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Research Article

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Phytochemical and *in vitro* antimicrobial efficacies of the crude flavonoids and saponins rootbark extract of *Combretum glutinosum* perrot. Ex. Dc.

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Abstract The methanol rootbark extract of *Combretum glutinosum* was subjected to preliminary phytochemical screening and *in vitro* antimicrobial tests. The methanol rootbark extract revealed the presence of flavonoids, tannins saponins, alkaloid and cardiac glycosides using standard procedures. The antimicrobial activity of the plant extract was assayed by the agar plate disc diffusion and nutrient broth dilution techniques. Test microorganisms which were laboratory isolates include *Escherichia coli*, *Shigella dysenteriae*, *Bacillus subtilis*, *Corynae bacterium* species and *Aspergillus niger*. The antimicrobial activities were shown to be concentration-dependent. The extract inhibited the growth of some of the test organisms; the highest diameter of inhibition zone of the crude flavonoids fraction was exhibited by *Corynae bacterium* species had 30.33±0.58 mm followed by *Shigella dysenteriae*; the crude saponins fraction was effective towards *Escherichia coli* with mean inhibition diameter of 19.67±0.58 mm. The extract couldn't inhibit the growth of *Bacillus subtilis* as well as *Aspergillus niger* at the tested concentrations of 100, 50 and 25 mg/ml. However, the crude flavonoids fraction inhibited the growth of *Bacillus subtilis* at all concentrations with mean values of 25.00±0.00, 22.67±0.58 and 19.33±0.58 mm respectively at concentrations of 100, 50 and 25 mg/ml. Therefore, it is pertinent to say that the activities against bacteria by this plant extract may be due to flavonoids part of the extractives. More so, this study has justified the traditional use of this plant for the treatment of some infectious diseases whose causative agents are some of the organisms tested in this study.

Keywords antimicrobial, rootbark, flavonoids, saponins, *Combretum glutinosum*

Introduction

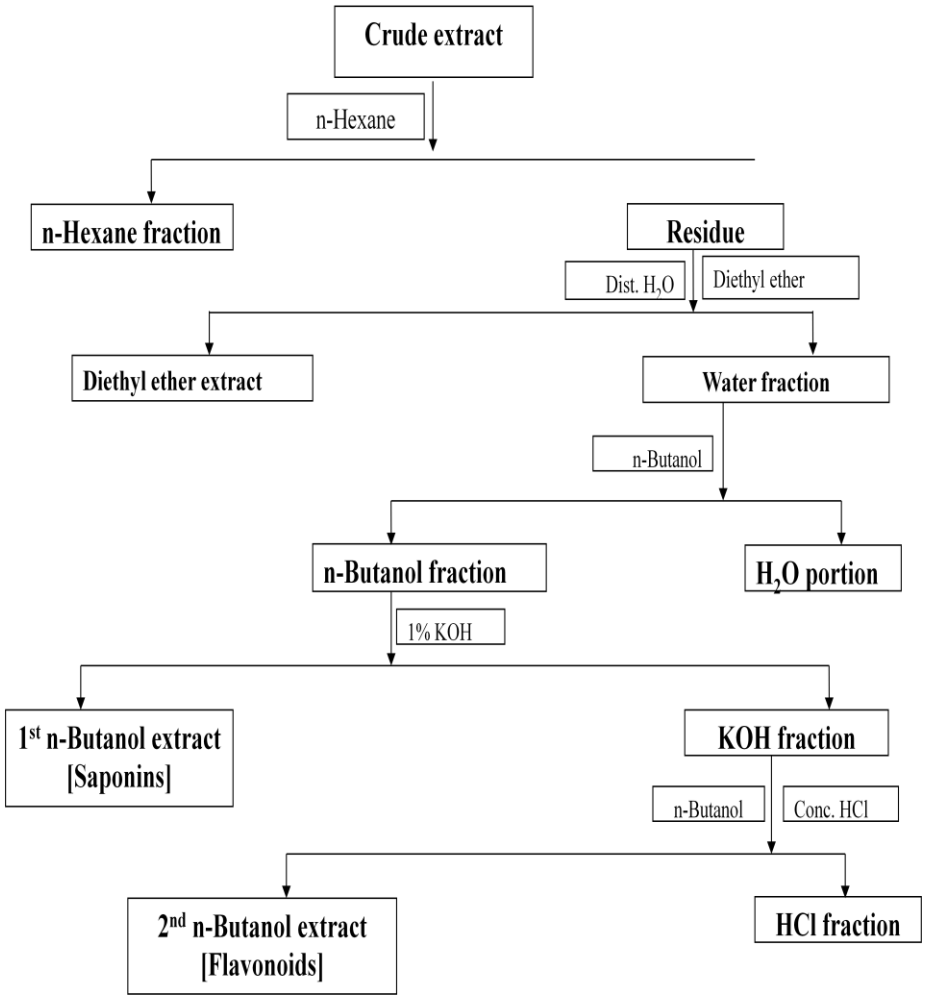
The use of plants as medicine is an ancient practice common to all societies especially the African society. This practice continued to exist in the developing nations. It is this basis that researchers keep on working on medicinal plants in order to produce/develops the best medicines for physiological uses [1].

