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Phytochemicals and Evaluation of Antibacterial Activities of *Combretum Fragrans* (Bush Willow)

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ABSTRACT

This work is designed to confirm the traditional claim of the antibacterial activity of the leaves and stem bark extracts of *Combretum, fragrans*. The antibacterial activity of the extracts of *Combretum fragrans* were examined against some selective bacterial strains such as *Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae and Escherichia coli*. The antibacterial activity was evaluated using agar well diffusion method. The antibacterial activity appeared to have inhibitory zones that ranged between 10 and 22mm of diameter. The antibacterial activity of the leaves and stem bark extracts was comparable with that of amoxicillin, standard. The leaves and stem bark of *C. fragrans* were also screened phytochemically for its constituents. The phytochemical revelation showed that both acetone and water extracts contain alkaloids, flavonoids, tannins, saponins, glycosides and resins. Quantitative analysis was also carried out which showed that alkaloids, tannins and flavonoids except saponins are higher in the leaf extracts than in the stem bark extracts. This result confirms the use of the plant in traditional medicinal treatment of dysentery, diarrhea, and chronic wounds. The presence of alkaloids, flavonoids, tannins, saponins and glycosides might be responsible for these activities.

KEYWORDS: *Combretum fragrans,* Phytochemical, Antibacterial Activity, and Medicinal

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Traditional medicine is an important source of potentially useful new components for the development of chemotherapeutic agents (Racio, *et al.,* 1989). History has shown that, medicinal plants have been paramount in the treatment of diseases for all people from commoners to those in power. Medicinal plants have been written about by scholars in the Bible and ancient manuscripts, and are as important to health care today as they were thousands of years ago. The idea that every plant has its medicinal use, as suggested by Paracelsus, the renowned 15th century physician, is fascinating, as is the idea that each and every illness and disease has its perfect natural cure. It was in the 15th century that the knowledge of medicinal practice became more widespread in the form of compilations known as herbals (Lewis, 2010). Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, Flavonoids and phenolic

compounds (Hill, 1952). Many of these medicinal plants are used as spices and food plants. They are also sometimes added to food meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999; 2001). *Combretum fragrans* is shrub-like or a small tree up to 10-12m high. The back is grey and reticulately fissured, the branches peeling to give dark reddish colour. Leaves are opposite or 3-4 verticillate, the lamina is broadly ovate-elliptic. The shoots are leafless during flowering. The flo#wers are greenish to white and fragrant, particularly at night. *C. fragrans* is common in deciduous woodland and wooded grassland associated with seasonally waterlogged clay soils and is sometimes also found growing on shallow, stony` soils (Wickens, 1973).

Materials and Methods

The plant sample was collected in June behind Barde Hostel, Adamawa State University Mubi. It was identified by Mr. Ayuba Samuel, a taxonomist at the Department of Natural Resource Conservation, Ministry of Environment, Mubi. The stem bark and the leaves of this plant were air dried and pound to fine powder and kept for further uses.

Preparation of Extraction

Aqueous Extract

Water was used for extraction to stimulate the condition in which the traditional medical practitioners generally use for the herbs. The 50g of the dry sample was extracted with 300ml distilled water for 24hrs at room temperature, with occasional shaking. The mixture was filtered and the filtrate, used for both qualitative and quantitative analysis as well as evaluation of antibacterial activities (Daniyan and Muhammad, 2008).

Acetone Extrac.

25g of the powdered sample was extracted with 100ml of the acetone using a soxhlet apparatus for 2hrs.The extract was concentrated over water bath.

Qualitative Analysis of Phytochemicals.

The chemical tests were carried out on the aqueous solution and on the powdered specimen using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harbone (1973).

Test for Tannins

About 0.5g of the dried powdered sample was boiled in 20ml distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. A blue black, precipitate was taken as an evidence for presence of tannins (Sofowara, 1993).

Test for Saponins

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test to these compounds (Rahaman, 2010). The 5ml of plant extract was strongly shaken in a test tube. Formation of a large amount of froths that lasted for about 30 minutes indicated the

presence of saponins. On mixing this froth with olive oil emulsion was formed (Harbone, 1973).

Test for Flavonoids

1ml of 10% NaOH was added to the 3ml of the aqueous extract. Appearance of yellow colour indicated the presence of flavonoids (Sofowara, 1993).

Test for Glycosides

The 5ml of the extract was mixed with 25ml of 50% H_2SO_4 in a test tube. The mixture was heated in boiling water for 15 minutes, cooled and neutralized with 10% NaOH and 5ml of fehling solution (A and B) was added followed by boiling. The appearance of brick red precipitate indicated the presence of glycosides (Trease and Evans, 1989)

Test for Alkaloids

3ml of the plant extract was mixed with 1ml of 10% HCl in a test tube and be heated for 20 minutes. This was allowed to cool and filtered; 1ml of the filtrate was treated with few drops of Mayer's reagent. Appearance of creamy precipitate indicated the presence of alkaloids (Trease and Evans, 1989).

