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

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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 IC₅₀ value. The IC₅₀ 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).**
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$_2$O$_2$. The decrease in absorbance of H$_2$O$_2$ at 285 nm was measured spectrophotometrically after 10 min against a blank solution containing the test sample in phosphate buffer solution (PBS) without H$_2$O$_2$ 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 
\[ \text{H}_2\text{O}_2 = \left( \frac{A_c - A_s}{A_c} \right) \times 100 \] 
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\(_2\)O\(_2\) 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/ml was made by dissolving 2.5 g of the 
extract in 10 ml 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 nutrient broth, 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

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Alkaloid</th>
<th>Phenol</th>
<th>Tannin</th>
<th>Saponin</th>
<th>Volatile oil</th>
<th>Flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLE</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ALE</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

Table 2. Antioxidant activity of *Commiphora kerstingii* leaves

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>methanolic extract</td>
</tr>
<tr>
<td>1</td>
<td>23.10</td>
</tr>
<tr>
<td>0.5</td>
<td>27.76</td>
</tr>
<tr>
<td>0.25</td>
<td>33.15</td>
</tr>
<tr>
<td>0.125</td>
<td>39.21</td>
</tr>
<tr>
<td>0.0625</td>
<td>52.85</td>
</tr>
</tbody>
</table>

IC₅₀: 0.33 0.54 0.49

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

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th><em>Escherichia coli</em></th>
<th><em>Streptococcus mutans</em></th>
<th><em>Proteus mirabilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>12 mm</td>
<td>23 mm</td>
<td>10 mm</td>
</tr>
<tr>
<td>200</td>
<td>7 mm</td>
<td>17 mm</td>
<td>6 mm</td>
</tr>
<tr>
<td>150</td>
<td>5 mm</td>
<td>8 mm</td>
<td>3 mm</td>
</tr>
<tr>
<td>100</td>
<td>3 mm</td>
<td>5 mm</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- : no inhibition

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

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th><em>Escherichia coli</em></th>
<th><em>Streptococcus mutans</em></th>
<th><em>Proteus mirabilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>8mm</td>
<td>7mm</td>
<td>8mm</td>
</tr>
<tr>
<td>200</td>
<td>6mm</td>
<td>5mm</td>
<td>6mm</td>
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<tr>
<td>150</td>
<td>4mm</td>
<td>3mm</td>
<td>3mm</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- : no inhibition
Table 5. Minimum inhibitory concentration (MIC) of the aqueous and methanolic leaves extract of Commiphora kerstingii

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Escherichia coli</th>
<th>Streptococcus mutans</th>
<th>Proteus mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>200</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<td>150</td>
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<td>++</td>
</tr>
<tr>
<td>100</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ : growth; - : no growth

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 contain 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 IC₅₀ (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 IC₅₀ 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 IC₅₀ 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

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Conflict of interest

The authors declared no conflict of interest.

References