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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL STUDIES OF THE LEAF EXTRACT OF *COMBRETUM LAMPROCARPUM* DIELS (*COMBRETACEAE*)

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ABSTRACT

The aim of this study was to determine the antibacterial activity of *Combretum lamprocarpum* leaf extracts on some clinically isolated bacteria species. The pulverized plant material was extracted by microwave-assisted extraction using chloroform, ethyl acetate and methanol. The Agar well diffusion methods was used to determine the antibacterial activity of the plant against *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* The phytochemical screening of the extracts revealed the presence of steroids, terpenes, alkaloids, carbohydrates, saponins, tannins, cardiac glycosides and flavonoids. The antibacterial screening of the extracts showed that all the fractions had significant activity against test organisms. The diameter of the zone of inhibition ranged between 14 and 29 mm, the minimum inhibitory concentration ranges between 3.13 and 50.00 mg/mL. The minimum bactericidal concentration was between 6.25 and 50.00 mg/mL. This study revealed that the leaves of *Combretum lamprocarpum* has a medicinal value in treating bacterial infections.

Keywords: *Combretum lamprocarpum, Combretaceae,* leaf extract, microwave-assisted extraction

INTRODUCTION

The use of medicinal plants in the treatment of diseases is as old as mankind. Despite the advances made by mankind in the production of pharmaceutical drugs, the decreasing effectiveness of synthetic drugs and the increasing contraindications of their usage, make the continued use of plants as important sources of medicinal agents (Petrovska *et al*., 2012). According to the World Health Organization, traditional medicine is "the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses" (WHO, 2005).

Plant have been used by people since time immemorial as source of medicine for the treatment of several ailments and diseases. *Combretum lamprocarpum* Diels belongs to the genus combretum and the family Combretaceae. It is commonly known as *Bauli* in Hausa, *Danehee* in Fulfulde, and *Ajantiro* in Yoruba speaking languages in Nigeria. In Nigeria, the leaf decoction of the plant has been reported as a remedy for diarrhea (Offiah, *et al,* 2012). The leaf of the plant also is reported to have been used in the treatment of wounds (Gibreel, 2008). The stem bark infusion of the tree has been used to stop vomiting (Adjakpa *et al.,* 2016).

MATERIALS AND METHODS

Collection and identification of plant material

The leaf of *Combretum lamprocarpum* Diels was collected from Makurdi, Benue State, Nigeria. It was authenticated by Mr. Namadi Sanusi of the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where a specimen voucher number (900743) was deposited. The leaf was air-dried and ground into powder using wooden mortar and pestle, sealed in polythene bag and kept until when required.

Microwave Assisted-Extraction (MAE)

The pulverized plant material (1 kg) was divided into three equal portions and placed in mason jars. N-hexane was added to the three portions until it just covered the top. The bottles were covered tightly and then irradiated in the microwave oven under lowest power for 3 minutes. The jars were removed from the oven and allowed to cool and vented. The process was repeated 5 times. The samples were washed 3 times with n-hexane and filtered through muslin cloth. The procedure was repeated using chloroform, ethyl acetate, and methanol one after the other. Each of the extracts were concentrated in a rotary evaporator at 40 °C.

Phytochemical screening

The extracts were tested for the presence of alkaloids, anthraquinones, carbohydrates saponins, cardiac glycoside, flavonoids, saponins, steroids/terpenoids and tannins.

Test for alkaloids

The extract (0.5 g) was stirred with 5 mL of 1% aqueous hydrochloric acid on a water bath and filtered. The filtrate (3 mL) was divided into three portions. To the first portion, few drops of freshly prepared Dragendoff's reagent was added and observed for the formation of an orange to brownish precipitate. To the second portion, 1 drop of Mayer's reagent was added and observed for the formation of a white to yellowish or cream coloured precipitate. To the third portion, 1 drop of Wagner's reagent was added to give a brown or reddish or reddish- brown precipitate (Silva *et al.,* 1998).

