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Antioxidant and Antibacterial Activity of *Commiphora kerstingii* Engl. Stem Bark Extract

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Abstract: Methanolic extract of *Commiphora kerstingii* Engl. stem bark was screened for antioxidant activity using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay to obtain an IC_{50} value of 26.27±0.24 μg cm^{-3} compared to ascorbic acid with an IC_{50} value of 33.59±0.21 μg cm^{-3} used as control. Antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were tested. The result showed that *Staphylococcus aureus* was the most inhibited by the methanolic extract with diameter of inhibition zone of 30 mm while *Pseudomonas fluorescens* was the least inhibited with diameter of inhibition zone of 16 mm. Phytochemical investigation showed the presence of alkaloids, saponins, anthraquiniones, cardiac glycosides, tannins and flavonoids in the methanolic extract of *Commiphora kerstingii* stem bark.

Key words: *Staphylococcus aureus*, *Commiphora kerstingii*, ascorbic acid, phytochemical screening, DPPH radical scavenging

INTRODUCTION

Oxidative stress is a problem in human beings, since it not ouly make our body cells age but cause diseases such as cancer that are difficult to treat. One of the ways to make the body healthier is to stop the ageing process. This can be done using certain chemicals and metals to prevent oxidation or mop up free radicals from the body known as antioxidant (Andlauer and Fürst, 1998). The human body produces endogenous antioxidants such as reduced glutathione (GSH), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), which are very important for counteracting or preventing oxidative stress (Sen, 1995), but the amount produced by the human body is insufficient. Therefore, supplementation had to be adopted in order to get enough antioxidants using natural exogenous antioxidants, such as vitamin C, vitamin E, flavone, beta-carotene, natural products in plants and so on (Diplok and Charleux, 1998; Rice-Evans *et al*., 1997). Exogenous antioxidants found in plants are safer compared to synthetic antioxidants (Grice, 1986; Sokmen *et al*., 2004) and they are considered to be useful agents for prevention of cardiovascular diseases (Duthie and Brown, 1994) and several kinds of cancer (Milner, 1994).

Due to the increasing interest in using plants as sources of safer antioxidants, the tropical plant *Commiphora kerstingii* was investigated. The plant is a tree about 9 m high distributed along the arid regions of Africa and it is often planted. Its stem bark is used traditionally in Northern Nigeria to treat fever, cancer, measles, asthma, rheumatism and venereal diseases (Mann *et al*., 2003). Earlier studies on *Commiphora kerstingii* showed that the plant contains classes of natural products like saponins, tannins and volatile oils. In addition, the plant was active against three bacteria namely *Bacillus subtilis*, *Candida albican* and *Escherichia coli* (Kubmarawa *et al*., 2007). The stem bark and leaves exudes resins (Mann *et al*., 2003).

The present study was carried out to investigate the antioxidant activity, phytochemical and antibacterial activity screening of *Commiphora kerstingii* in order to validate its claimed traditional medicine uses.

MATERIALS AND METHODS

General

Absorbance data were measured with a UV spectrometer Spectronic 20D+ instrument.

Extraction of Plant Materials

Stem bark of *Commiphora kerstingii* was collected at Ahmadu Bello University, Zaria in July, 2007. The specimen was authenticated by Mallam Gallah, Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria, through comparison with a voucher specimen deposited at the herbarium unit of the department.

Twenty five grams of powdered air-dried stem bark of *Commiphora kerstingii* was extracted with 250 cm^3 of methanol in a covered container left to stand for 4 days. The methanol extract was concentrated in vacuum using a rotatory evaporator to yield 2.20 g of residue. The residue was stored in a desiccator until when needed for analysis.

Antioxidant Activity Assay

Preparation of DPPH

0.004% 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in methanol was prepared and stored in the dark before use. The solution gives a deep purple color.

Preparation of Sample Solution

Various concentrations of the residue (extract) were prepared using methanol as solvent. The concentrations of the samples are 10, 20, 40 and 80 μg cm^{-3}, respectively.

