Phytochemical Screening and Antibacterial Potential of the Trunk Bark of *Ochthocosmus Africanus*

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ABSTRACT

An estimated 57 million deaths are recorded worldwide every year out of which infectious diseases are responsible for 17 million; one-third of the overall mortality rate. To face this crucial issue, the search for new anti-infective agents that could be used by needy populations appeared primordial. The present study focused on *Ochthocosmus africanus* (*Ixonanthaceae*) known to be traditionally used in disease caretaking. The phytochemical screening of the hydroalcoholic extract from the plant trunk bark was followed by the determination of its antibacterial potential by macro-dilution in a liquid medium. The minimal inhibitory and bactericidal concentrations (MIC and MBC, respectively) were assessed on six Gram-negative rods (*Klebsiella pneumoniae, Enterobacter aerogenes, Moraxella* spp., *Escherichia coli, Serratia odorifera, and Shigella sonnei*) and three Gram-positive rods (*Lactobacillus bulgaricus, Clostridium* spp., *and Bacillus* spp.). The Phytochemical screening revealed the presence of secondary metabolites like flavonoids, anthocyanins, tannins, saponosides, triterpenes, cardiotonic heterosides, and reducing sugars. The antibacterial tests further revealed inhibitory and bactericidal features of the extract with MIC values ranging from 25 through 100 mg/mL; while the MBCs were recorded between 50 and 200 mg/mL. Bactericidal activity was observed on *Escherichia coli, Enterobacter aerogenes, Shigella sonnei, Clostridium* spp, and *Lactobacillus bulgaricus* (CMB/CMI=2) and bacteriostatic activity on the others. These findings could justify, at least partially, the use of this plant in infectious; but additional efforts on toxicity are needed for safer healthcare.

Key Words: *Ochthocosmus africanus, Phytochemical screening, CMI, CMB, Bacteria*

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INTRODUCTION

In many developing countries, access to quality medicines remains limited to large cities while infectious diseases are rampant in remote areas. In connection with the paucity of mobility facilities, the high cost of pharmaceutical drug specialties and various other socio-economic constraints, lots of community members in these countries rely on drugs from doubtful origins and traceability to manage disease in general. The available drugs generally have poor quality in most cases of common pathologies. The high expectations that followed the Penicillin introduction in the 1940s to improve human lifespan rapidly faded away with their abusive use that resulted in the selection and dissemination of adapted microbial strains otherwise referred to as microbial resistance [1]. Microbial resistance makes the control of microbial conditions more and more costly and is associated in these areas with low purchasing power and

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ancestral traditional disease control practices.

Trends of resistance to conventional drugs and reliance on non-conventional alternatives stimulated the new paradigms about effective anti-infective agents available and affordable to the majority of needy populations. This makes traditional medicine a very important component of the cultural heritage with the abundance of natural resources that could be used as raw materials. Accordingly, and based on the fact that more than 80% of African populations used resources from traditional medicine, the WHO encouraged research in that field acknowledging that scientific knowledge of traditional drugs could significantly improve their efficacy in managing human disorders. These conditions include infectious diseases (IDs) which represent one of the most common public health issues throughout the world [2]. Several related studies have been conducted in Cameroon in that vein [3-6] with extracts from several species of plants belonging to various botanical families. The present investigation focuses on *Ochthocosmus africanus* a plant species from the *Isonanthaceae* family. *Ochthocosmus africanus* is widely used in traditional medicine for severe stomach-aches [7], as an expectorant, in diarrheal conditions, in the management of sexual impotence, and in breastfeeding stopping [8-10].

