In vitro evaluation of antimicrobial, anti-Gardia activities and phytochemical screening of Helioptropium sudanicum used in traditional medicine

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ABSTRACT

The present study was designed to investigate the antimicrobial, and antioxidant activities of Helioptropium sudanicum. Phytochemical study was piloted to detect the bioactive compounds, which are responsible for biological activities. The ethanol plant extract were seasoned against standard bacteria strains, such as Staphylococcus aureus (ATCC25923), Pseudomonas aeruginosa (ATCC27853), Klebsiella, Escherichia coli (ATCC25922) and fungi Candida albicans (ATCC7596), using cup-plate agar diffusion method. The anti-Gardia activity was conducted via In vitro susceptibility assays using the sub-cultured method. Ethanolic extract showed good activities against klebsella, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Candida albicans. Potential anti-Gardia activity was presented by ethanol via susceptibility assays, which gave inhibition of 97 and 95% in concentrations of 100 and 50% after 72 h. The results of phytochemical screening showed that the extract of studied plant contain alkaloids, phenols, tannins, saponins, flavonoids, and terpenoids. This study showed the antimicrobial, anti-Gardia properties of the studied plant, and interesting correlation with the phytochemical constituents and biological activities.

Key words: Helioptropium sudanicum, antimicrobial and anti-Gardia properties, phytochemical screening.

INTRODUCTION

Traditional obsess in folk medicinal plants is well known since thousand years ago. Commonly, the ailment incidence in the rural area is treated with local plants that contain many pharmaceutical constituents (Sofowora, 1982). Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; barks, leaves, stems, roots, flowers, fruits and seeds, etc., that is, any part of the plant body may contain active components (Ugochukwu et al., 2013). About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants collected from central Sudan between January 2016 and February 2016. The plant was identified and authenticated by the taxonomists of International University of Africa. The plant parts were air-dried, under the shadow with good ventilation and then ground finely in a mill until their uses for extracts preparation.

Plants extraction

50 g of the powdered plant material was soaked in about 500 ml of ethanol solvent. The mouth of the flask was covered with aluminum foil and shaken thoroughly and allowed to get soft for overnight. Then the mixture was filtered using filter paper with funnel, filtrate was then collected by vacuum flask and allowed to be dry at normal room temperature for about 5 days. The residues were collected by scratching with new sterilize blades and was

MATERIALS AND METHODS

The aerial parts of Helioptropium sudanicum were
Preparation of standard bacterial suspensions

One milliliter aliquots of a 24 h broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 h. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in a small volume of normal saline to produce a suspension containing about 108-109 colony-forming units per ml. The suspension was stored in the refrigerator at 4°C until use.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline and 0.02 ml volumes (one drop) of the appropriate dilutions were transferred by digital pipette (Finnpipette Adjustable Volume) onto the surface of dried nutrient agar plates. The plates were allowed to stand for 2 h at room temperature for the drops to dry, and then incubated at 37°C for 24 h. After incubation, the number of developed colonies in each drops were counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and the dilution factor to give the viable count of the stock suspension expressed as the number of colony forming units (C.F.U) per ml of suspension. Each time a fresh stock suspension was prepared, all the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of standard fungal suspensions

The fungal cultures were maintained on sabouraud dextrose agar, incubated at 25°C for seven days. The fungal growth was harvested and with sterile normal saline and finally suspended in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until use.

In vitro testing of extracts for antimicrobial activity

The cup-plate agar diffusion method was adopted according to Kavanagh (1972) with some minor modifications to assess the antibacterial activity of the prepared extracts. One milliliter of the standardized bacterial stock suspension was thoroughly mixed with 150 ml of the sterile molten Mueller-Hinton agar which was maintained at 45°C. 10 ml aliquots of the inoculated Mueller-Hinton agar were distributed into sterile 15 Petri-dishes. The agar were left to dry and in each of these plates, 5 cups (10 mm in diameter) were cut using a sterile cork borer(No.4) and agar agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic Microliter-pipette, and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for each extracts against each of the test organisms. After incubation, the diameters of the resultant growth inhibition zones were measured and the values were tabulated.

