Antimicrobial and Antioxidant activities of *Citrullus lanatus* var. *citroides*

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Abstract Extracts obtained from leaves, stem, fruits pulp and seeds of *C. lanatus* var. *citroides* were screened for their antimicrobial activity against five standard bacteria and two strains of pathogenic fungi using disc diffusion method. The results showed that the crude extracts of the four different morphological organs exhibited various degrees of antimicrobial effects. The chloroformic extract of the fruit pulp showed the highest antibacterial activity with notable effect on *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. The ethanolic extract of the fruit exerted the highest activity against the two tested fungi species. The chloroform, ethylacetate and butanol extracts of the fruit were screened for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. The butanol extract exhibited a potent antioxidant activity, while the chloroform and the isolated compounds did not show significant activity.

Key words ABST. Antimicrobial activity. *Cucurbitaceae* DPPH. *Citrullus lanatus* var. *citroides*, Radical-scavenging.
Introduction

Plants are considered as the oldest source of pharmacologically active compounds that have the most significant contribution in the field of disease treatments throughout the history of mankind (Nicole, 2004). Sudan is a country with a population with basic curative medicinal attentions (Galal et al., 1991).

Infectious diseases, particularly those involving the gastrointestinal tract, are a serious problem worldwide, mainly among third world children (Watson, 1992). On the other hand, some of the drugs currently in use result in adverse side effects (Covington, 1988). Therefore, the search for new antimicrobial substances exhibiting minimal side effect is warranted (Khalid al., 1994). One of the most promising areas in the search for new biological active compounds are plants used in traditional medicine (Alonso al., 1995).

Antioxidants, which scavenge active oxygen species (free radicals), are found in a variety of foodstuffs and are commonly referred to as scavengers. (Beckman et al, 1990; Bohme et al, 1993 Antioxidants play an important role in human health because the biologic defense mechanisms cannot operate under severe oxygen stress. According to recent research, activated oxygen is thought to be a major factor in aging, hardening of the arteries,
diabetes, cancer and tissue injury skin (Ito and Hirose, 1989; Beckman, 1994).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are largely employed as preservatives by pharmaceutical, cosmetic and food industries, even if they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals (Madhaavi and Salunkhe, 1995). The need to replace synthetic antioxidants with natural and properly safe ones, together with the interest of food industry and preventive medicine in the development of bioactive naturally-occurring antioxidants, has fostered research on the screening of plant sources, especially the inexpensive residue sources from agricultural industries (Moure et al., 2001).

Plants containing cucurbitacin were early recognized in folk medicine to have biological values. Scientific studies mainly refer to Middle East and Asia where cucurbit plants were used actively as herbal remedies. Citrullus lanatus var. citroides belongs to the family Cucurbitaceae. Members of this family are generally dioecious herbs which prostrate or climbing by means of tendrils. The fruit, eaten when fully ripe or even when almost putrid, is used as a febrifuge (Grieve, 1984). The fruit is also diuretic, being effective in the treatment of dropsy and renal stones (Chiej, 1984). The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes (Duke and Ayensu, 1985). In Northern Sudan is often used for burns, swellings, rheumatism, gout and as laxative (Basheer, 2007).
**Materials and Methods**

**Plant material:**

Different morphological organs of *Citrullus lanatus* var. *citroides* were collected from Al- Musawarat, Northern Sudan on February 2006.

The taxonomic identification of this plant was carried out at Medicinal & Aromatic Plants Research Institute, National Center for Research by W.E.A/Ala. A voucher specimen was deposited at the herbarium of the institute.

Fifty grams of the dried plant materials were extracted were extracted at the beginning with hexane in conical flask for 24 hours and filtered. The filtrates were collected together and the residue was brought to dryness and extracted with chloroform following the same methods as for hexane. The filtrates were collected together and the residue was brought to dryness and extracted with 150 ml 90 % ethanol following the same method as for hexane and chloroform. The ethanol extract was modified to aqueous extract, which was extracted successively with equal volumes of two organic solvents of increasing polarity (ethyl acetate and butanol).

The different extracts obtained were evaporated under room temperature and brought to complete dryness to yield crude extracts.

