IBBJ

Summer 2016, Vol 2, No 3

Original Article

Phytochemical Screening, Antioxidant and Antibacterial Activities of *Commiphora kerstingii*

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Submitted 23 Oct 2016; Accepted 11 Nov 2016; Published 05 Dec 2016

Phytochemical screening, antioxidant and antibacterial activities of aqueous and methanolic crude extracts of *Commiphora kerstingii* leaves were investigated. The antioxidant and antibacterial properties of different solvent extracts of *Commiphora kerstingii* plant were scrutinized. The phytochemical screening of the various extracts was carried out using standard methods and the results revealed the presence of alkaloids, phenols, tannins, saponins and volatile oils. The antioxidant activity of the extracts was screened using hydrogen peroxide free radical scavenging assay to obtain an IC_{50} value. The IC_{50} values of ascorbic acid (standard drug), methanolic and aqueous extracts were 0.49, 0.33 and 0.54 mg/ml, respectively. The antibacterial activity was tested using *Streptococcus mutans*, *Escherichia coli*, and *Proteus mirabilis*. The results showed reasonable zones of inhibition at almost all concentrations used against tested organisms; with *Streptococcus mutans* being most inhibited (diameter of inhibition of 23 mm) and *Escherichia coli* being least inhibited (3mm) with the methanolic extract. In contrast, *Escherichia coli* was the most inhibited (8 mm) and *Streptococcus mutans* showed least inhibition (3 mm) in the presence of aqueous extract. Minimum inhibitory concentration (MIC) was 0.05 g/ml on the average. The results, thus support the use of the plant traditionally to treat dental caries, diarrhea, and urinary tract infection, and suggest its usage in the formulation of new antioxidant and antibacterial drugs.

Keywords: *Commiphora kerstingii, Streptococcus mutans, Escherichia coli, Proteus mirabilis,* antioxidant activity

Medicinal plants represent a rich source from which antioxidant and antibacterial agents may be obtained. Plants are used medicinally and can be a potent source of many drugs (1). Herbal medicine has been shown to be effective recently, the world health organization (WHO) estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care and there are reports from various researchers on natural substances of plant origin which are biologically active with desirable antimicrobial and antioxidant properties (2).

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Oxidative stress is defined as the disturbance of oxygen (free radical) species production (3). Reactive oxygen species (ROS) are a problem in human beings, since they not only make our body cells age but also cause diseases such as cancers that are difficult to treat. The chain reaction caused by ROS can lead to cross-linking of atomic structures. In cases where the ROS-induced chain reaction involves base pair molecules in a strand of DNA, the DNA can become cross-linked (4). DNA cross-linking can in turn lead to various effects on aging, especially cancer (5). Antioxidants neutralize the effect of ROS through different ways and may prevent the body from various diseases. Antioxidants may be synthetic or natural. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been reported to be dangerous for human health (6). In nature, there are wide varieties of natural antioxidants with different composition, physical and chemical properties, and various mechanisms and/or site of action (7). Superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPX) etc… which are enzymes also present in plasma, may act as antioxidant as they can transform ROS and reactive nitrogen species into stable components (8). Vitamin A, C and E are among popular antioxidants, which play a crucial role in preventing oxidation damages in biological systems (9-10).

The present research investigated the antibacterial and antioxidant activities of *Commiphora kerstingii* leaves extract. *Commiphora kerstingii* is a tropical tree with 10 m high, widely spread in the arid regions of Africa where it is often planted. Earlier studies on this plant showed that it contains classes of natural products like saponins, tannins, and volatile oils. In addition, *Commiphora kerstingii* has been confirmed to be active against bacteria and fungi, e g; *Bacillus subtilis, Candida albicans and Escherichia coli* (11). The stem bark of this plant is used in Northern Nigeria to treat fever, cancer, measles, asthma, rheumatism and venereal diseases (12).

Materials and methods

Plant extract preparation

Fresh leaves of *Commiphora kerstingii* were collected from Vimtim, Mubi North, Adamawa State in October, 2015. The leaves were identified by Baba Taina at Department of forestry, Ministry of environment, Mubi, through comparison with a voucher specimen deposited at the herbarium unit of the department with FHI number 177.

50 g of the air-dried *Commiphora kerstingii* leaves were pulverized and extracted with 500 ml of either methanol or water. The solutions were allowed to stand for 24 h, after which they were filtered and concentrated using rotary evaporator as described by Akinyemi et al. (13).

Phytochemical screening

The extracts were analyzed for the presence of alkaloids, tannins, flavonoids, phenols, saponins, and volatile oils. The methods previously described (14-17) were adopted.

