16.5±0.1	12.0±0.2	16.6±0.5	17.5±0.5
GGLM	14.0+0.2	12.5+0.5	15.0+0.8	11.4+ 0.3	12.5+0.3	13.5+0.6
AMP	20.4±0.2	25.5± 0.8	11.2±0.1	10.0±0.0	10.0±0.2	15.5±0.2
CONT	10.0+0.0	10.2+0.1	10.0+0.0	10.0+0.1	10.0+0.0	10.0+0.0

P < 0.05

a. BCSH = B. coriaceae stem hexane; GGSH = G. gynandra stem hexane; GGLH = G. gynandra leaf hexane; BCLH = B. coriaceae leaf hexane ; GGSM = G. gynandra stem methanol; GGLM = G. gynandra leaf methanol; BCSM = B. coriaceae stem methanol; BCLM = B. coriaceae leaf methanol ; AMP = Ampicillin (2.5ug/ml); CONT = control (methanol).

b. Solutions of extracts were made in methanol at a concentration of 200mg/ml and results are in triplicates.

The result of the antifungal activities of the eight extracts of both plants at a concentration of 200mg/ml is presented in Table 3. Five clinical strains of human pathogenic fungi were used and these consisted of : *Candida albicans*, *Penicillium sp*, *Fusiparum oxyposirum* (yeasts); *Aspergillus niger* and *A. flavus* (molds). *C. albicans* was the most sensitive, this was closely followed by the *Penicillium sp*. *A. niger* was the least sensitve in the assay. Generally, *B. coriaceae* extracts were more active than those of *G. Gynandra (*Table 3).

Table 3
Antifungal Activities of *G. gynandra* and *B. coriaceae* extracts

Extracts/ Drugs[a,b]	**Mean Diameters of zones of Inhibition of fungi in mm (+ SEM)**				
	C. albicans	*Penicillium sp*	*F. oxyposirum*	*A. flavus*	*A. niger*
BCSH	24.1±1.2	12.5±0.5	14.0±0.7	15.5±0.6	19.6±0.3
GGSH	10.0±0.0	12.0±0.7	13.5±0.5	13.5±0.5	10.2±0.2
GGLH	11.5±0.5	14.5±0.5	10.1±0.2	10.2±0.3	10.0±0.1
BCLH	13.5±0.4	17.1±0.4	11.5±0.4	10.0±0.1	10.0±0.5
BCSM	10.4±0.2	14.4±0.6	11.1±0.2	17.0±0.6	12.4±0.2
BCLM	14.5±0.6	17.5±0.5	10.0±0.0	16.6±0.4	10.5±0.5
GGSM	16.0±0.7	18.0±0.8	13.0±0.2	10.0±0.4	13.0±0.5
GGLM	17.5+0.5	12.7+0.3	10.0+0.0	11.5+0.2	12.4+0.4
TIO	15.0±0.	25.0±1.2	18.7±0.3	12.4±0.6	10.0±0.3
CONT	10.0+0.2	10.0+0.0	10.0+0.0	10.0+0.0	10.0+0.0

P < 0.05

a. BCSH = B. coriaceae stem hexane; GGSH = G. gynandra stem hexane; GGLH = G. gynandra leaf hexane; BCLH = B. Coriaceae leaf hexane; GGSM = G. Gynandra stem methanol; GGLM = G. Gynandra leaf methanol; BCSM = B. coriaceae stem methanol; BCLM = B. coriaceae leaf methanol;; AMP = Ampicillin (2.5ug/ml); CONT = control (methanol). b. TIO = tioconazole (1mg/ml), concentration of extracts = 200mg/ml.

DISCUSSION

Prescence of alkaloids, cyanogenetic glycosides, steroidal nucleus and reducing sugars were indicated to various extends in the four plant materials screened for secondary metabolites. This is not suprising for plants of the Capparidaceae family (Kjaer and Thomson, 1973; Lakshimi and Chanhan, 1977). Cardiac glycosides and tannins were not detected in the present studies (Table 1)

From the result of the antibacterial studies as shown in Table 2 all the extracts exhibited appreciable antibacterial properties inhibiting the growth of all the fungi at 200mg/ml. Extracts of *B. coriaceae* stem hexane, leaf hexane and stem methanol displayed overwhelming activities, inhibiting the growth of *B. cereus* and *B. subtilis* up to 25cm in diameter. In most instances, activities were greater than the standard therapeutic agent, ampicillin. Various sentivities were observed for the bacteria. For details, see Table 2. However, it is worthwhile to note that *E. coli* and *P. aeruginosa* used in the study were insensitive to ampicillin and extracts of both plants inhibited the growth of these two microorganisms.

In the antifungal assay, the title plants have again displayed high antifungal activities. Once more, *B. coriaceae* stem hexane extract showed the highest activities, inhibiting the growth of *C. albicans*

up to 21mm. This was folowed by *G. gynandra* stem methanol extract. The least active of the extracts was *G. gynandra* leaf hexane extract. The hexane extract of *B. coriaceae* stem inhibited the growth of *A. niger* (19.6mm), a most insentive mold chemotherapeutically while tioconazole was inactive.

Conclusively, all extracts have displayed antimicrobial activities from both studies. This has further confirmed the use of the plants in African ethnopharmacology for treatment of bronchitis, boils, earache, eye wash, disinfectant and nasal congestion (Burkhill,1985; Ainsle, 1937; Kerharo and Bouquet, 1950; Irvine, 1961). There is need for the development of new antibiotics due to acquired resistance, more importantly from natural sources as this delays resistance. The two plants used for the study provide good opportunities for drug development in this area. In the light of the above, *B. coriaceae* stem hexane extract being the most active of the studied extracts was analysed by thin layer chromatography (silica gel; F_{254}) whereby six main compounds were detected, two of them alkaloids (Dragendoff positive). This was subjected to column chromatography and fractions 29 - 36 (100ml each),eluted with increasing hexane/ethyl acatate mixtures; contained three compounds (Rf 0.21, 0.28 and 0.38; hexane : ethyl acetate; 60:40). Further purification and bioactivity is in progress and will be communicated later.

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