DPPH Free Radical Activity

In the activity testing 3 cm^3 of sample solution is poured into 3 cm^3 of DPPH solution and allowed to stand in the dark for 30 min. After 30 min the absorbance is measured at 517 nm and recorded. This experiment was carried out for each concentration of sample solution that is 10, 20, 40 and 80 μg cm^{-3}, respectively in triplicates (Gupta *et al*., 2003).

The percentage inhibition of DPPH by methanolic extract was then determined by calculation as follows:

$$100 - \frac{[(A_{DPPH} - A_{sample}) \times 100]}{A_{DPPH}}$$

Where:

A_{DPPH} = Absorbance of DPPH ouly

A_{Sample} = Absorbance of DPPH and methanolic extract combined in the same vessel

From the result obtained a plot of percentage inhibition of DPPH against concentration of methanolic extract is made and the IC_{50} determined. Control experiment was performed using ascorbic acid as sample and its IC_{50} also determined.

Antibacterial Activity Test

Preparation of Test Samples

In the study of the antibacterial activity, the methanol extract obtained as described above in extraction of plant material was used. It was diluted in dimethyl sulfoxide (DMSO): methanol (1:1, v/v) solvent. As a precaution not to miss trace amount of antibacterials for preliminary screening, a relatively high concentration of 200 mg cm^{-3} of the extract was prepared for bioassay.

Test Microorganisms

The methanolic extract of the plant was assayed for antibacterial activity against 6 clinical bacteria isolates, which were obtained from the Department of Medical Microbiology, Ahmadu Bello University, Zaria-Nigeria. The bacteria included *Bacillus subtilis* and *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. The bacteria were rejuvenated on Mueller Hinton agar medium (MHM, Merck, Germany) and subculture as needed.

Antibacterial Bioassay

Agar Well Diffusion Method

For bioassay suspension of approximately 1.5×10^{8} cells cm^{-3} in sterile normal saline were prepared as described by Forbes *et al*. (1998) and about 1.5 cm^{3} of it was uniformly seeded on MHM in 12×1.2 cm glass Petri dished, left for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm diameter and about 2 cm apart were punctured in the culture media using sterile borers. Respective concentrations of the extracts were administered to fullness in each well. Culture plates were incubated at 37°C for 48 h. After 48 h bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm using a transparent ruler. Each experiment was done in triplicates and the mean of the diameter of inhibition zones was calculated. Controls included use of solvent without test compounds, although no antibacterial activity was noted in the solvent used for the test.

Phytochemical Screening

A qualitative chemical analysis of methanol extract of the plant was carried out to detect the presence of some classes of natural products like alkaloids, tannins, flavonoids, saponins, anthraquiniones and cardiac glycosides using earlier described methods by Harborne (1998) and Oyewale *et al*. (2001).

RESULTS AND DISCUSSION

The methanol extract of *Commiphora kerstingii* was assayed for antioxidant activity using DPPH at various concentrations of the extracts in methanol as solvent. Table 1 shows the absorbance values obtained for DPPH only and that of methanolic stem bark extract of the plant at various concentrations. Table 1 also contains the percentage inhibition of DPPH by the methanol extract

Table 1: Antioxidant activity assay of *Commiphora kerstingii* stem bark methanolic extract

Concentration (μg cm^{-3})	Absorbance sample	Absorbance control	Inhibition sample (%)	Inhibition control (%)	p-value
DPPH	0.462±0.004				0.600**
DPPH+sample					
80	0.389±0.006	0.424±0.025	84.1	91.8	
40	0.312±0.011	0.269±0.014	67.5	58.2	
20	0.325±0.008	0.242±0.012	70.3	52.3	
10	0.201±0.002	0.136±0.005	43.5	29.5	