In vitro testing of extracts for anti-parasite activity

Giardia sample collection

Fecal samples were collected from patients presented to Al-Basha’ir hospital, Khartoum February 2017. Convenient sampling method was used. The examination of positive sample showed the presence of cysts and trophozoites which were subsequently used in this study.

Preparation of Girdia cultures from fresh trophozoite samples

The trophozoites obtained from two fresh fecal samples were inoculated in flasks with each of them containing 5ml of Thioglycollate media, antibiotics (gentamycin) and 10% Human serum and were incubated at 37°C anaerobically. Motile trophozoite (as inoculum) Cultures were examined using light microscope at 40x magnification and the parasite were counted using Neubauer chamber.

In vitro susceptibility assays of Giardia

In vitro susceptibility assays used the sub-cultured method of Cedillo et al. (2002) which has described as a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in Entamoeba histolytica, Giardia intestinalis and Trichomonas vaginalis (Arguelloet al., 2004). 5 mg from each extract was dissolved in 50 um of (DMSO) at Eppendorf tube containing 950 µl D.W in order to reach a concentration of 5 mg/ml (500 ppm). The concentrations were stored at 20°C for further analysis. Sterile 96-well microtite plate wells (8 column 12 rows) were chosen for each extracts, 80 µl (micro-litres) of an extracts solution (5 mg/ml) were added to the first column wells C-1: On the other hand, 40 µl of complete Thioglycollate medium were added to the other wells in the second column and third column (C-2 and C-3). Serial dilutions of the extracts were obtained by
taking 40 µl of extract to the second column wells and taking 40 µl out of the complete solution in C2 wells to C3 wells and discarding 40 µl from the total solution of C3 to the remaining 40 µl serial solutions in the successive columns. 160 µl of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 200 µl.

In each test metronidazole (a trichomonocide), pure compound [1-(2-hydroxyethyl)-2-methyle-5 nitroimidazole] was used as a positive control with concentration of 312.5 µg/ml, whereas untreated cells were used as a negative controls (culture medium plus trophozoites). For counting, the samples were mixed with eosin in equal volume. The final number of parasites was determined by using light microscope at 40x magnification and the parasite were counted by using Neubauer chamber after 24, 48 and 72 h. The mortality % of parasite for each extracts activity was carried out according to the following equation (Al-kaissi, 2010):

\[
\text{Mortality of parasite (\%)} = \frac{\text{Death cells}}{\text{Death + Living cells}} \times 100
\]

**Phytochemical screening**

Plant extracts were screened for presence of phytochemical constituents. The phytochemical screening was performed using standard procedures as described by Trease and Evans (1989) and Odebiyi and Sofowora (1978).

**Alkaloids**

Wargner's reagent: To 0.5 ml of the extract, 2 ml of Wargner's reagent was added and the reaction mixture was observed for the formation of brown precipitate

Mayer reagent: 1 ml of Mayer reagent was added to 1 ml filtrate. A slight yellow color was observed to appear.

**Phenols**

To 1 ml of extract, 2 ml of distilled water were added followed by few drops of 10% ferric chloride (FeCl₃). Appearance of blue or green color indicates presence of phenols.

**Glycosides**

0.5 g of solvent extract was dissolved in 2.0 ml of glacial acetic acid containing one drop of FeCl₃ solution. This was then under laid with 1.0 ml of concentrated H₂SO₄. A brown ring obtained at the interface indicated the presence of glycosides.

**Tannins (Ferric chloride reagent)**

0.5 ml of the extract was boiled with 10 ml of distilled in a test tube. Thereafter, few drops of 0.1% Ferric chloride solution was added and then the reaction mixture was observed for blue or greenish black color change.