Previous investigations on *Ochthocosmus africanus* revealed the presence of ochtofridelane, stigmasterol, N-p-trans-coumaroyltyramide, and taraxerol [11]. Scientific pieces of evidence related to the bioactivity of plants from the *Isonanthaceae* family have been reported on extracts and/or isolated compounds [12, 13]. To present knowledge, however, no studies on antimicrobial potential have yet been performed on members of the *Ochthocosmus* genus. The present work aims at detecting a few phytochemical compounds it contains and determining the antibacterial potential of the hydroethanolic extract from the trunk bark of *Ochthocosmus africanus*. More especially, it will investigate through the minimal inhibitory and minimal bactericidal concentration (MIC and MBC, respectively) of the extract on common etiologies of bacterial diseases. In the short run, the findings will primarily serve traditional practitioners in their daily activities. In the intermediate and long runs, standards will be produced for more effective use of this plant's resources in addition to isolation of active secondary metabolites that could be used as concentrated bioactive in the control of IDs.

MATERIALS AND METHODS

Plant material and extraction

The plant material used consisted of the trunk bark of *Ochthocosmus africanus*, harvested on November 1st, 2016 in Batouri (East Cameroon). The identity was subsequently confirmed at the National Herbarium of Cameroon under reference Voucher 45453 HNC.

The extraction was carried out with 70% ethanol. In the process, 2.2 kg of powder from the bark of the plant's trunk was macerated in 8 L 70% ethanol for 72 hours. The filtrate obtained through Whatman® No. 1 paper was concentrated by rotative evaporation (Heldolph®) at 65°C and 200 mBars. The total extract obtained was subsequently dried in an oven at 40°C.

Phytochemical screening

The aqueous test-solution was prepared by homogenizing with a magnetic stirrer 1 g of the total extract in 20 mL of distilled water. The preparation obtained was then subjected to phytochemical screening for the detection of secondary metabolites according to Bruneton 1999 [14].

Alkaloids test

In a test tube containing 5 mg of the extract dissolved in 1 mL of methanol, 1 mL of 1% H_2SO_4 was added. The resulting preparation was heated to ebullition in a water bath for 5 minutes. After cooling and filtration, 5 drops of Mayer's reagent were added to the filtrate. The development of a precipitate indicated the presence of alkaloids.

Anthocyanins test

In a test tube containing 5 mg of the extract dissolved in 1 mL of methanol, five drops of concentrated hydrochloric acid were added. The development of the orange color was characteristic of the presence of anthocyanin in the extract.

Anthraquinones test

In 1 mL of ether-chloroform (1:1), 5 mg of extract was dissolved. The mixture was treated with 4 mL of 10% sodium hydroxide. The development of a red color testified to the presence of anthraquinones.

Flavonoids test

For this test, 5 mg of extract was dissolved in 1 mL of methanol. The mixture was then treated with 0.05 g of magnesium shavings and 3 drops of concentrated H_2SO_4. The presence of flavonoids was characterized by the development of the following colors: yellow for flavones, red for flavonols, and pink for flavanones.

Saponins test

In a test tube containing 5 mL of distilled water, 5 mg of extract was dissolved, then heated to ebullition in a water bath for 5 minutes. After cooling the preparation was stirred vertically for 15 seconds and then allowed to stand. The appearance of more than one centimeter's high persistent foam reflected the presence of saponins.

Tannins test

In this test, 5 mg of extract was dissolved in 1 mL of ethanol. Then, 3 drops of 10% Iron Chloride III were added. The development of a blue-violet or greenish color was characteristic of the presence of the tannins.

Triterpenes and steroids tests

In a test tube containing 1 mL of methanol, 5 mg of the extract was dissolved. To the resulting mixture, 0.2 mL of each of the following reagents was added: chloroform, glacial acetic acid, and concentrated H_2SO_4. Development of a purple or greenish color indicated the presence of triterpenes or steroids, respectively.

Test for reducing sugars

To 2 mL of the extract in a test tube, 1 mL of an equal volume of Fehling A and B solutions was added. The mixture was heated to ebullition for 3 to 10 minutes. The development of a brick-red precipitate indicated the presence of reducing sugars.

Cardiotonic heterosides test

2 mL of the alcoholic filtrate, 1 mL of glacial acetic acid, 1-2 drops of $FeCl_3$, and 1 mL of concentrated H_2SO_4 were added. Cardiotonic heterosides were characterized by the development of a brown ring at the interface. A purple ring could also appear under the brown ring.