**Antimicrobial activity test:**

The crude extracts of leaves, stems, fruit bulb and seeds of *Citrullus lanatus* were subjected to antibacterial and antifungal tests. The bacteria
used were of the American type culture collection (ATCC). They were obtained from the stock culture of National Sanitary Laboratory and Biotechnology laboratory, Department of Chemistry. Strains maintained for tests were *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, *Bacillus subtilis* (NCTC8236) and *Proteus vulgaris* (ATCC474021). The fungi species used were *Aspergillus niger* (ATCC9763) (As.) and *Candida albican* (ATCC7596).

The media used for antibacterial screening tests were nutrient broth and Mueller Hinton agar. For antifungal activity test, nutrient broth and Sabouroud dextrose agar were used. Twenty seven grams of Mueller Hinton agar were suspended in 650 ml of distilled water and heated on a boiling water-bath to dissolve the media completely and were then divided into 20 ml portion in small vials. The prepared media were sterilized by autoclaving at 121 C° (at atmospheric pressure of 15 pound).

**Preparation of standard bacterial suspension:**

Each 10 ml portion of sterilized nutrient broth was inoculated with a loopful of each of the bacterial slant agar culture and was incubated for 18-24 hours at room temperature. 10 % dilution from each liquid culture was prepared in sterilized normal saline and kept in a refrigerator.

**Preparation of dilutions of the extracts:**

The dilution used for all extracts was 100 mg/ml. Hexane extracts for leaves, stems and fruit were redissolved in hexane solvent to prepare 100 mg/ml. The Hexane extract of seeds (oil) prepared as 1:9 (v/v) extract & hexane solvent respectively. The chloroform extracts was redissolved in a
mixture of petroleum ether and methanol (8:2 v/v) and a concentration 100 mg/ml was prepared for use. Chloroform was found to be inhibitory to the growth of the tested bacteria and hence it was not used as a solvent in applications meant for tested antibacterial activity. Also, the ethanolic extracts was redissolved or suspended in methanol solvent and 100 mg/ml was prepared for use.

**Antibacterial assay:**

The cup-plate agar diffusion method adopted in this study was that of Murray (1995), with some minor modifications to assess the antibacterial activity of the prepared plant extracts. From each of the standard bacterial stock suspension, 1 ml was thoroughly mixed with 20 ml of sterile Molten Muellur Hinton agar (45°C - 50°C). They were distributed into sterile Petri-dishes and left to solidify on a plain surface. Then, four cup-shape wells (10 mm diameter) were made in each plate using sterile cork-borer (No. 9). The agar disks were removed and four alternate cups were filled with 8 ml sample of each extract using sterile adjustable pipettes. Four Petri-dishes with two alternate cups were used with the respective solvent instead of the extracts as Control.

The plates were then incubated in upright position for 18-24 hours at room temperature. Two replicates were carried out for each extract against each of the tested organisms. After incubation period, the inhibition zones diameters were measured and the mean values were tabulated.

**Antifungal assay:**

The cup-plate agar diffusion method was adopted with some minor modifications to assess the antifungal activity of prepared extracts (Murray,
From each of the fungal stock suspension, 2 ml was thoroughly mixed with 20 ml of sterile molten Sabourout Dextrose Agar (45-50 °C), distributed into sterile Petri-dishes and left to solidify on a plain surface. Then, four cup-shaped wells (10 mm diameter) were made in each plate using cork-borer (No.9). The agar disks were removed and alternate cups were filled with 1ml sample of each extract using sterile adjustable pipettes. The plates were then incubated in the upright position for 24-48 hours at a 37°C. Two replicates were carried out for each extract against each of the tested organism. After incubation periods, the inhibition zones diameter were measured and the mean values were tabulated. (Table 3.3)

**Antioxidant screening (Free Radical Scavenging Activity (DPPH)): Assay by UV Spectrophotometry:**

The method was carried out as described by Brand and Hawaii, (1989) with some minor modifications. Each sample stock solution (1mg/ml) was diluted to final concentrations of 1000, 500, 100, 50 and 10 µg/ ml in methanol. A total of 3.8 ml of 50 µM DPPH methanolic solution (1mg/ 50 ml) was added to 0.2 ml of sample solution of different concentrations and allowed to react at room temperature. After 30 min, the absorbance of the reaction mixture was measured at 517 nm (Shimadzu UV-VIS 1601PC). The DPPH solution and methanol were used as control while vitamin C was used as standard antioxidant. The absorbance of control was measured immediately at 0 min. The percentage inhibition was calculated using the following formula:
Percentage Inhibition (%) = \( \frac{\text{Abs (DPPH)}}{\text{Abs (DPPH + sample)}} \)