Antioxidant activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the methods described by Nabavi et al. (18-19). Briefly, a solution of hydrogen peroxide (2 ml) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen was determined by absorption at 285 nm using a UV/Visible spectrophotometer. The samples at '1, 0.5, 0.25, 0.125, and 0.625 mg/ml' were added to H_2O_2. The decrease in absorbance of H_2O_2 at 285 nm was measured spectrophotometrically after 10 min against a blank solution containing the test sample in phosphate buffer solution (PBS) without H_2O_2 and blank solution conTaining PBS without hydrogen peroxide (positive control). All tests were performed in triplicate. The

percentage of hydrogen peroxide scavenged by the extract was calculated as follows: % scavenged H_2O_2 = $[(A_c - A_s) / A_c] \times 100$ where A_c is the absorbance of the control and A_s the absorbance in the presence of the sample of extract and standard (17-18). The values of percentage of inhibition were obtained from the above equation. For 50% inhibitory concentration (IC_{50}) evaluation of the extract, graphs showing the concentration of the samples (water and methanol extracts) versus % inhibition (% H_2O_2 reduction) were plotted.

Antibacterial activity

The aqueous and methanolic extracts of plant were assayed against 3 clinical bacteria isolates, which were obtained from Microbiology laboratory of Modibbo Adama University of Technology, Yola. The bacteria include *Streptococcus mutans, Escherichia coli* and *Proteus mirabilis*. The Agar disc diffusion method was adopted for antibacterial activity assessment. The culture medium was prepared and the bacteria under test were grown in the nutrient agar in an incubator at 37 °C for 24 h. The concentrations were modified and determined as earlier described by Vollekova et al. (20) with some modification by Usman et al. (21). In this test, the microorganisms were prepared using the broth dilution technique. The stock extract concentration of 250 mg/mlml was made by dissolving 2.5 g of the extract in 10 mlml of sterile distilled water and the working concentrations prepared by two-fold serial dilution techniques that ranged from 200 mg/ml to 50 mg/ml using nutrientbroth, and were later inoculated with 0.2 ml suspension of the test organisms. After 24 h incubation at 37 °C, the plates were observed for turbidity considering the diameter of disc and zones of inhibition.

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. It`s also defined as the lowest concentration where no visible turbidity was observed in the test tubes. The concentrations were determined as earlier described by Vollekova et al. (20) with some modification by Usman et al. (21). The MIC was determined for microorganisms that showed sensitivity to the test extracts. After 24 h incubation at 37 °C, the tubes were observed for turbidity. The lowest concentrations where no turbidity was observed, was determined and noted.

Results

Antioxidant activity

The phytochemical screening of water and methanolic extracts (Table 1) showed that the plant contains some secondary metabolites which were tannins, phenols and volatile oils. Alkaloids and saponins were found only in methanolic extract whereas flavonoids were below detectable levels in both extracts.

The ability of the extract to scavenge hydrogen peroxide was assessed. In hydrogen peroxide radical method, the inhibition percentage of methanolic extract showed in Table 2 was in the range of 23.11% - 52.85%. Similarly, both the ascorbic acid (Vitamin C) and aqueous extract showed an increase of inhibition percentage with decreasing the amount of ascorbic acid and extract. (Table 2 and Fig. 1).

Antimicrobial activity

The antibacterial activity of both methanolic and aqueous extracts have shown a reasonable zone of inhibition at almost all the concentrations ranging from 250-150 mg/ml against the tested organisms. But the concentration of 100 mg/ml and 50 mg/ml have shown no growth except for the methanolic extract which at 100 mg/ml showed little inhibition effect on *Escherichia coli and Streptococcus mutans* (Tables 3 and 4).

Table 1. Phytochemical screening of aqueous and methanolic extracts of *Commiphora kerstingii* leaves

Solvent	Alkaloid	Phenol	Tannin	Saponin	Volatile oil	Flavonoid
MLE	+	+	+	+	+	-
ALE	-	+	+	+	-	-

MLE: methanolic leaf extract; ALE: water leaf extract; +: present; -: below detectable levels.

Table 2. Antioxidant activity of *Commiphora kerstingii* leaves

Concentration (mg/ml)	Percentage inhibition (%)		
	methanolic extract	aqueous extract	ascorbic acid
1	23.10	21.09	28.6
0.5	27.76	33.08	36.1
0.25	33.15	40.65	41.6
0.125	39.21	41.13	48.4
0.0625	52.85	52.09	57.1
IC_{50}	**0.33**	**0.54**	**0.49**

Table 3. The inhibition zone of of methanolic extract at different concentrations against some bacteria

Concentration (mg/ml)	*Escherichia coli*	*Streptococcus mutans*	*Proteus mirabilis*
250	12 mm	23 mm	10 mm
200	7 mm	17 mm	6 mm
150	5 mm	8 mm	3 mm
100	3 mm	5 mm	-
50	-	-	-

- : no inhibition

Table 4. The inhibition zone of aqueous extract at different concentrations against some bacteria