According to WHO as many as 80% of World population depends on traditional medicine for their primary healthcare needs today and that 25% of the drugs are based on plants and their derivatives [2]. Most of the pathogen causing enteric infection have developed resistance to the commonly prescribed antibiotics which increase the likelihood to increase the length of stay in the hospital [3] and increase used of a particular antibiotics could lead to increased bacterial resistance [4].

Combretum is a very large genus comprising of about 250 species found in both temperate and tropical region of the world [5]. *Combretum glutinosum* is a bushy shrub or small tree growing up to 12 m and a deciduous species sprouting in the middle of the dry season. The trunk is usually twisted and low branched with a rounded open crown. The lower branches characteristically point down wards. The bark is grey black and may be smooth or rough [6].
The root and stem barks of *Combretum glutinosum* are used in the treatment of scrotal elephantiasis, dysentery, ring worms, syphilis, typhoid-fever; eye sore and ear ache [7]. This study is embarked upon to evaluate the antimicrobial potential of the crude flavonoids and saponins root bark extracts of *Combretum glutinosum.*

Materials and Methods

Collection, Identification and Extraction of the Plant Material



Scheme 1: Fractionation of crude flavonoids and saponins [8; 9].

The fresh root bark of *Combretum glutinosum* was collected from Potiskum Local Government Area, Yobe state Nigeria in July, 2014 with proper identification from the Department of Chemistry University of Maiduguri, Borno State Nigeria; where a voucher specimen numbered 14/CHM/010 was deposited.
The fresh roots were collected and air-dried under room temperature constantly monitored for 4 days and later ground into coarse powder using wooden mortar and pestle. About 230 g of powdered sample was taken into a round bottom flask; a sufficient amount of 80% methanol was added to the powdered plant material and shaken vigorously. The mixture was then refluxed for 6 hours. The extract was filtered using muslin cloth and later filtered

through Whatmann No. 1 filter paper after which the extract was concentrated to dryness and the extract so obtained was then fractionated into the crude flavonoids and saponins as shown in Scheme I below as described by [8] as adopted by [9]; the crude methanol extract and fractions were then kept safely and aseptically in a desiccator until use.

Phytochemical Screening

Phytochemical evaluation was determined using conventional methods of analyses as described earlier by several authors [10-13].

Test Microorganisms

The Gram positive organisms used were *Bacillus subtilis* and *Corynae bacterium* spp.; Gram negative bacteria were *Escherichia coli* and *Shigella dysenteriae;* while *Aspergillus niger* was the fungal species used. These organisms were clinical isolates obtained from the Department of Veterinary Medicine, University of Maiduguri, Nigeria.

Susceptibility Test:

The fractions of the methanol rootbark extract of *Combretum glutinosum* were subjected to preliminary antimicrobial studies against 2 Gram positive and 2 Gram negative bacteria and a fungal spp. using the hole-in-plate disc diffusion technique as described earlier [14; 15; 16]. The extract was made in different stock concentration prepared by dissolving 2.5 g of flavonoids and saponins extract in 5 m1 of distilled water. The microorganisms were maintained on agar slant until use. The inoculum were then prepared by subjecting the test organisms in nutrient agar and incubated for 24 hours at 37 ^{o}C. After incubation, the broth culture was dilute to 1:3000 for both Gram positive and Gram negative bacteria, 1 ml of the diluted culture was inoculated into 39.2 g/1400 m1 sterile molten nutrient agar (48^{o}C) and sabouraud dextrose agar prepared according to manufacturer's specification was also poured into sterile Petri dishes; they were gently swirled and allow to solidify. Inculcated nutrient agar plates were bored using sterile number V cork borer. All the holes were filled with equal volume of each portioned portion equivalent to 100 mg/hole, 50 mg/hole and 25 mg/hole. The extracts were allowed to diffuse into the agar for an hour. Thereafter, plates were then incubated over night at 35 ^{o}C and 37 ^{o}C. At the end of incubation period, inhibition zones were recorded in millimeters as the diameter of clear zone around the bored holes using transparent meter rule.