\( \text{Abs (DPPH)} \)

Were Abs\( \equiv \) absorbance

The IC\(_{50}\) value was determined as the concentration of each sample required to give 50 % of the absorbance shown by the control. All tests and analyses were carried out in triplicates and averaged. The tests carried out for chloroform, ethyl acetate and butanol crude extracts.

**Results and Discussions**

The screening of bioactive agents from plants is one of the most intensive areas of natural products research today, yet the field is far from exhausted. Extracts obtained from leaves, stem, fruits and seeds of *C. lanatus* var. *citroides* were screened for their antimicrobial activity against five standard bacteria namely; *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Proteus vulgaris* and two fungi species namely; *Candida albican and Aspergillus nigar* at concentration 100 mg/ml. The results of preliminary screening were of summarized in Tables (3-3) and (3-4).

The means of diameter of the growth inhibition zones obtained in the experiment have been shown in Tables (3-3) and (3-4).

On the basis of the results obtained with standard chemotherapeutic agents against the same standard tested organisms, plant extracts resulting in more than 18 growth inhibition zones are considered to possess relatively high antibacterial activity, and those resulting in 14-18 mm inhibition are of
intermediate, and those resulting in zones below 14 mm inactive (Cruickshank et al., 1975).

All the tested bacteria were resistant to hexane extracts of the leaves and fruits, while they were intermediate to hexane extract of stems. *S. aureus* was susceptible towards all extracts (Table 3.3).

The five standard bacteria were sensitive to chloroform and ethanol extract of the fruits which showed inhibition zone (36 mm) and (33 mm) respectively. The ethanol and chloroform extracts of the leaves exhibited the same activity (33 mm), followed by the chloroform extracts of the stems (29 mm) and seeds (26 mm) respectively. Our results are in accordance with the result of previous study of *C. colonthysis* (synonym of *C. lanatus* var. *citroides*) by Usman et al., (2003) who had demonstrated antibacterial activity of ethanolic extracts fruits, stems, leaves and roots against Gram positive bacteria *Bacillus pumilus* and *Staphylococcus aureus*, while fruit and root extracts in double strength gave positive results against Gram positive bacillus (*bacillus subtilis*). The Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* showed no response.

*Bacillus subtilis* showed more susceptibility towards chloroform extracts of all studied organs. The fruits and leaves gave very high inhibition zones (37 mm), as well as stems (36 mm), followed by seeds (27 mm) respectively. Also the chloroform extracts of all organs showed the highest inhibition zones against *E. coli* when compared with the hexane and ethanol extracts. The fruits and stems exerted the highest activity (37 mm) and (36 mm) respectively, followed by stems leaves (31 mm) and seeds (28 mm).
Proteus valgaris showed the highest sensitivity (29 mm) to the ethanol extract of the fruit. Moreover this bacterium was almost equally susceptible to ethanol extract of the stems and chloroform extracts of the fruits, stems and leaves with inhibition zones in the range of 22-23 mm. The chloroform extract of the seeds, hexane extract of stems and ethanol ones of leaves and seeds also exerted good activity against P. valgaris but with less inhibition zones in the range of 16-18 mm.

Compared to the other tested bacteria, P. aeruginosa was less susceptible to extracts of different organs of C. lanatus var. citroides. The chloroform and ethanol extracts exhibited inhibition zones between 15 and 20mm, and 16 to 18 mm respectively. Only the hexane extract of stems gave activity with inhibition zone 16 mm.

The antibacterial activities of different organs were compared with antibiotic Gentmicin at a concentration of 40 µg /ml. S. aureus was susceptible to the ethanol and chloroform extracts of the fruits and the chloroform extract of the leaves, more than tested antibiotic. The growth inhibition zones of B. subtilis and E. coli obtained by the chloroform extracts of all organs with the exception of that of the seeds were greater than the zones recorded with the tested antibiotic. However, Gentmicin exerted greater inhibitory effect on Pr. valgaris and P. a erguinosa than that obtained by the different extracts except for the ethanol extract of the fruits.

