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Phytochemical screening and antibacterial evaluation of extract and fractions of *Combretum bauchiense* leaves

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Abstract
Objective: *Combretum bauchiense* as claimed in folkloric medicine is used for treatment of infectious diseases, feverish condition and as a tonic. There is no documented scientific report on its biological activity as at the time of this research. This research work evaluates the phytochemical constituents and antibacterial activity of extract and fractions of *Combretum bauchiense* leaves against six pathogenic microorganisms.
Methods: Cold maceration and vacuum liquid chromatographic methods were employed for extraction and fractionation respectively. The phytochemical screening was carried out using standard methods. *In vitro* antibacterial study was carried out to determine the minimum inhibitory concentration using agar dilution method.
Result: The phytochemical screening indicated the presence of terpenoids, flavonoids, saponins, tannins, cardiac glycosides and reducing sugar in extract whereas hexane fraction contained terpenoids and saponin, ethyl acetate fraction contained of terpenoids, flavonoids, saponins, tannins, cardiac glycosides and reducing sugar and butanol fraction contained flavonoids, saponins, tannins, cardiac glycosides and reducing sugar. The results the minimum inhibitory concentration value of extract and fractions ranged from 0.3125 - 5mg/mL against the test organisms.
Conclusion: *Combretum bauchiense* leaves have demonstrated reasonable antibacterial activity comparable to commonly prescribed synthetic antibiotic agent. The leaves of the plant may possibly be a putative source of antibiotic agent in combating infectious diseases thus substantiating its folkloric claims.

Keywords: Phytochemicals, *Combretum bauchiense*, methanol and antimicrobial

1. Introduction
The use of plants with useful medicinal properties have attained a commanding role in the health systems globally for the treatment of infectious diseases as well as maintenance of good health [1]. Medicinal plants contain phytocompounds which play a specific therapeutic role in the body system making it a viable alternative medicine. The screening of plant extracts for biological activity has in most cases involved higher plants, many of which have shown clinical relevance as a source of potential for development of chemotherapeutic agents [2]. Previous researchers have reported the presence of alkaloids, terpenoids, flavonoids, tannin, saponins, steroids, volatile oils, carbohydrate and glycosides in various medicinal plants which are responsible for the observed therapeutic effects [3-5]. Moreover, due to potentials in medicinal plants-based medicine most pharmaceutical companies have renewed interest in sourcing drug molecule from plant. This has resulted in development of notable therapeutic agent from medicinal plants currently in use such as digoxin from *Digitalis purpurea*, silymarin from *Silybum marianum*, berberine from *Berberi vulgaris*, morphine from *Papaver somniferum*, taxol from *Taxus brevifolia*, vinblastine and vincristine from *Catharanthus roseus* [6]. Despite the progress made in development of antibacterial agents for treatment of infectious diseases, cases of drug resistant bacteria emerge continually. Consequently, it reduces the effectiveness of commonly prescribed antibiotics, increases death rate and places high burden on the cost of health care. Therefore, there is need to search for an innovative antibacterial agent from medicinal plants to overcome the soci-economic and health impacts caused by multidrug resistant pathogens [7]. *Combretum bauchiense* Hutch. & Dalziel belongs to the family of Combretaceae of the order Myrtales [8]. The genus *Combretum* contains useful plants that are widely used in African traditional medicine for the treatment of several disease conditions such as*:* fever, malaria, diabetes, jaundice, backache, oedema, dysentery,

constipation, and abdominal pain [9-11]. Some of the Combretum species that have been reported to possess excellent antimicrobial properties include: *Combretum sordidum* [12], *Combretum collin* and *Combretum erythropholeum* [13], *Combretum hétérogènes*, *Combretum microphyllium* and *Combretum molle* [14], *Combretum album* pers. [15] *Combretum laxum* and *Combretum imberbe* [16-17]. Whilst several studies have been previously published in literatures on combretum species, there is no any published work on the phytochemicals and antibacterial of *Combretum bauchiense* leaves as at the time of this research.

This study aimed to evaluate the antibacterial activity of the extract and fractions of the leaves of *Combretum bauchiense* against some clinical selected pathogens.

2. Materials and Methods

2.1 Plant collection

Combretum bauchiense leaves were collected from Ezeani in Nsukka Local Government Area of Enugu State, Nigeria on February, 2020. It was identified by Mr. Felix Nwafor, a taxonomist in charge of the herbarium unit of the department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria. The voucher number is PCG/UNN/0398.

2.2 Preparation of plant material

The fresh plant sample was collected from the wild fields and the leaves were detached from the unwanted parts of the plant. The fresh leaves samples were then obtained and washed under a running tap water to remove soil particles and other contaminants. The leaves were air dried under a shade at room temperature for 14 days. After air-drying under a shade, the leaves were pulverized to a coarse powder using an electrical blender.