Extract antibacterial potential

Bacteria types used

Nine bacteria types were chosen for their frequent implications in human pathologies and their ubiquitous distribution. All the isolates were provided by the Laboratory of Microbiology at the Université des Montagnes Teaching Hospital. These included six Gram-negative rods (*Klebsiella pneumoniae, Enterobacter aerogenes, Moraxella* spp., *Escherichia coli, Serratia odorifera, and Shigella sonnei*) and three Gram-positive rods (*Lactobacillus bulgaricus, Clostridium* spp., *and Bacillus* spp.).

Before the tests were conducted, all the isolates were grown on Mueller Hinton agar at 37°C for 18-24 h. The colonies from this fresh bacterial population were then used for the preparation of a suspension in 5 mL of sterile physiological saline. The resulting preparation was thereafter adjusted to a turbidity standard comparable to that of 0.5 on the McFarland scale.

Dilution range of the extract and susceptibility tests

The macro-dilution in liquid medium technique was used in this investigation. The stock extract solution was prepared at 800 mg/mL. This was obtained by dissolving 6 g of the powder in 7.5 mL of Mueller Hinton broth. The mixture was homogenized by vortexing. For the dilution range, 1500 µL of Mueller Hinton broth was dispensed from the first to the last tube in the range, as well as in the 3 control tubes. Then an equal volume (1500 µL) of the mother solution above prepared was added to the first tube of the dilution range. From this arrangement, a serial dilution (order 2) followed in the others tubes of the series and resulted in a concentration range found between 400-0.39 mg/mL. In each of the dilution tubes (except the negative and reagent control), 15 µL of the bacterial inoculum was added. The preparations were incubated at 37°C for 18 to 24 hours. Upon completion of incubation, the turbidity was first assessed visually, and then the tubes were centrifuged at 5000 rpm for 5 minutes to detect the sediment in case of growth.

Antibacterial's potential parameters

- *Minimal inhibitory concentration (MIC)*

The MIC was identified from the first tube (lowest extract concentration) of the range in which no growth (absence of turbidity) was recorded and for which no bacterial sediment was observed upon centrifugation. The experiment was repeated three times in each case.

- *Minimal bactericidal concentration (MBC)*

In each of the tubes for which no bacterial growth was recorded and in the controls of the concentration range carried out for the MIC, about 5 µL of re-homogenized bacterial suspension were streaked on Mueller Hinton agar. The set was incubated overnight at 37°C. The CMB of the extract was detected from the first dilution (lowest in the range) in which no culture was recorded. The operation was repeated three times.

- *MBC/MIC ratio*

This report confirms the bacteriostatic or bactericidal character of a substance. When the value is greater than or equal to 4, the substance is said to be bacteriostatic.

If it is less than 4, the substance is regarded as bactericidal. If it is equal to 1 then it is said to be absolutely bactericidal.

- *Inhibition diameter: Method by diffusion in agar medium*

The inhibition diameter was assessed by the disc diffusion method carried out on extract concentrations equal to the MIC and CMB values recorded. This test was carried out on all bacterial subjects.

The culture was performed by swabbing the agar surface with the above-prepared bacterial suspension (0.5 McFarland). Sterile 6 mm diameter discs cut from sterilized Whatman No.3 paper were firmly adjusted to the swabbed preparation. On the surface of these discs, 15 µL of the extract at the MIC and CMB values were delicately dispensed. The preparations were incubated at

room temperature for 15 minutes on the bench top, then allowed to an overnight incubation at 37°C. Upon completion of incubation the inhibitory diameters around the discs were recorded in millimeters. The test was carried out 5 times in each case.

RESULTS AND DISCUSSION

The semi-quantitative result from the phytochemical screening of the hydroethanolic extract of the trunk bark (8.36%) is shown in **Table 1**.