Test for anthraquinones glycosides

a) Free anthraquinones

The extract (0.5 g) was shaken with 10 mL of benzene, the content was filtered, and 5 mL of 10% ammonia solution was added to the filtrate, the mixture was shaken. Presence of a pink, red or violet colour in the ammoniacal layer (Lower phase) indicates the presence of free anthraquinone (Trease and Evans, 2002).

b) Combined anthraquinones

The extract was boiled with 10 ml of aqueous sulphuric acid and filtered hot. The filtrate was shaken with 5 mL benzene, the benzene layer was separated and half its own volume, 10 % NH_4OH was added. A pink, red or violet colouration in the ammonia phase (lower phase) indicates the presence of combined anthraquinone or anthraquinone derivative (Trease and Evans, 2002).

Carbohydrates (Molisch Test)

Each of the extracts (0.5 g) was mixed with Molisch reagent and concentrated H_2SO_4 was added along the sides of the test tube to form layers. A ReddishViolet ring was observed, the interference indicated the presence of carbohydrates (Ladan *et al.,* 2014).

Test for cardiac glycosides (Keller-Killiani test)

Exactly 1 mL of concentrated H_2SO_4 was put in a test tube. The aqueous extract (5 mL) from the plant sample was mixed with glacial acetic acid (2 mL) containing 1 drop of $FeCl_3$. The above mixture was carefully added to the 1 mL of concentrated H_2SO_4. The appearance of a brown ring indicated the presence of cardiac glycosides (Ayoola *et al.,* 2008).

Test for flavonoids

Shinoda Test: A small quantity of magnesium powder and a few drop of conc. HCl was added to an alcoholic solution of each plant extract (2 mL). The appearance of an orange, or pink or red to purple colour indicated the presence of flavonoids (Sofowora, 1993).

Sodium Hydroxide Test: The extract (2 mL) was dissolved in 10 % aqueous sodium hydroxide solution and filtered to give yellow colour, a change in colour from yellow to colourless on the addition of dilute HCl indicates the presence of flavonoids (Cannell, 1998).

Test for saponins glycosides

Distilled water (5 mL) was added to each extract (0.5 g) in a test tube and shaken vigorously for 30 seconds then allowed to stand for 45 minutes. A honey-comb froth that persisted for 30 minutes or more was observed indicative of the presence of saponins (Silva *et al.,* 1998)**.**

Test for steroids and terpenoids

The Salkowski's test for steroids: The extract (0.5 g) was dissolved in chloroform and concentrated sulphuric acid (2 mL) was carefully added down the side of the test tube to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring (Sofowora, 1993).

The Liebermann-Burchard's test: The extract (0.5 g) was dissolved in chloroform (2 mL) and filtered into a clean, dry test tube. Acetic anhydride (2 mL) was added to the filtrate and shaken. Few drops of concentrated sulphuric acid were added carefully down the side of the tube to form a lower layer. A brownish-red or violet ring at the interface of the two liquids and the upper layer turning green denotes the presence of sterols and terpenes (Tajudeen *et al.,* 2015).

Test for Tannins

The dried powdered sample (0.5 g) 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 which shows the presence of tannins (Trease and Evans, 2009).

The Antibacterial screening

Preparation of stock solution for antibacterial studies

Exactly 1.0 g each of the n-hexane, ethyl acetate, chloroform and methanol extract were separately weighed into Eppendorf tubes and dissolved in 10 mL dextrose saline to produce extract solutions of 100 mg/ML and further diluted to 50 mg/mL, 25 mg/mL, and 12.5 mg/mL stock solution. These concentrations were used for the *in vitro* studies (Eze *et al.,* 2013).

Biological studies

The antibacterial activities of the ethyl acetate, chloroform and methanol extracts of *Combretum lamprocarpum* were determined using microbial strain obtained from the Medical Microbiology laboratory of the Ahmadu Bello University Teaching Hospital, Zaria, they were sub-cultured on sterile nutrient broth and incubated at 37°C for 24 hours. The test microorganisms were; Gram-positive bacteria: *Staphylococcus aureus*, the Gram-negative bacteria; *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*.

Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration (MIC) of the extracts was determined using the tube dilution method as outlined by CLSI (2014). Dilution of concentration of extract that exhibited sensitivity against the test organisms was prepared in the tube containing the Mueller Hinton Broth (MHB). The organisms were inoculated into each of the tubes containing the dilution extracts. The tubes were inoculated at 37 °C for 24 hours for bacteria. The lowest concentration in the series showing no visible growth of the test organism was considered the MIC.

Minimum Bactericidal Concentration (MBC):

The minimum bactericidal concentration of the extract was determined as outlined by CLSI (2014). The MBC was determined by assaying the test tube content of the MIC determinations. A loop of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then inoculated at 37 °C for 24 hours and 25 °C for 48 hours, for bacteria and fungi respectively after which it was observed for microbial growth. The lowest concentration of the subculture with no growth was considered as the MBC.

RESULTS AND DISCUSSION

The result of the preliminary phytochemical screening of the crude extract revealed the presence of secondary metabolites which are known to possess biological activity. Steroids and terpenes were observed in all the four extracts. The presence of alkaloid, carbohydrates, cardiac glycosides and flavonoids

was observed in ethyl acetate, chloroform, and methanol extracts. Anthraquinones were absent in all the extracts. Saponins were present in methanol extract only. Tannins were present in ethyl acetate and methanol extracts (Table 1).

The results of determination of zone of inhibition of the plant extract (Table 2) ranges from 14-29 mm against the test organisms. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) (Table 3,4) of ethyl acetate, chloroform, and methanol extracts of the leaf of *Combretum lamprocarpum* Diels showed that all the plant extracts inhibited and completely kill the microorganisms tested at various concentration (MIC= 3.13 – 50.00 mg/mL, MBC=6.25-50.00 mg/mL). Therefore, all the extracts had significant activity against the test microorganisms.

Table 1: Result for phytochemical screening of the plant extracts

Phytochemical	**H.E**	**C.E**	**E.E**	**M.E**
Alkaloids	-	+	+	+
Anthraquinones	-	-	-	-
Carbohydrates	-	+	+	+
Cardiac glycosides	-	+	+	+
Saponins	-	-	-	+
Steroids	+	+	+	+
Tannins	-	-	+	+
Terpenes	+	+	+	+
Flavonoids	-	+	+	+

Key: H.E= Hexane Extract, E.E= Ethyl acetate Extract, C.E= Chloroform Extract, M.E= Methanol Extract, Positive (+)=Present, Negative (-) = Absent

Table 2: Result of zone of inhibition of the extracts (mm)

Test organisms	C.E	E.E	M.E
E. coli	14	14	16
P. aeruginosa	18	17	29
S. aureus	17	21	26
S. typhi	16	14	19

C.E= Chloroform extract, E.E= Ethyl acetate extract, M.E= Methanol extract

Table 3: Result of Minimum inhibitory concentration (MIC) of the crude extracts (mg/mL)

Test organisms	**C.E**	**E.E**	**M.E**
E. coli	50.00	50.00	12.50
P. aeruginosa	25.00	25.00	3.13
S. aureus	25.00	12.50	3.13
S. typhi	25.00	25.00	6.25

Key: ND= Not determined, C.E= Chloroform extract, E.E= Ethyl acetate extract, M.E= Methanol extract

Table 4: Result of the Minimum Bactericidal Concentration of the extracts.

Test organisms	**C.E (mg/mL)**	**E.E (mg/mL)**	**M.E (mg/mL)**
E. coli	ND	ND	25.00
P. aeruginosa	50.00	50.00	6.25
S. aureus	50.00	25.00	6.25
S. typhi	50.00	50.00	12.50

Key: ND= Not Determined, C.E= Chloroform extract, E.E= Ethyl acetate extract, M.E= Methanol extract

CONCLUSION

Phytochemical screening of the leaf of *Combretum lamprocarpum* revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids which are important metabolites. Several studies confirmed the presence of these phytochemicals to have medicinal as well as physiological activity. The findings from this study showed that the plant possess antimicrobial activity against gram-negative and gram-positive bacteria. Therefore, the leaf extracts from this plant could be a potential source for useful drug.

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