2.3 Preparation of the plant extract

1.02Kg of the air dried, coarsely pulverized leaves sample was soaked in 4.5litres of methanol and left for 72 hours at room temperature with constant agitation. The mixture was filtered first, using a cotton wool clogged funnel and then filtered severally through Whatman No. 1 filter papers. The filtrate was concentrated under reduced temperature and pressure using a rotary evaporator to obtain methanol extract. The extract was then transferred into an air-tight container and stored at 4º± 2 ºC in a refrigerator until when needed.

2.4 Fractionation of the Methanol Extract

40g of the dried methanol extract was dissolved in 100mL of methanol. The dissolved extract was transferred into porcelain mortal and triturated with a sufficient quantity of dry silica gel for fixation using a glass pestle. After obtaining a well homogenized triturated sample, the sample was spread and allowed to dry before loading into the VLC column (5cm diameter and 45cm long) packed with silica gel of 200-400 mesh size. The mixture was fractionated using successive volumes of solvents starting with n-hexane, ethyl acetate and finally, n-butanol. Each of the solvents was allowed to run down the VLC column until a clear solution was obtained after each addition. The different fractions of each solvent were collected at each point and labelled appropriately. These fractions were then concentrated under reduced temperature and pressure using rotary evaporator to afford n-hexane fraction (HFCB), ethyl acetate fraction coded (EFCB) and n-butanol fraction coded (BFCB) respectively. The fractions were stored in airtight containers in a refrigerator at 4º± 2 ºC until when needed.

2.5 Qualitative phytochemical screening of the extract and fractions

The tests used for the qualitative phytochemical screening were described previously [18-19].

2.6 Collection of test organisms

The pathogenic microorganisms used in this study were obtained from different patients that visited Adoni Biomedical Laboratory Research Centre, Nsukka Enugu State. The test organisms were six in numbers comprising of two (2) gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*), three (3) gram negative bacteria (*Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumoniae*). The bacteria were cultured on nutrient agar medium and incubated at 37 °C for 24h and subsequently sub-cultured again for another 24h at 37 °C.

2.7 Determination of Minimum Inhibitory Concentration

In this study two (2) fold serial dilutions of 100 mg/mL each of the test agents MECB, HFCB, EFCB and BFCB were used to obtain concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL. From these different dilutions of the test agents, different agar dilutions of each of the test agents were prepared by diluting 1mL of a 100 mg/mL of each test agent in 20mL of the molten agar to obtain a concentration of 5 mg/mL. Also, 1ml of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL of the test agents were diluted with 20mL of the molten agar to obtain the following concentrations 5 mg/ml 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.3125 mg/mL and 0.15625 mg/mL. Nutrient agar was used as the culture media for the bacteria isolates. Standard inoculum of each of the respective test organisms were delivered onto the surface of the agar containing different concentrations of the test agents. The plates were properly labeled for identification and then incubated for 24hours at 37 ºC. The minimum inhibitory concentration (MIC) was determined by establishing the presence of any visible growth of the test microorganism after the incubation period. The MIC is the lowest concentration of the test agents that will be able to inhibit the growth of the tested microorganisms.

3. Result

3.1 Percentage Yield of Extract and Fractions

The percentage yield of MECB was 22.18%. Among the solvent fractions, EFCB gave the highest yield (53.25%) while HFCB gave the lowest yield (14.28%) and BFCB gave (24.83%) as shown in Table 1.

Table 1: Percentage yields of extract and fractions

Extract/ Fractions	Mass of pulverized sample / extract	Mass of extract / fractions recovered	% Yield
MECB	1020	226.21	22.18
HFCB	40	5.71	14.28
EFCB	40	21.30	53.25
BFCB	40	9.93	24.83

3.2 Phytochemical screening

The results of the phytochemical screening are present in varying amount in each of the fractions. MECB contains flavonoids, reducing sugar, tannins, saponins, cardiac glycosides, terpenoids except alkaloids. Among the fractions EFCB which is moderately polar contains more phytoconstituents followed by BFCB whereas HFCB which is nonpolar constituent contains terpenoids and saponins only as shown in Table 2.

Table 2: Phytochemical Constituents of Extract and Fractions

Phytocompounds	Phytochemical Test	MECB	HFCB	EFCB	BFCB
Reducing sugars	Fehling	++	-	++	+
Flavonoids	Ethyl acetate	++	-	++	++
Terpenoids	Salkowski	++	+++	+++	-
Saponins	Frothing	+++	+	+++	+++
Tannins	Ferric chloride	+++	-	+++	++
Alkaloids	Meyer Dragendorff	-	-	-	-
Cardiac glycosides	Keller-Killiani	++	-	+	+

Key: +: Low colour intensity; ++: Moderate colour intensity; +++: High colour intensity; and -: no colour change noticed

3.3 Minimum Inhibitory Concentrations determination

The minimum inhibitory concentrations (MIC) ranges from 0.3125-5 mg/ml for MECB, 1.25-5 mg/ml for HFCB, 0.625-5 mg/ml for EFCB and 5 mg/ml for BFCB against test microorganisms as shown in Table 3.