Results and Discussion

Table 1: Phytochemical contents of rootbark methanol extract of *Combretum glutinosum*

Constituents	Inference
Test for Tannins	
1% ferric chloride	+
10% Lead acetate test	+
Test for Glycosides	
Free Anthraquinone	-
Combined Anthraquinone	-
Test for Cardiac Glycoside	
Salkowski's Test	+
Liebermann-Burchard's test (steroidal nucleus)	+
Saponins	
Frothing test	+
Test for Flavonoids	
Shinoda's test	+
Ferric chloride test	+
Lead acetate test	+
Sodium hydroxide test	+
Test for Alkaloids	
Dragendorff's reagent	+
Mayer's reagent	+

Key: + = present; - = absent

Table 2: Antimicrobial susceptibility of the crude flavonoids and saponins of the rootbark extract of *Combretum glutinosum*

Extract fraction/ Conc. (mg/ml)	Test microorganisms/Diameter of inhibition zone (mean ± SEM) mm				
	Bacillus subtilis	***Corynaebacterium species***	***Escherichia coli***	***Shigella dysenteriae***	***Aspergillus niger***
Crude flavonoids 100	25.00±0.00	30.33±0.58	24.00±0.00	27.33±0.58	0.00±0.00
Crude Saponins 100	0.00±0.00	18.00±0.00	19.67±0.58	12.67±0.58	0.00±0.00
Crude flavonoids 50	22.67± 0.58	26.33±0.58	17.67±0.80	14.67±0.58	0.00±0.00
Crude saponins 50	0.00±0.00	13.67±0.58	13.67±0.58	10.00±0.00	0.00±0.00
Crude flavonoids 25	19.33±0.58	23.33±0.58	15.33±0.58	19.00±0.00	0.00±0.00
Crude saponins 25	0.00±0.00	11.67±0.58	10.33±0.58	8.00±0.00	0.00±0.00

The photochemical screening of the crude methanol rootbark extracts of *Combretum glutinosum* revealed the presence of flavonoids, tannins, saponins, alkaloids and cardiac glycosides were found to be absent. These classes of compound are known to show curative activity against several pathogens and therefore could explain its use traditionally for the treatment of wide array of illness [1, 17]. Alkaloids, cardiac glycosides, flavonoids and tannins were detected as shown in Table 1. The presence of saponins, alkaloids and flavonoids corroborated to the results on the stem bark earlier by [18], although this part of the plant contains no anthraquinones as equally found in the stem bark [18].

The *in vitro* antimicrobial screening presented in Table 2 showed the susceptibility expression against some studied microorganisms. The extract exhibited considerable level of inhibition against some of the test organisms with the highest activity on *Corynaebacterium species* and no activity was recorded against *A. niger*. Similar results were shown by [18]; that methanol extract of the stem bark of *Combretum glutinosum* showed the highest level of inhibition on *Salmonella typhi* and *Escherichia coli* while the aqueous extract showed less response. The high antibacterial activities of this extracts could be connected to the presence of the plant secondary metabolites detected. More so, it has been shown that the flavonoids fraction is apparently the most effective part compared to the saponins fraction. According to [9]; that the presence of phenolic compounds could be responsible for the broad activity by flavonoids which are believed to act as cytoplasmic poison that inhibit enzymes such as peptidase on the bacterial cytoplasm. It has been reported that terpenoids inhibits the growth of micro organisms [19] and tannins has been widely used as an application to sprains, bruises, and superficial wounds [20].

Conclusion

The result of this study shows that methanol rootbark extract of *Combretum glutinosum* possesses significant antibacterial activity and thereby supports its traditional usage by rural dwellers and traditional medicine practitioners from Potiskum, Yobe State, Nigeria.

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