Table 1. Phytochemical composition of the hydroethanolic extract

Phytochemical group	Reagent	Result obtained
Alkaloids	Valser-Mayer	-
Anthocyanin	H_2SO_4 + NH_4OH	+ + +
Anthraquinones	10%NaOH	+ + +
Cardiotonic heterosides	glacialCH_3COOH + $FeCl_3$ + H_2SO_4	++
Flavonoids	1%$FeCl_3$	+ + +
Saponosides	None	+ + +
Reducing sugars	Fehling A and B solutions	+ + +
Steroids	$CHCl_3$ + glacialCH_3COOH +H_2SO_4	+
Tannins	10%$FeCl_3$	+
Triterpenes	$CHCl_3$ + glacialCH_3COOH +H_2SO_4	+

+++: Highly concentrated; ++: Averagely concentrated; +lowly concentrated; -: not detected

It comes out that the *O. africanus* raw material contains 90% of the target secondary metabolites. About 55% of these metabolites are also highly concentrated and include anthocyanin, anthraquinones, flavonoids, saponosides, and reducing sugars. Relatively low concentrations of cardiotonic heterosides, sterols, triterpenes, and tannins were observed in addition.

Susceptibility test

Antibacterial activity of the extract was observed on all the isolates subjected. Based on the extract concentrations that were necessary for the activity recorded, the minimal inhibitory concentrations (MIC), the minimal bactericidal concentrations (MBC), and the MBC/MIC ratio (R) were presented as displayed in **Table 2**.

Table 2. MIC, MBC, MBC / MIC ratio and inhibition diameters

Bacterial type	MIC (mg/mL)	MBC (mg/mL)	Inhibition Diameter (mm)		Ratio (MBC/MIC)
			CMI	CMB	
Escherichia coli	50	100	11.66	12.66	2
Enterobacter aerogenes	50	100	11.33	12.33	2
Shigella sonneii	50	100	10.66	11.66	2
***Clostridium* spp.**	25	50	11	13.33	2
Serratia odorifera	25	100	12	13.33	4
***Bacillus* spp.**	25	100	11.33	12.33	4
***Moraxella* spp.**	50	200	12	12	4
Lactobacillus bulgaricus	50	100	11	12	2
***Klebsiella* spp.**	100	200	12.33	12.33	2

MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; R: MBC/MIC

It reveals that the lowest MIC and MBC values were obtained on the *Clostridium* with 25 mg/mL for the MIC and 50 mg/mL for the CMB, while the highest MBC was recorded with *Klebsiella* (200 mg/mL). Five out of the 9

isolates had identical MICs (50 mg/mL). In addition, the bactericidal potential was observed in 67% of cases while in 33%, the bacteriostatic effect was recorded. Globally, the inhibition diameter values ranged from 11.66 to 13.33 mm.

For the present investigation, the choice of *Ochthocosmus africanus* was based on its use in traditional medicine for the care-taking of intestinal disorders in Batouri, East Cameroon.

Phytochemical screening of the hydroalcoholic extract of the trunk barks revealed the presence of flavonoids, anthocyanins, saponosides, triterpenes, cardiotonic heterosides, and reducing sugars. The relative concentrations in anthocyanins, anthraquinones, flavonoids, saponosides, and reducing sugars were higher than those of tannins, triterpenes, and steroids. Previous work on the same part of this plant [11] reported the presence of ochtofridelane, stigmasterol, N-p-trans-coumaroyltyramide, and taraxerol. According to certain sources, these compounds have antibacterial and antiparasitic potentials [15].

The biological activity of these metabolites is essential in research and development related to pharmaceutical industries. This is the case, for instance, of highly concentrated phenolic compounds which associate a very large set of substances that are difficult to define in time and space because of the large diversity and environmental variations regulating growth and responses to local stressors. The fundamental structural element which characterizes these metabolites is the presence of a benzene nucleus, directly linked to at least one hydroxyl group, free or engaged in other chemical groups like ethers, esters, or heterosides [16, 17]. These associations could play important and differential roles in the degree of activity.

Investigating the antimicrobial potential of the hydroalcoholic extract from the trunk bark of *O. africanus*, it appeared that it is active on all the isolates subjected to varied concentrations, regardless of the bacterial Gram type. Though yet to be accurately demonstrated, these findings could be attributed to the inherent metabolites association discussed above. This activity could also be justified by microbial characteristics, the overall chemical composition of the extract, the stability of the antimicrobial agents, or specific cellular organization [18].

