EVALUATION OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF SOME SUDANESE MEDICINAL PLANTS

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ABSTRACT

The aims of this study to evaluate the phytochemical constituents and antimicrobial activities of some Sudanese medicinal plants (Combretum hartmannianum leaves (Habiel), Hydnora abyssinica rhizome (Tartous), Sesbania leptocarpa leaves (Surib) and Ficus vasta leaves (Gom'aiz)). Preliminary phytochemical screening of different extracts of the plants is carried out by conventional chemical tests using precipitation and color test. In vitro antimicrobial activity is assessed by using cup-plate agar diffusion method. The preliminary phytochemical screening exhibit the absence of saponins, and sterols from the ethanolic extract of C. hartmannianum leaves. H. abyssinica rhizomes extract show the presence of tannins, cardiac glycosides, flavonoids and reducing sugars. Ficus vasta extract shows absence of tannins, sterols and anthraquinones glycosides. Sesbania leptocarpa leaves extract show absence of alkaloids, cardiac glycosides and flavonoids glycosides. All the screened plants (concentration 100 mg/ml) exhibit positive effects against tested bacterial strains except Sesbania leptocarpa has negative effects against Staphylococcus aureus bacteria. The most prominent activity against tested bacteria is showed by H. abyssinica and Ficus vasta ethanolic extracts. Sesbania leptocarpa (100mg/ml) has no antifungal activity against the tested fungal strains. H. abyssinica, C. hartamannianum, and Ficus vasta show promising antifungal activity. The tested plants have promising antibacterial and antifungal activities, which require further phytochemical studies to assess its active constituents responsible for those biological actions.

KEYWORDS: Sudanese medicinal plants, Antimicrobial activity, Phytochemical screening.
INTRODUCTION

Sudan is rich with medicinal plants, which contain biologically active compounds but not thoroughly evaluated. A number of naturally occurring compounds (such as alkaloids, tannins and flavonoids) have been shown to possess antimicrobial activities against many pathogens. [1] Synthetic antibiotics are considered as core base for therapy of bacterial infections. Though, misuse of antibiotic has become the major factor for the emergence of multi-drug resistant strains of several microorganisms. [1] Antimicrobial agents obtained from plants are considered as best alternative to synthetic ones. [1] Researchers focus their attention on plants as a source of new leads to develop better drugs against resistant -microbial strains. [1] Hydnora abyssinica (Fam. Hydnoraceae) known locally as ‘Altartous’ is a parasitic, fungus-like plant with a fleshy subterranean rhizome. [2] It is widely distributed in Sudan. [2] It contains large percentage of tannins. [2] In Sudan, the plant is found effective against severe infectious diarrhea. [2]

Table 1. Plants involved in the study.

<table>
<thead>
<tr>
<th>No.</th>
<th>Latin name /Family</th>
<th>Local name</th>
<th>Solvent used</th>
<th>Plant part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Hydnora abyssinica</em> Fam. Hydnoraceae</td>
<td>Tartous</td>
<td>Ethanol</td>
<td>Rhizome</td>
</tr>
<tr>
<td>2</td>
<td><em>Sesbania leptocarpa</em> Fam. Leguminosae</td>
<td>Surib</td>
<td>Ethanol</td>
<td>Seeds</td>
</tr>
<tr>
<td>3</td>
<td><em>Combretum hartmannianum</em> Fam. Combretaceae</td>
<td>Habel</td>
<td>Ethanol</td>
<td>Leaves</td>
</tr>
<tr>
<td>4</td>
<td><em>Ficus vasta</em> Fam. Moraceae</td>
<td>Gom’aiz, Shagar eltartar</td>
<td>Ethanol</td>
<td>Leaves</td>
</tr>
</tbody>
</table>

Surib (*Sesbania leptocarpa*) of the family Leguminosae is a wild plant widely spread in Gezira scheme in Sudan. Surib seeds sometimes unavoidably mix with machinery-harvested crops especially wheat. The phytochemical screening of the seeds shows the presence of tannins, saponins, alkaloids, terpenes flavonoids and anthraquinone glycosides. [3]

The leaves of Habeel (*Combretum hartmannianum*) of the family Combretaceae were used as an antipyretic, diuretic and for various diseases such as yellow fever and hepatic disorder. [4] Plants belonging to the family Combretaceae have been reported to have antiparasitic activities and contain triterpenes, stilbenes, and methoxylated flavonoids. [4]
Gom’aiz, *(Ficus vasta)* of the family Moraceae was used traditionally in rheumatism, pains, and intestinal worms.\(^5\) The plant was approved to have anti-anthelmintic, antimicrobial and cytotoxic activity.\(^5\) The poultice of burned leaves and barks were used as anti-tumor.\(^6\)

**Objectives**

To determine the phytoconstituents and antimicrobial activities of *Hydnora abyssinica* rhizomes, *Sesbania leptocarpa* seeds, *Combretum hartmannianum* leaves, and *Ficus vasta* leaves.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