Test for Oils

5ml of the extract was shaken with dilute HCl.There was no appearance of a white precipitate which indicated the absence of oils (Lawal, *et al.,* 2010)

Test for Resins

2ml of the extract was added equal volume of acetic anhydride solution and drops of concentrated H_2SO_4. Violet colouration was taken as an indication for the presence of resins (Sofowara, 1993)

Quantitative Phytochemical Analysis.
Akaloids Determination using Harborne (1973) Method

5g of the sample was weighed into 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and extract, concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate collected was washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid which was dried and weighed.

Tannin Determination by Van -Burden and Robinson (1981) Method

500mg of the sample was weighed into 50ml plastic bottle. 50ml of distilled water was added and shaken for 1hr. This was filtered into a 250ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1m $FeCl_3$ 0.1N HCl and 0.008m potassium ferrocyanide.

The absorbance was measured at 120nm within 10 minutes. Tannin content was calculated as tannic acid equivalent from the standard graph.

Saponins Determination by Obandoni and Ochuko (2001) Method

20g of the sample was put into a conical flask and 100cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4hrs with continuous stirring at about 55^{0}c. The mixture was filtered and the residue was re - extracted with another 100ml 20% ethanol. The combined extract was reduced to 40ml over hot water bath at about 90^{0}c. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated.

60ml of n - butanol was added. The combined n – butanol extracts was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight; the saponins content was calculated as percentage i.e.

$$\frac{\text{Mass yielded}}{\text{Initial mass}} \times 100\%$$

Flavonoid Determination by Bohamad and Kocipai (1994) Method

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered. The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Blood Base Agar Medium

This was prepared by weighing 1.0g nutrient agar dissolved in 20cm^3 of distilled water. The solution was sterilized by autoclave at 121^{0}c for 15 minutes. It was allowed to cool and 5ml of blood was added. It was mixed and poured into a sterilized petri – dish to solidify (Murray, *et al.,* 2003).

Nutrient Agar Medium

Nutrient agar was prepared by dissolving 0.56g of nutrient agar in 20cm^3 of distilled water. The solution was sterilized at 121^{o}C for 15 minutes. It was cooled and poured into a sterilized petri – dish to solidify (Murray, *et al.,* 2003)

Preparation of Culture and Inoculation

Stool and mucus samples were collected from patient attending Medical Center, Adamawa State University Mubi, was used to inoculate the different media. Stool sample was used on salmonella and Shigella medium, while mucus was used in blood based agar medium. The plates were incubated for 24 hrs at 37 ^{o}C. Pure cultures of *E. coli*, *S. dysentariae, S. aurcus and S. typhi* were obtained (Murray, *et al.,* 2003).

The pure cultures were used separately to inoculate nutrient agar medium in petri – dishes. This was done by streaking the surface of the plates and covered. The inoculated plates were incubated at 37^{0}c for 24hrs (Murray, *et al.,* 2003).

Assay of Extracts

The extracts were dissolved in 5ml of water to produce 0.5% m/v dilution to give 0.5% standard drug (amoxicillin) as positive control and water as negative control. Wells (6mm in diameter) were punched into the nutrient agar containing the bacteria by a sterile borer and filled with 0.5ml of the test extract dilution and control solutions (water and amoxicillin). The plates were incubated for 24hrs at 37^0C and were examined for clear zone of inhibition. The presence of zones of inhibition indicated the activities in millimeters. These were recorded as diameters of the activities (Murray, *et al.,* 2003).

Results and Discussion.

The results of the qualitative and quantitative phytochemical screening of the leaves and stem bark of *C. fragrans* are presented in Tables 1 and 2. Table 3 shows the result of antibacterial test of the leaves and stem bark extracts.

Table 1: Qualitative analysis of leaves and stem bark of *C. fragrans*

Constituents	Stem bark		Leaf	
	WEs	AEs	WEs	AEs
Alkaloids	+	+	+	+
Flavonoids	+	_	+	+
Tannins	+	+	+	+
Saponins	+	–	+	+
Glycosides	+	_	+	+
Oils	–	–	–	–
Resin	+	+	+	+

Key: WEs = Water extract; AEs = acetone extract; + = Presence; – = Absence

Table 2: Quantitative analysis of leaves and stem bark extratcs of *C. fragrans* (in %).