The antifungal activity against C. albican and A. nigard is presented in Table 3-4. The ethanolic extracts of all organs showed the highest antifungal activity, however chloroform extracts also showed relatively high activity. The ethanolic extract of the fruit pulp showed the highest antifungal effects
compared to the other organ extracts exerting maximum effect (41mm) on *C. albican* and (35 mm) on *A. nigar*. The ethanolic extract of the stem showed similar inhibitory effects against *C. albican* and slightly less activity on *A. nigar* (31mm).

The hexane extracts of all studied organs (leaves, stems, fruits and seeds) showed no activity against the two tested fungi species.

The inhibition zones displayed by the ethanol extracts of the fruits pulps and stems were comparable to that exhibited by the antibiotic Clotrimazolo against *C. albican*. However, ethanol extracts (except that of the seeds) as well as the chloroform of the seeds showed higher activity against *A. nigar* than Clotrimazolo.

The antimicrobial effects of this plant extracts against the studied bacteria suggest that, different organs of *C. lanatus* var. *citroides* may possess remarkable therapeutic action in the treatment of bacterial and fungal diseases such as gastrointestinal infection, diarrhoea, respiratory and skin diseases. The high potency of *C. lanatus* var. *citroides* against these microbes gives scientific basis for it uses in folk medicine and could provide an example of prospecting for new compounds.
Table 3.3: Antibacterial activity of *Citrullus lanatus* var. *citroides* extracts

<table>
<thead>
<tr>
<th>Part of Plant</th>
<th>Extraction Solvent</th>
<th>Inhibition zone (mm)</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ps</td>
<td>Pr</td>
</tr>
<tr>
<td>Leaves</td>
<td>Hexane</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Fruit</td>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seeds</td>
<td>Ethanol</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>Fruit</td>
<td>Ethanol</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Seeds</td>
<td>Ethanol</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Leaves</td>
<td>Ethanol</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>Fruit</td>
<td>Ethanol</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Seeds</td>
<td>Ethanol</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Gentlecin</td>
<td>23</td>
<td>25</td>
</tr>
</tbody>
</table>

S.a.: *Staphylococcus aureus*, B.s: *Bacillus subtilis*, E.c: *Escherichia coli*

P. r: *Proteus vulgaris*, P.s: *Pseudomonas aeruginosa*
Table 3.4: Antifungal activity of *Citrullus lanatus* var. *citroides* extracts.

<table>
<thead>
<tr>
<th>Inhibition zone (mm)</th>
<th>Organ</th>
<th>Extraction Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Aspargellus nigra</strong></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Leaves</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Stem</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Fruit</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Seeds</td>
</tr>
<tr>
<td>18</td>
<td>30</td>
<td>Leaves</td>
</tr>
<tr>
<td>17</td>
<td>25</td>
<td>Stem</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>Fruit</td>
</tr>
<tr>
<td>37</td>
<td>37</td>
<td>Seeds</td>
</tr>
<tr>
<td>37</td>
<td>30</td>
<td>Leaves</td>
</tr>
<tr>
<td>35</td>
<td>41</td>
<td>Stem</td>
</tr>
<tr>
<td>31</td>
<td>41</td>
<td>Fruit</td>
</tr>
<tr>
<td>25</td>
<td>37</td>
<td>Seeds</td>
</tr>
</tbody>
</table>
Determination of Antioxidant Activity:

Results of antioxidant activity of different extracts as well as isolated compounds were presented in Table 3-10. The antioxidant potentials of tested samples were compared with ascorbic acid. The butanol extract showed a high effective free radical scavenging in the DPPH assay at all concentrations. It was effective in reducing the stable radical DPPH to the yellow colored diphenylpicrylhydrazine suggesting a high free radical scavenging activity. In fact, the butanol extract exhibited a remarkable antioxidant effect with extraordinary scavenging rates of 42.34, 43.89, 55.98, 96.22 and 102.25 % at 10, 50, 100, 500 and 1000 µg/ml respectively. Ascorbic acid which was used as positive control, recorded scavenging rate of 45.02, 47.87, 69.86, 120.54 and 129.65 % at 10, 50, 100, 500 and 1000 µg/ml respectively (Table 3.10) and Figure 3.