Concentration (mg/ml)	*Escherichia coli*	*Streptococcus mutans*	*Proteus mirabilis*
250	8mm	7mm	8mm
200	6mm	5mm	6mm
150	4mm	3mm	3mm
100	-	-	-
50	-	-	-

- : no inhibition

Table 5. Minimum inhibitory concentration (MIC) of the aqueous and methanolic leaves extract of *Commiphora kerstingii*

Concentration (mg/ml)	*Escherichia coli*	*Streptococcus mutans*	*Proteus mirabilis*
250	++	++	++
200	++	++	++
150	++	++	++
100	++	++	++
50	-	-	-

++: growth; - : no growth

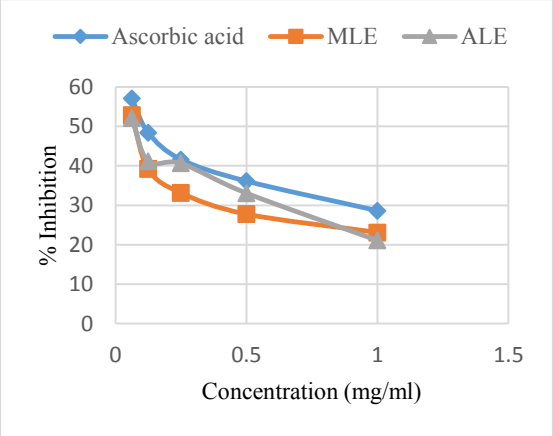

Figure 1. Hydrogen peroxide radical scavenging activity. % inhibition of ascorbic acid, methanolic extract (MLE) and aqueous extract (ALE) are shown.

The tube with the least inhibition inducing Concentration was taken and recorded to determine the minimum inhibitory concentration (MIC). According to Table 5, 50 mg/ml is the MIC of both extracts.

Discussion

The methanolic leaf extract in the present study gave a more active component and showed higher zones of inhibition in the antibacterial activity, than the aqueous extract. Literature reports showed a high correlation between antioxidant activity and phenolic compounds (22). This implies that compounds that have tannins in nature are most likely to exhibit antioxidant activity although other phenolic compounds like flavonoids also possess antioxidant activity and they are known to be in synergistic relationship with tannins in plants (23).

Table 1 showed that the plant extracts contains phenolic as well as tannins that are very good antimicrobial agents (24).

The lowest concentration of methanolic extract (0.0625 mg/ml) showed the highest percentage of inhibition value (52.85%). There was a characteristic increase in inhibition as the extract concentration decreased. This is in agreement with the work of Odeja et al. (25) but contrary to the work of Musa (26). The IC_{50} (the concentration of the sample required to scavenge 50% of the peroxide radicals) was actually used to examine the antioxidant effectiveness of the samples. The lower the IC_{50} the greater the overall effectiveness of suspected antioxidant sample in question. From the results obtained, the methanolic and water extracts and ascorbic acid had the IC_{50} values of 0.33, 0.54, and 0.49 respectively. Therefore, the methanolic extract was a more effective antioxidant than water extract although, they are both good antioxidants. Hence, *C. kerstingii* leaves is said to have antioxidant property which may be due to the presence of phenolic compounds of the plant and this agrees with the work of Musa (26) which revealed the antioxidant property of *C. kerstingii* stem bark.

Results presented in Tables 3 and 4 have clearly shown that crude extracts of *Commiphora kerstingii* have a dose dependent antibacterial activity. This is in agreement with previous studies (27-29) which demonstrated that several *Commi-*

phora species had considerable antibacterial activity against some gram positive and gram negative bacteria. In the present study, the antibacterial test in methanolic extract revealed that gram positive (*S. mutans*) bacteria showed significantly higher growth inhibition than the gram negative (*E. coli* and *P. mirabilis*) bacteria. This is in agreement with the *in vitro* studies by Paraskeva et al. (30) using selected African *Commiphora* species. The methanolic extract ranked higher in inhibiting the growth of the tested bacteria, with largest inhibition zone against *S. mutans,* followed by *E. coli and P. mirabilis*. Similar studies by Musa (26) demonstrated a good activity of *C. kerstingii* stem bark extract against tested bacteria. It had been said that phenolic compounds possess significant antioxidant activity, which is likely due to the presence of tannins detected and the antibacteria results of the present research showed that the plant *C. kerstingii* is active against the three (3) clinical bacteria isolates tested. Thus, in addition, other phytochemicals should be present.

Acknowledgement

The authors wish to thank the laboratory technologists (Mr. Umar & Mr. Madu Joshua) of Microbiology laboratory, Modibbo Adama University of Technology, Yola, and the research assistant (Mr. Mohammad Yahya Falalu) of American University of Nigeria, Yola for their assistance in the analyzes.

Conflict of interest

The authors declared no conflict of interest.

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