Antimicrobial activity tests revealed MICs values for all the isolates subjected in the present survey; ranging from 25 to 100 mg/mL. The lowest one (25 mg/mL) was recorded on *Clostridium* spp., *Serratia odorifera*, *Bacillus* spp., and the highest (100 mg/mL) on *Klebsiella*. The higher value on *Klebsiella* could be linked to the bacterial capsule known to play an important role in cellular protection from external constraints compared to those recorded on other Gram-negative and Gram-positive rods. Spore-forming *Bacillus*is behaved differently than *Klebsiella*. The difference in the inhibitory concentrations between these two bacterial types may be related to the composition of their outermost coverage; the capsule for *Klebsiella* and the spore for *Bacillus*. More specifically, the role of their chemical composition might reasonably be pointed out, though the Gram type might be key in a typical cell organization. In fact, the capsules and spores have chemically different compositions. The combined influence of both bacterial components should, however, not be ruled out. This development is supported by the values recorded with *Bacillus* spp., *Lactobacillus bulgaricus*, and *Clostridium* spp., on one hand, and *Klebsiella* capsule on the other. The MBCs obtained varied between 50 and 200 mg/mL. The lowest value (50 mg/mL) was recorded on the *Clostridium* and the highest (200 mg/mL) on *Moraxella*. Previous findings reported the antibacterial potential of other plant extracts on several microorganisms including *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia* [19-22]; in agreement with the findings from the present one. Accordingly, it could reasonably be anticipated that the potential of the hydroalcoholic extract of the trunk bark of *O. africanus* observed in the present survey likely extends to other clinical and other environmental hosts; further indicating how plants from the *Ixonanthaceae* family could be used in disease control and prevention [12, 13].

A glance at the MBC/MIC ratio values showed bacteriostatic activity on *Serratia odorifera*, *Bacillus* spp., and *Moraxella* spp. (MBC/MIC $\geq$ 4); and bactericidal potential on *Escherichia coli*, *Enterobacter aerogenes*, *Lactobacillus bulgaricus*, and *Klebsiella pneumoniae* (MBC/MIC$\leq$ 4). This activity might theoretically be associated with the secondary metabolites in the extract which act either individually or in combination. Flavonoids, for instance, are known for their antibacterial potential on many bacterial types. It has also been reported that several mechanisms could be enacted simultaneously [23-25]. Some other findings highlighted sets of interactions between flavonoids and mammalian cells [26], justifying the necessity for additional research initiatives in connection with their toxicity, and their preservation the adequate dosages during caretaking. This development is true for triterpenes [27] and tannins which are largely used in traditional medicine. Triterpenes act by altering bacterial proteins through precipitation and/or making the nutritional proteins unavailable [28, 29]. In other words, as a combination of many antibacterial agents that could act individually or collectively, the activity recorded with the extract used in the present study could be evidently defensible. Acknowledging, however, the role of combinations in the survival of plants under various natural environmental influences, it is likely that

these secondary metabolites act together in a complex of interactions that are not predictable to current knowledge. The inhibition parameters (MIC and MBC) were evidence to confirm, quantify and compare the activities on one hand and appreciate isolate action-specific trends on the other. The results of this *in vitro* investigation provide an important basis for the use of the extract of *O. africanus* in the control of infections associated with the microorganisms subjected and their likes, provided that studies on toxicity are conducted. These developments justify future related surveys for standardization that is necessary to appropriately use the virtues of this plant. This would take into account massive experiences resulting in the production of comparable standards for the type of extract in connection with other conventional antibacterial agents.

CONCLUSION

The present study revealed that *O. africanus* contains phytochemicals with valuable antimicrobial potential etiologies of bacterial infections. This could serve in the development of Improved Traditional Medicines, provided that future steps are taken in the research on other key characteristics with patients, did not suggest any significant difference.

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