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage (Aurand, 1977). The percentage of hydroxyl radical scavenging increased with the increasing concentration of fruit extract and isolated compounds.

The ethyl acetate extract revealed a non significant free radical scavenging activity with scavenging rates of 2.35, 9.57 and 15.66 % at 100, 500 and 1000 µg/ml respectively. Also, the chloroform extract showed no antioxidant effect. Concerning the antioxidant activity of pure compounds, only compound (5) exhibited moderate activity at concentrations 500 and 1000 mg ml with scavenging rates of 54.49 and 72.80 % respectively.
Preliminary phytochemical screening of *C. lanatus* showed the presence of large amounts of phenolics and flavonoids. This result promoted us to evaluate antioxidant activity of the fruit. Antioxidant activity may be due to phenolic compounds in fruit methanolic extract, but further work should be done on the isolation and identification of other antioxidant components of *Citrullus lanatus* var. *citroides*.

Flavonoids and phenolic compounds are the main antioxidative compounds of fruits and vegetables (Huang *et al*., 1998). However, from the phytochemical screening the antioxidant property of *C. lanatus* var. *citroides* fruits pulps could be attributed to the presence of these compounds. However, the magnitude of antioxidative potency varies with the type of extracts. The active principles in these vegetal extracts are principally water soluble or lipophilic antioxidant molecules. Indeed, most of these plant extracts contain various amounts of Vitamin E, Vitamin C, β-carotene, and other flavonoids (Aruoma, 1994; Aruoma 2003) and were used as potential antioxidant prophylactic agents for both health and disease management. In this investigation the antioxidant molecules seem to be water soluble, so isolation and characterization of antioxidantly active compound(s) from Butanol and Hexane extract is warranted.

Shu-Jing (2008) reported that both ethanol and water extracts of wild bitter melon (*Momordica charantia*) were effective in reducing the stable radical DPPH. The water extract (IC$_{50}$ = 129.94 µg/ml) demonstrated a stronger DPPH radical scavenging than ethanol one (IC$_{50}$= 156.78 µg/ml). Interestingly, they demonstrated that both extracts showed a stronger scavenging activity than vitamin E (IC$_{50}$ = 172.21) which reflects potent free radical scavenging activity in polar extracts.
Table 3.10: Free radical scavenging of the crude extracts and isolated compounds from *Citrullus lanatus* fruit

<table>
<thead>
<tr>
<th>sample</th>
<th>10 µg/ml</th>
<th>50 µg/ml</th>
<th>100 µg/ml</th>
<th>500 µg/ml</th>
<th>1000 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BuoH.Extract</td>
<td>41.34</td>
<td>43.89</td>
<td>55.98</td>
<td>96.22</td>
<td>102.25</td>
</tr>
<tr>
<td>EA. Extract</td>
<td>0.0</td>
<td>0.0</td>
<td>2.35</td>
<td>9.57</td>
<td>15.66</td>
</tr>
<tr>
<td>Chl. Extract</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.02</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>45.02</td>
<td>47.87</td>
<td>69.86</td>
<td>120.54</td>
<td>129.65</td>
</tr>
</tbody>
</table>
Conclusion

This study can be considered as the first detailed *in vitro* antimicrobial and antioxidant features of *Citrullus lanatus* var. *citroides*. Extracts prepared from different organs of *C. lanatus* showed variable antibacterial activity. Tested bacteria were most susceptible to chloroform extract of the fruit pulps and tested fungi were most susceptible towards ethanolic extract. In addition, the butanol extract of the fruit pulps possessed potent antioxidant and free radical scavenging activity, which could be derived from compounds such as flavonoids and phenols. Thus, this plant could be a source of striking antioxidant agents, which provide prophylaxis against various diseases like heart stroke, arteriosclerosis and cancers diseases.

The present results support the use of *C. lanatus* var. *citroides* in the treatment of diseases like abscesses and wound healing in traditional medicine.

In conclusion, *Citrullus lanatus* var. *citroides* has displayed promising biological activities and could form a good basis for its selection for further investigation in the potential discovery of new natural bioactive compounds.

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