Table 3: Minimum inhibitory concentrations (mg/mL) of MECB, HFCB, EFCB, and BFCB

Test samples	MICs on each of the test organisms				
	Staphylococcus aureus	***Salmonella typhi***	***Klebsiella pneumoniae***	***Escherichia coli***	***Streptococcus pneumoniae***
MECB	0.3125	0.3125	2.5	5	0.3125
HFCB	1.25	2.5	5	5	1.25
EFCB	0.625	2.5	5	5	2.5
BFCB	1.25	0.3125	2.5	2.5	0.625

4. Discussion

The pathogenic microorganisms used in this study are frequently incriminated in community acquired infections as well as hospital acquired infections hence, the rationale behind sourcing the microorganisms from infected subjects. As observed, the MECB gave significant amount of yield which showed that methanol was the right solvent for the initial extraction. The EFCB ethyl acetate fraction gave the highest yield when compared to the other fractions while the HFBC n-hexane fraction gave the lowest yield whereas the yield of BFCB was reasonable Table 1. The phytochemical analysis showed the presence of phytoconstituents of medicinal importance in the MECB, HFBC, EFCB and BFCB respectively table 2. These phytoconstituents observed is in agreement with phytochemical present in other combretum species as reported [20-21]. over the years, previous researchers have been able to isolate and characterize some bioactive compounds present in the Combretum genus. Among these are flavonoids and saponoidal triterpenes. These phytochemical compounds have been showed to possess excellent antibacterial activity against some selected bacterial strains. Three novel flavonoids with antibacterial activity have been isolated from the leaves of *Combretum apiculatum*: 2-hydroxy-4, 4', 6'-trimethoxyalchone (flavokawain–a chalcone); 5-methoxy-7-hydroxyflavanone (alpinetin–a flavanone); and 5, 7-dihydroxyflavanone (pinocembrin–a flavanone) [22]. It was noted that the chalcone "flavokawain-A" has a better anti-staphylococcal activity than the flavanones alpinetin and pinocembin. Similarly, five well known flavonoids with antibacterial property have been isolated from *Combretum erythrophyllum* [12]. These flavonoids include: genkwanin; rhamnocitrin; quercetin-5,3'-dimethylether; rhamnazin; and 5-hydroxy-7,4'-dimethoxy-flavone. The saponoidal triterpenes isolated and characterized from combretum species genus are thirteen novel cycloartane type triterpenes from the leaves of *Combretum quadrangulare* [23]. While eleven bioactive pentacyclic triterpenes have been isolated from the stem of *Combretum laxum* [15]. Pentacyclic triterpenes imberbic acid and hydroxyimberbic acid as well as their gylcosidic derivatives derived from *Combretum imberbe* have been shown to exhibit an excellent antibacterial activity [16]. *Combretum bauchiense* has shown to contain antibacterial property just like other combretum species. The result of minimum inhibitory concentration revealed that the MECB possess good antimicrobial activity with mics of 0.3125mg/ml against *S. aureus*, *S. typhi* and *S. pneumonia* whereas *K. pneumonia* and *E. coli* have mic of 2.5 and 5 mg/ml. Among the fractions, HFBC has mics of 1.25 mg/ml against *S. aureus* and *S. pneumonia*, 2.5 mg/ml for *S. typhi* while for *K pneumonia* and *E. coli* 5 mg/ml. EFCB exhibited mics of 0.625 mg/ml for *S. aureus*, 2.5 mg/ml for *S. typhi* and *S. pneumonia* and 5 mg/ml for *K pneumonia* and E. coli. The BFCB exhibited mic of 0.3125 mg/ml for *S. typhi*, 0.625 mg/ml for *S. pneumonia* 1.25 mg/ml for *S. aureus*, 2.5 mg/ml for *K pneumonia* and *E. coli*. In attempt to compare the activity of the fraction, both EFCB and HFCB showed no significant difference in their mics against test organisms whereas BFCB showed best mics against the test organisms which implies that most potent antibacterial agent resides more in the BFCB. The most likely reasons for these variations in potencies may be thought to be associated with the type of *in vitro* assay used and / or presence of different functional groups present in these phytochemical compounds which may confer lipophilicity or hydrophilicity to these compounds thereby, influencing the selectivity and thus, sensitivities of these bacterial and fungal strains tested.

5. Conclusion

Combretum bauchiense leaves possesses potential antibacterial agents due to the presence of many bioactive constituents. However, the use of the plant's parts in treatment for infectious diseases as claimed in folkloric medicine has been established. Therefore, further research needs to be carried out to isolate and characterize the bioactive constituent from the n-butanol fraction which exhibited best activity against the tested organisms.

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