- Acetic anhydride GP, Bios Europe-UK
- Ammonia solution LR, Loba cheme, India
- Aluminum chloride GPR, Loba chemie, India
- Benzene LR, Loba chemie, India
- Bismuth subnitrate, Breckland scientific supplier, UK
- Dimethyl sulfoxide (DMSO), Sigma-Alorich, China
- Chloroform LR, Loba chemie, India
- Copper (II) sulphate LR, Breckland scientific supplies, UK
- Ethanol GPR, Bios Europe-UK
- Ferric chloride anhydrous LR, Labtech, chemicals
- Glacial acetic acid LR, CDH, India
- Hydrochloric acid, Loba chemie, India
- Hexane LR, Loba chemie, India
- Iso-amyl alcohol LR, CDH, India
- Mercuric chloride, Hopkin and William, England
- Magnesium ribbon, Hopkin and William, England
- Methanol GPR, Bios Europe-UK
- 1-naphthol LR, S.d.fine-chem, Ltd, India
- Petroleum ether LR, Loba chemie, India
- Potassium iodide LR, CDH, India
- Potassium sodium(+) tartarte LR (Rochelle salt), CDH, India
- Sulphuric acid LR, Loba chime, India
- Sodium hydroxide, CDH, India

**Plants Samples Collection**

*Combretum hartmannianum* leaves, *Hydnora abyssinica* rhizome, *Sesbania leptocarpa* Leaves and *Ficus vasta* leaves are collected from White Nile state in Sudan during January 2013. They are authenticated in Medicinal and Aromatic Plants Research Institute (MAPRI) and the voucher specimen are deposited in the herbarium.

**Extraction of Plant Material**

Thirty grams from the required powdered plant’s parts are separately macerated in 300 ml of ethanol, methanol, chloroform and distilled water for 48 hours in a conical flask at room temperature with intermittent shaking then each extract was filtered, concentrated (20% solution) and kept in a refrigerator until use.
Preliminary phytochemical screening of different extracts of the plants

As described by Mosa et al., (2013) the following tests are carried out.

**Test for Tannins**

To 2 ml water extract of all plant parts, 2 ml of 10% ferric chloride solution was added in a test tube. Blue-black precipitate indicates the presence of tannins.

**Test for Alkaloids**

To 2 ml methanolic extract of all plant parts, 1 ml of 1% hydrochloric acid was added in a test tube, and heated in a water bath for 10 minutes. 1 ml from each solution was taken and 6 drops of Dragendorff’s reagent / Wagner’s reagent / Mayer’s reagent were added and mixed separately. Appearance of Orange precipitate, brownish-red precipitate and/or creamish precipitate respectively indicates the presence of alkaloids.

**Test for Saponins**

To 0.5 ml methanolic extract of all plant parts, 5 ml distilled water was added in a test tube and vigorously shaken. Persistent froth volume produced, checked each 10 minutes for 30 minutes, and indicates the presence of saponins.

**Test for Cardiac Glycosides (Keller-Kiliani test)**

To 2 ml methanolic extract of all plant parts, 1 ml glacial acetic acid, 6 drops of 10% ferric chloride solution and 6 drops of concentrated sulphuric acid were added in a test tube. Green-blue color indicates the presence of cardiac glycosides.

**Test for Steroids and Terpenes (Liebermann-Burchard reaction)**

To 2 ml chloroform extract of all plant parts, 2 ml acetic anhydride and few drops concentrated sulphuric acid were added in a test tube. Blue-green ring between layers indicates the presence of steroids and pink-purple ring indicates the presence of terpenes.

**Test for flavonoids:**

a- **Shinoda’s test**

To 2 ml ethanol extract of all plant parts, 0.5 ml concentrated hydrochloric acid and few pellets of magnesium turning were added in a test tube. Pink-tomato red color indicates the presence of flavanoids.

b- To 2 ml ethanol extract of all plant parts, 1 ml of 1% potassium hydroxide solution was added in a test tube. Dark yellow color indicates the presence of flavanoids.
c- To 2 ml ethanol extract of all plant parts, 1 ml of 1% aluminum chloride in methanol was added in a test tube. Yellow color indicates the presence of flavanols, flavanones and/or chalcones.

d- To 2 ml ethanol filtrates all plant parts, 0.5 ml concentrated Hydrochloric acid and few drops of amyl alcohol were added in a test tube and shaken. Red color indicates the presence of flavonoidal glycosides.

**Test for Anthraquinones Glycosides**
To 2.5 g powdered material of all plant parts, 10 ml of 20% sulphuric acid and 2 ml of 2% ferric chloride solution were added in a test tube, boiled on a water bath (refluxed) for 30 minutes, allowed to cool, and filtered. The solution then extracted with 10 ml chloroform in separating funnel. Chloroform layer separated and concentrated to about 4 ml and 2.5 ml of 10 % ammonia solution added. Pink-red color acquired by the alkaline layer indicates the presence of anthraquinone glycosides.