Constituents	Stem bark	Leaf
Alkaloids	24 ± 0.2	40 ± 0.2
Saponins	16 ± 0.2	10.5 ± 0.2
Flavonoids	12 ± 0.2	20 ± 0.2
Tannins	11 ± 0.2	24 ± 0.2

Table 3: Antibacterial activity of leaves and stem bark extracts of *C. fragrans.*

Pathogens	Stem		Leaves			
	AEs	WEs	AEs	WEs	WC	AMXC
Shigella dysentariae	NS	NS	20	18	NS	10
Staphylococcus aureus	12	14	16	15	NS	22
Salmonella typhi	NS	NS	NS	NS	NS	18
E. coli	NS	15	14	12	NS	12

Keys: WEs = Water extract; AEs = Acetone extract; WC = Water as negative control; AMXC = Amoxicillin as positive control; NS = Non sensitive.

Discussion

Phytochemical Screening of Leaves and Stem Bark Extracts of C. fragrans.

Table 1 presents the results of the phytochemical screening for the presence of alkaloids, flavonoids, saponins, tannins, glycosides, resins and oils. All of these were present in both the leaves and stem bark extracts except saponins, flavonoids and glycosides which were not present in the acetone extracts of stem bark and oils were not present in either of the extracts. These results agree with the work of Pia, (2007) which showed that the family of *Combretacae* contains alkaloids, tannins, glycosides and saponins. The presence of these secondary metabolites may attribute to the activity against microorganisms.

Leven, *et al.,* (1979) reported that, these secondary metabolites are responsible for the antimicrobial properties of plants. The absence of these secondary metabolites in the extracts supports the absence of inhibition of these microorganisms. Hassan et al (1980) reported that, the inhibitory effect *Carica papaya* was due to the presence of alkaloids, tannins, terpenes and glycosides.

Quantitative Analysis of the Leaves and Stem Bark Extracts of C. fragrans

The quantitative analysis of leaves and stem bark extracts of *C. fragrans* revealed that the leaves extracts contain more aikaloids (40%), tannins (24%) and flavonoids (20%) than the stem bark extracts which contains 24%, 11% and 12% of alkaloids, tannins and flavonoids respectively. However, saponins content of the stem bark extracts (12%) was higher than that of the leaves extracts (10.5%).

Antibacterial Screening

The acetone and water extracts from the leaves and stem bark of *Combretum fragrans* were considerably active against microorganisms evaluated. Four bacteria were chosen based on the prevalence of these microbes in the locality. These are *Escherichia coli, Shigella dysenteriae, Salmonella typhi* and *Staphylococcus aureus,* gram positive and gram negative bacteria. *E. coli* an enterobacteria, known to cause diarrhea, dysentery, urinary tract or surgical wound infections and skin diseases etc.

These organisms are known to be resistant to many antibiotics including amino glycosides, ciprofloxacin, tetracycline and beta lactams etc. (IPAM, 2003). Preliminary antibacterial activity have been detected under test conditions with acetone and water extracts using an agar well diffusion method. The water acetone extracts of the leaves gave activities against *S. desynteriae* (20 and18mm), *S. aureus* (16 and 15mm), and *E. coli* (14 and 12mm). *S. aureus* was susceptible to all the extracts. Amoxicillin, a control drug inhibited all the pathogens including *S. typhi* which was not susceptible to the crude extracts. The antibacterial activity of this plant agrees with the work of Chhabra *et.al* (1984) which showed that *C. fragrans* gives inhibition against *S. aureus*. The zone of inhibitions of the acetone and water extracts of this plant (Table 4.1.3) Indicates that the extracts contain promised antimicrobial agents. Stem bark and leaves decoction of *C. fragrans* have been used for many ailments, such as skin infections, dysentery, swollen glands, wounds and syphilis (Iwu, 1993). The performance activity may be due the presence of active constituents in the extracts.

Flavonoids for example, is known to inhibit tumor growth and protect against gastric infections which gives credence to some of the ethnomedical uses of plants (Harborne and Baxter, 1993). This result also shows that this plant can be used as pain reliever, insecticides as a function of alkaloids which is contained in plant.The presence of resins justifies the use of leaf paste traditionally which compresses swollen parts and heal them.

The results show therapeutic activity of *C. fragrans* against human pathogens and can therefore be used to remedy some of the common diseases which these pathogens are the causative agents.

Conclusion

The result of this research work have shown that *C. fragrans* contains alkaloids, flavonoids, saponins, tannins, glycosides and resins. Due to the presence of the above constituents in the plant, its extracts give significant activity against human pathogens.

Acetone and water seems to be good solvents for extracting the biologically active components which are responsible for the inhibition or retardation of bacterial growth and metabolism. The claim of water normally used as solvent by herbalist to cure several diseases is confirmed as the extracts of *C. fragrans* gave an appreciable activity against some bacteria. The method used by herbalist can be justified also from the work of Edeoga, (2005) who showed that saponins, tannins and alkaloids inhibit bacterial growth.

The study therefore concludes that *C. fragrans* has antibacterial potential due to the presence of bioactive constituents. Hence the plant is a potential source of natural antibacterial which could be useful in physiological and pathological medicine and of great interest to drug manufacturing industries.

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