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PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF *COMMIPHORA PEDUNCULATA* (ENGL) STEM BARK EXTRACTS

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ABSTRACT
The extracts from the stem bark of Commiphora pedunculata, a plant used in Northern Nigeria for the treatment of infectious diseases, were subjected to phytochemical as well as antimicrobial screening using standard procedures. The antimicrobial activity against S. aureus, B. cereus, S. typhii, E. coli and C. albicans was carried out using the disc diffusion and broth micro dilution methods as outlined by the NCCLS. Preliminary phytochemical screening of the petroleum ether, ethyl acetate and methanol extracts revealed the presence of cardiac glycosides, anthraquinones, saponins, triterpenes, steroids, flavonoids, tannins and alkaloids. The results of the antimicrobial activity as indicated by the zone of inhibition of growth of the test microorganisms ranged from 17 to 28 mm, the MIC results ranged from 3.125 to 12.5 mg/mL and the MBC results ranged from 6.25 to 25.0 mg/mL for the petroleum ether, ethyl acetate and methanol extracts. The MIC of 12.50 mg/mL exhibited by the petroleum ether extract against both Gram-positive and Gram-negative bacteria indicates broad spectrum activity of Commiphora pedunculata. The results from this study showed that the extracts from the stem bark of the plant contain antimicrobial components worthy of further investigation and lends credence to the use of the plant for the treatment of infectious diseases.
Keywords: Phytochemistry, Commiphora pedunculata extracts, antibacterial activity, MIC, MBC.

INTRODUCTION

It is evident that even though scientific advances have been made in the quest to understand the physiology of the body, biotechnology and the treatment of diseases, natural products remain a crucial, cheap and uncontroversial component of the comprehensive health care strategy for the future (Patwardhan, 2005). Medicinal plants occupy a central position in traditional medicine and have been and still remain a viable source of medication and drug discovery (Sani *et al,* 2013). The world health organization (2000), defines traditional medicine as the diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and or mineral based medicine, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being as well as to treat, diagnose or prevent illiness (Timmermans, 2003). The study of plants used in traditional medicine requires the effective integration of information on chemical composition of extracts, pharmacological activities of isolated compounds as well as indigenous knowledge of traditional healers (Pareskeva, 2007).

Commiphora pedunculata is a savannah shrub of about 4 – 6m height but may sometimes grow to between 12 and 15m. The bark of most *Commiphora pedunculata* is papery and peels off into papery flakes, revealing a green bark underneath. The leaves are mostly compound. The fruit of *Commiphora pedunculata* greatly enhances the identification of the specie. When ripe, the fruit splits into halves revealing a brightly colored pseudo-aril. This fleshy appendage completely or partially encompasses the seed as part of an attachment around part of the seed. The shape of the pseudo-aril differs from species to species. The flowers may be uni or bisexual, with the unisexual flowers only being semi developed with non-functional stamens (Steyn, 2003).

Commiphora pedunculata stem bark has been reported to treat infected wound, the root is usually chewed for treating cough and it is also used for treating jaundice, nausea and yellow fever, the decoction of the leaves and stem bark is used for the treatment of dysentery and diarrhea (Baba maiwada, Personal communication). To our knowledge, there is no reported work on the phytochemical constituents and antimicrobial activity of the stem bark of *C. pedunculata*. The aim of this study therefore was to establish the phytochemical constituents as well as to validate the claims of the antimicrobial effects of the stem bark of the plant.

MATERIALS AND METHODS

The Plant

Fresh plant material was collected in the month of June from the bushes around Zaria. The leaves were identified by Mallam U.S Galla of the Herbarium Unit in the Department of Biological Sciences Ahmadu Bello University, Zaria. The voucher number 219 was assigned to the plant.

Extraction of the stem bark

The stem bark was removed from the stem, air-dried for 21days and crushed to coarse powder. The dried powder (500 g) was macerated successively in n-hexane, ethyl acetate and methanol exhaustively until complete extraction.

Phytochemical screening

Preliminary phytochemical screening of the plant was determined using standard methods described by Trease and Evans (1989) and Sofowora (1993).

Preparation of the different extract concentrations

The extracts (1 g each) were weighed and dissolved in 10 mL of DMSO to give stock solutions of 100m/mL. 5 mL of the stock solution was transferred into 5mL of sterile distilled water to give 50 mg/mL solution. The process of serial dilution was continued until 5 different concentrations (100 mg/mL to 6.25 mg/mL) were obtained.

The test organism

The test organisms were *Basillus cereus, Salmonella typhi, Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.* They were obtained from the Department of Medical Microbiology Ahmadu Bello University Teaching Hospital Shika, Zaria. All the isolates were checked for purity and maintained on nutrient agar slants (Harley, 2002).

Culture media

The culture media used were Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB). All the media were prepared according to manufacturer's instruction.

Preparation of inoculums of test organisms

Suspensions of the organisms were made in sterile distilled water and compared with the McFarland turbidity standard, until the opacity matched with the scale number 1 standard, which corresponded to 1.5 x 10^8 cfu/mL (Harley, 2002).

Sensitivity test of the extract using agar well diffusion method

The agar well diffusion method was used (Nostro *et al.,* 2000). The standardised inocula of the isolates were uniformly streaked onto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (8 mm in diameter) five appropriately labelled wells were punched into each agar plate. 0.2 mL of the appropriate extract concentration was placed in each well and then allowed to diffuse into the agar. The plates were incubated at 37ºC for 24hours. While for the fungi, Sabouraud Dextrose Broth was used and the incubation period was 48hours at 25ºC. The antimicrobial activities were expressed as diameter of inhibition zones produced by the plant extracts.