**Test for Carbohydrates (Molisch’s test)**
In this method, to 2 ml ethanol extract of all plant parts, 2 drops of Molisch’ test reagent (α-naphthol in ethanol) was added in a test tube and mixed thoroughly. Gently 5 ml of concentrated Sulphuric acid were added. Purple color at the interface indicates the positive test.

**Test for Reducing Sugars (Fehling’s test)**
In this method, to 2 ml of Fehling’s reagent (copper sulphate/sodium potassium trratrate in water) in an empty test tube, 3 drops ethanol extract of all plant parts were added and boiled on water bath. Green suspension and red precipitate indicates the positive test.

**In vitro Antibacterial Activity**
The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modification to assess the antibacterial activity of the prepared extracts. Four wells (10 mm in diameter) were made in each plate. Alternate cups were filled with 0.1 ml sample of each extracts using automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 18 hours. Each extract was tested against organisms in three replicates. After incubation the diameters of the clear zones of growth inhibition were measured.
**In vitro Antifungal Activity**

The same method as for antibacterial activity was adopted. Instead of Muller Hinton agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25\(^\circ\)C for two days for *Candida albicans* and three days for *Aspergillus niger*.

**RESULTS AND DISCUSSION**

As shown in Table 2, the preliminary phytochemical screening exhibit the absence of saponins, and sterols from the ethanolic extract of *C. hartamannianum* leaves. *H. abyssinica* rhizomes extract show the presence of tannins, cardiac glycosides, flavonoids and reducing sugars. *Ficus vasta* extract shows absence of tannins, sterols and anthraquinones glycosides, and this result similar to that obtained by Khaled and Lucy (2013) for the methanolic extract of aerial parts of *Ficus vasta*.  

*Sesbania leptocarpa* leaves extract show absence of alkaloids, cardiac glycosides and flavonoids glycosides.

**Table 2. Preliminary phytochemical screening of the screened plants.**

<table>
<thead>
<tr>
<th>Test</th>
<th><em>Combretum hartamannianum</em> (leaves)</th>
<th><em>Hydnora abyssinica</em> (rhizomes)</th>
<th><em>Ficus vasta</em> (leaves)</th>
<th><em>Sesbania leptocarpa</em> (leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mayer’s reagent</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Wagner’s reagent</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Dragendorff’s reagent</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shinoda’s test</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Amyl alcohol</em> (Flavonoids glycosides)*</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>KOH</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

As shown in Table 3, all the screened plants (concentration 100 mg/ml) show positive effects against tested bacterial strains except *Sesbania leptocarpa* has negative effects against *Staphylococcus aureus* bacteria. The most prominent activity against tested bacteria is showed by *H. abyssinica* and *Ficus vasta* ethanolic extracts. The antibacterial activities
obtained for *Ficus vasta* leaves are similar to that results done by Al-Fatimi *et al.*, (2007) for methanolic extract of fruits of the same plant except the antibacterial activity against *E. coli* is opposite. [9] The *H. abyssinica* ethanolic extract exhibit higher antibacterial activity when compared to the result obtained by Mohamed *et al.*, (2005) for the water extract of the same plant. [10] This may be due to use of ethanol as solvent in this study.

Table 3. *In vitro* antibacterial activity of the screened plants.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Antibacterial activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracts</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><em>Sesbania leptocarpa</em> (100mg/ml)</td>
<td>13±1.00</td>
</tr>
<tr>
<td><em>Hydnora abyssinica</em> (100mg/ml)</td>
<td>19.5±0.71</td>
</tr>
<tr>
<td><em>Combretum hartmannianum</em> (100mg/ml)</td>
<td>17.5±0.71</td>
</tr>
<tr>
<td><em>Ficus vasta</em> (100mg/ml)</td>
<td>17.5±0.71</td>
</tr>
</tbody>
</table>

As shown in Table 4, *Sesbania leptocarpa* (100mg/ml) has no antifungal activity against the tested fungal strains. *H. abyssinica, C. hartamannianum, and Ficus vasta* show promising antifungal activity. The *H. abyssinica* ethanolic extract show higher antifungal activity when compared to the result obtained by Mohamed *et al.*, 2005 [10] for the water extract of the same plant. The positive antibacterial and antifungal activities of the extracts of these tested plants may be directly related to the presence of terpenes, flavonoids and tannins.

Table 4. *In vitro* antifungal activity of the screened plants.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracts</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td><em>Sesbania leptocarpa</em> (100mg/ml)</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Hydnora abyssinica</em> (100mg/ml)</td>
<td>23.5±0.71</td>
</tr>
<tr>
<td><em>Combretum hartmannianum</em> (100mg/ml)</td>
<td>17.5±0.71</td>
</tr>
<tr>
<td><em>Ficus vasta</em> (100mg/ml)</td>
<td>19.5±0.71</td>
</tr>
</tbody>
</table>

CONCLUSION

Due to the presence of phenolic compounds, terpenes and alkaloids, these tested plants have promising antibacterial and antifungal activities, which require further phytochemical studies to assess its active constituents responsible for those biological actions.

REFERENCES