IC_{50} values presented are the SEM of three assays for each concentration of extract determined, **p-value shown in table was determined when means of percentage inhibition sample was compared to percentage inhibition control with Students' t-test. $p\leq0.05$ implies significant difference, while $p\geq0.05$ implies not significant difference

Table 2: Antibacterial activity of methanolic extracts of *Commiphora kerstingii* stem bark

Bacteria	Diameter of Inhibition Zone (DIZ) in mm
Staphylococcus aureus	30
Streptococcus pyogenes	24
Pseudomonas æruginosa	21
Pseudomonas fluoresceus	16
Bacillus subtilis	19

DIZ >15 mm indicates very good antibacterial activity

Table 3: Phytochemical components of methanolic extract of *Commiphora kerstingii* stem bark

Class of natural product	Response
Alkaloids	+
Saponins	++
Anthraquinones	+
Cardiac glycosides	++
Tannins	+++
Flavonoids	+++

+++: Very high quantity, ++: Moderate quantity, +: Low quantity

(sample) and that of ascorbic acid used as control at various concentrations. The concentration of the methanol extract that causes 50% inhibition of DPPH, which is known as IC_{50} was determined to be 26.27±0.24 μg cm^{-3}. This value is less than the IC_{50} of control which is 33.59±0.21 μg cm^{-3}. Despite this development, the statistical analysis result shows that the plant is as effective as the control when used as an antioxidant. A p-value of 0.600 (two-tail) was obtained using Microsoft Excel Program Data Analysis Toolpak as shown in Table 1. This p-value is greater than $\alpha = 0.05$, which implies that there is no significant difference between the inhibition of sample (that is the methanolic extract of *C. kerstingii*) and inhibition of control.

Table 2 shows that the crude methanolic extract was active against all the bacteria tested with *Staphylococcus aureus* having the highest diameter of inhibition zone (30 mm), while the lowest is shown by *Pseudomonas fluorescens* with a diameter of inhibition zone (16 mm). This result indicates that the plant *Commiphora kerstingii* is highly effective in suppressing the growth of *Staphylococcus aureus* and the other bacteria tested. However, the result justifies the traditional medicine use of this plant for the treatment of fevers and asthma because of its antibacterial activity against all the 6 clinical bacteria isolates tested.

However, Table 3 shows that the plant has tannins as one of the classes of natural products detected during phytochemical screening. Tannins are mostly made up of phenols and phenolic compounds and they are classified into two classes, which are hydrolysable and condensed tannins (Cowan, 1999). Literature shows that there is high correlation between antioxidant activity and phenolic compounds (Odabasoglu *et al*., 2004). This implies that compounds that are tannins in nature are most likely to exhibit antioxidant activity. Although other phenolic compounds like flavonoids, which were found in the plant extract as shown in Table 3, also possess antioxidant activity and they are known to be in synergistic relationship with tannins in plants (Rice-Evans *et al*., 1997).

The positive result as shown in Table 3 shows that the methanolic plant extract contains phenolic compounds like tannins that are very good antimicrobial agents (Scalbert, 1991). In the above paragraph, it had been said that phenolic compounds possess significant antioxidant activity, which is likely due to the presence of tannins detected and the result of this experiment shows that the plant *C. kersingii* antibacterial agents in it that are highly active against six clinical bacteria isolates. Thus, it maybe summarized that the class of natural product present in the compound that exhibit both antioxidant and antibacterial activity are likely due to the presence of tannins, although other classes of natural products detected can also exhibit antioxidant and antibacterial activity.

CONCLUSION

The result reported in this study may be considered as the preliminary report on the *in vitro* antibacterial and antioxidant activities of *Commiphora kersingii*. Since the plant showed significant antibacterial and antioxidant activities, which are suspected to be phenolic compounds such as tannins from result obtained from the phytochemical screening, it become necessary to expand the work so as to carry out chromatographic separation to isolate the active compounds and characterize their structures using IR, UV, Mass and NMR spectroscopic methods. In addition toxicity assay is required to determine the safety level of the plant extract.

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