Minimum inhibitory concentration

The minimum inhibitory concentrations of the extracts were determined using the broth dilution method (Vollekova *et al.,* 2001). Varying concentrations of the extracts (1.5625-25 mg/mL) were prepared, by serial dilution, from the least concentration that exhibited activity against the tested organisms in test tubes containing Mueller Hinton broth (MHB). The organisms (0.1 mL) were inoculated into each tube containing the extracts. The tubes were incubated at 37ºC for 24 hours for bacteria and 48hours at 25 ºC for fungi. The lowest concentration in the series showing no visible growth of the test organisms was considered to be the minimum inhibitory concentration (MIC).

Minimum bactericidal concentration

The contents of the MIC tubes in the serial dilution were sub cultured onto appropriately labeled Mueller Hinton agar plates and incubated at 37ºC for 24 hours, then they were observed for colony growth. The lowest concentration of the sub culture with no growth was considered as the minimum bactericidal concentration (Vollekova *et al.,* 2001).

RESULTS

The results of the phytochemical screening of the Stem bark of *C. pedunculata* are as shown in Table 1.0 Steroids and triterpenes were found to be present in the petroleum ether. Cardiac glycosides, Saponins, flavonoids and tannins were present in the ethyl acetate and methanol extract. Anthraquinones were absent in all three extracts, while only the methanol extract had alkaloids.

Table 1: Phytochemical constituents of the stem bark of *C. pedunculata*.

Phytochemical Constituent	n-petroleum ether extract	Ethyl acetate extract	Methanol extract
Anthraquinones	-	-	-
Cardiac glycosides	-	+	+
Saponins	-	+	+
Steroids and terpenes	+	-	-
Flavonoids	-	+	+
Tannins	-	+	+
Alkaloids	-	-	+

Key: + = Present, - = Absent.

The results of the antimicrobial susceptibility tests were expressed in terms of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and diameter of zones of inhibition of the test organism. The results obtained are shown in Tables 2 - 4.

The results from the sensitivity test showed that the plant extracts had remarkable activity against the tested microorganisms with inhibition zones ranging from 17 mm to 28 mm. The petroleum ether extract was active against all the tested microorganisms, while the ethyl acetate and methanol extracts were active against all the microorganisms except *E.coli* and *C. albicans*. The MIC values ranged from 3.125 mg/mL to 12.50 mg/mL while the MBC values ranged from 6.25 mg/mL to 25.0 mg/mL for all the plant extracts against the tested microorganisms.

Table 2: Sensitivity profile of the microorganisms to varying concentrations of the stem bark extract of *C. pedunculata*

Test organisms	Mean Zone of inhibition (mm)			
	MT	EA	PE	Ciprofloxacin 10µg/disc
S. aureus	28	25	23	30
B. cereus	20	23	24	40
E. coli	-	-	20	35
S. typhi	25	20	24	27
C. albicans	-	20	17	-

Key: MT = Methanol, EA = Ethyl acetate, PE = Petroleum ether, - = No activity

Table 3: Minimum inhibitory Concentration (MIC) of the stem bark extracts of *C. pedunculata*.

Test organisms	Methanol extract (mg/mL)	Ethyl acetate extract (mg/mL)	Petroleum ether extract (mg/mL)
S. aureus	3.125	6.25	6.25
B. cereus	6.25	12.50	12.50
S. typhi	6.25	6.25	12.50
E. coli	-	-	12.50
C. albicans	-	6.25	12.50

Key: - = No activity

Table 4: Minimum bactericidal/fungicidal Concentration (MBC/MFC) of the stem bark extracts of *C.pedunculata*.

Test organisms	Methanol extract (mg/mL)	Ethyl acetate extract (mg/mL)	Petroleum ether extract (mg/mL)
S. aureus	6.25	12.50	12.50
B. cereus	12.50	25.00	25.00
S. typhii	12.50	12.50	25.00
E. coli	-	-	25.00
C. albicans	-	6.25	12.50

Key: - = No activity

DISCUSSION

The results of the phytochemical analysis of the methanol and ethyl acetate extracts of the stem bark of *C. pedunculata* revealed the presence of saponins, alkaloids, flavonoids and tannins, while the petroleum ether extract had steroids and terpenes as the major constituents (Table 1). Most of these plant constituents have been reported in literature to have antimicrobial properties *in vitro*, (Cowan, 1999; Sibanda and Okoh, 2007). Both the ethyl acetate and methanol extracts inhibited the growth of *S. aureus, B.cereus*, and *S. typhii*. The petroleum ether extract inhibited the growth of all tested microorganisms (*S. aureus, B. cereus*, *E. coli* and *S. typhi*) indicating broad spectrum antibacterial activity as well as anti fungal activity (*C. albicans*). Although the mechanism of action of these phytochemical constituents of the plant extracts being studied may be difficult to speculate; however, many antibacterial agents may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Franklin *et al.,* 1987). It is possible that the antimicrobial agents in the plant extracts act through some or all of the above mechanisms.

The fact that pathogenic strains of *S. aureus* can cause localized abscesses, sepsis, pneumonia and boils in humans (Melissa and William, 2012) implies that the methanol extract of *C. pedunculata* can be employed for the treatment of such infections.

The sensitivity of *B. cereus* to both the ethyl acetate and methanol extracts implies that the use of the plant for the treatment of dysentery and diarrhea is justified since the bacteria are responsible for such illness (Kotiranta *et al.,* 2000).

Both the methanol and ethyl acetate extracts showed activity against *S. typhi,* the bacteria responsible for salmonellosis. Both extracts could serve as source of compounds that may be effective in the management of this condition.

CONCLUSION

The results obtained from the phytochemical and antimicrobial studies of the stem bark of *C.pedunculata* show that the use of the plant in the treatment of infectious diseases is justified and the methanol, ethyl acetate and petroleum ether extracts could be investigated further for the development of antibacterial agents.

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