**Saponins (Frothing test)**

0.5 ml of the extract was added to 5 ml of distilled water in test tube. The solution was shaken vigorously and observed for the stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**Flavonoids**

- 1 ml extract was added to 1 ml of 10% lead acetate, it was gently shaken. A muddy brownish precipitate indicates the presence of flavonoids.
- 1 ml extract was added to 10% FeCl₃. The mixture was shaken. A wooly brownish precipitate indicates the presence of flavonoids.

**Terpenoids/ steroids (Salkowski test)**

To 0.5 ml of each extract, 2 ml of chloroform was added and then 3 ml of the concentrated H₂SO₄ was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids/steroids.

**RESULTS AND DISCUSSION**

The screening results of the antimicrobial activity of *H. sudanicum* are shown in Table 1 and Figure 1. The *in vitro* anti-parasite activities of *H. sudanicum* crude extracts against *Giardia lamblia* are shown in Table 2. While Figure 2 shows the mortality percent of *G. lamblia* trophozoites exposed to different concentrations of the ethanolic extract of *H. sudanicum*.

**Phytochemical screening test for the studied plant**

Phytochemical screening indicated that the ethanolic extract of *H. sudanicum* abundantly contain alkaloids, Phenols, Tannins, Saponins, flavonoids, and terpenoids compounds (Table 3).
Table 1: Screening antimicrobial activity of *Heliotropium sudanicum*.

<table>
<thead>
<tr>
<th>Plants extract</th>
<th>Conc. (%)</th>
<th>Test organisms used</th>
<th>Inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heliotropium sudanicum</em></td>
<td>Ethanol</td>
<td>S.a</td>
<td>P.s</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>-</td>
<td>12.5</td>
</tr>
</tbody>
</table>

*S.a* = *Staphylococcus aureus*; *Ps* = *Pseudomonas aeruginosa*; *E.c* = *Escherichia coli*; *klebs.* = *klebsella*; *cand.a* = *candida albicans*

M. D. I. Z mm = Mean diameter of growth inhibition zones, in mm.

- = No inhibition zones.

![Figure 1: Antimicrobial activity of *Heliotropium sudanicum* extract.](image)

Table 2: *In vitro* anti-parasite activities of *Heliotropium sudanicum* crude extracts against *Giardia lamblia*.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Time</th>
<th>Treatment and concentrations used</th>
<th>Positive control</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heliotropium sudanicum</em></td>
<td>24 h</td>
<td>100% 34 26 14 4 47</td>
<td>312.5 µg/ml</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>79 53 39 18 98</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>97 95 53 53 100</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

CONCLUSION

The present study clearly highlighted and evidenced that the ethanolic extracts from *H. sudanicum* under study exhibited remarkable antimicrobial, anti-parasite activities against the tested organisms. These observations should provide a way to further research on the ethnobotanical uses of this plant for the treatment of some pathogenic human organisms, particularly in the case of infections with emerging resistance. These results supported the claimed use of this plant in ethnomedicine for treatment of some diseases.

ACKNOWLEDGEMENTS

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Figure 2: Mortality percent of *G. lamblia* trophozoites exposed to different concentrations of the ethanolic extract of *Heliotropium sudanicum* at different times of exposure in comparison with both negative and positive controls *in vitro*.

Table 3: The result of the phytochemical screening test for *Heliotropium sudanicum*.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Test</th>
<th>Reagents</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaloids</td>
<td>Wagner's reagent</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s reagent</td>
<td>+ve</td>
</tr>
<tr>
<td><em>Heliotropium sudanicum</em></td>
<td>Phenols</td>
<td>10% FeCl₃</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Glycosides</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>0.1% Ferric chloride</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Saponins</td>
<td>Frothing test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; reagent</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; reagent</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Terpenoids/ Steroids</td>
<td>Salkowski test</td>
<td>+ve</td>
</tr>
</tbody>
</table>

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REFERENCES


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