General Phytochemical Screening and Antioxidant Activity of Some Sudanese Medicinal Plants

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(Received: July 27, 2014; Accepted: September 20, 2014)

Abstract— Medicinal plants are widely spread in Sudan and their biological and phytochemical properties are not thorough evaluated. The reactive oxygen species (ROS) which are produced in living cells are responsible for pathogenesis of many diseases. Antioxidants obtained from natural resources gained high research interest to face these types of diseases generated by ROS. In this work, the crude extracts of some Sudanese medicinal plants; Combretum hartmannianum (leaves), Hydnora abyssinica (rhizome), Striga hermonthica (whole plant), Ficus vasta (leaves), Guiera senegalensis (leaves) were screened to determine their active chemical constituents using conventional chemical tests (precipitation and color reagents) where applicable, while possible antioxidant activities were determined using the DPPH radical scavenging method. All the tested plants showed the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and sterols. The plants revealed promising antioxidant activity, and require further studies to throw light on their chemical composition and antioxidant properties.

Index Terms — Sudanese medicinal plants, Radical scavenging activity (RSA), Preliminary Phytochemical Screening.

I. INTRODUCTION

The reactive oxygen species (ROS) such as oxygen ions and peroxides are produced in living cells in response to inflammation, ultra violet (UV) and gamma radiation and metabolism of xenobiots by cytochrome P450. ROS are responsible for pathogenesis of many diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes mellitus and cancer [1]. Antioxidants obtained from natural resources gained high research interest to face these types of diseases generated by ROS [1].

Combretum hartmannianum, family Combretaceae, known locally in Sudan as Habeel. Leaves are used as an antipyretic, diuretic and for various diseases such as yellow fever and hepatic disorder [2]. The methanolic extracts of different parts of Combretum hartmannianum possess significant activity against the chloroquine-sensitive Plasmodium falciparum strain. Antiparasitic activities have been reported for plants belonging to the family Combretaceae [2]. In addition to this, different Combretum species were reported to contain triterpenes, stilbenes, and methoxylated flavonoids. Guiera senegalensis family Combretaceae known locally in Sudan as Ghibaish, leaves were used for leprosy prevention, the root decoction is used for diarrhea and dysentery [2]. In Ghana, the leaves of Guiera senegalensis were used to treat dysentery, diarrhea, gastro-intestinal pains and disorders, rheumatism and fever [3]. Guiera senegalensis was reported to have small prostaglandin inhibitory activity [3]. The methanolic extract of the dried leaves contain flavonol aglycones beside flavonol glycosides. Hydnora species are subterranean root holoparasites that used in traditional medicine of Sudan and other African countries to treat diarrhea, piles, acne, menstrual problems, stomach cramps and to stop bleeding [4]. H. abyssinica aqueous extract was reported to have intestinal relaxant effect and antimicrobial action [4]. This work also establishes the safety of aqueous extract of the plant rhizome [5]. Striga hermonthica (Scrophulariaceae) was known in Sudan as Al-buda [7]. It is a semi-parasitic plant that infests the roots of sugar cane (Saccharum officinarum), sorghum (Sorghum bicolor) and millet (Pennisetum americanum). It is used traditionally in treatment of dermatosis, leprosy, jaundice [6]. In Sudan the powdered whole plant is mixed with sesame oil and used against leukoderma [7]. This plant also reported to have an antibacterial, contraceptive, antioxidant and weak antiplasmodial activities [6]. Apigenin and 5-hydroxy-6, 5-dimethoxyflavone-7, 4-O-diglucoside have been previously isolated from the whole plant extract [6]. Ficus vasta (Moraceae) is a fig tree of dry North Africa including Sudan. It is used traditionally in rheumatism, pains, and intestinal worms [8]. In Sudan, the plant known as Gom’aiz, and Shagar el-tartar. The poultice of burned leaves and barks were used as anti-tumor [7]. This plant was approved to have anti-anthelmintic, antimicrobial and cytotoxic activity [8]. Plants containing terpenes, flavonoids and other phenolic compounds have been reported to have antioxidant properties [6].

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The above mentioned plants are widely spread in Sudan and their biological and phytochemical properties are not through evaluated. In this work, the whole extracts of these plants were screened to determine their active chemical constituents and possible antioxidant activity.

II. MATERIAL AND METHODS

Plants samples collection
Combretum hartmannianum leaves, Hydnora abyssinica rhizome, Striga hermonthica whole plant, Ficus vasta leaves, and Guiera senegalensis leaves were collected from White Nile state in Sudan during January 2013. They were authenticated in Medicinal and Aromatic Plants Research Institute (MAPRI) and the voucher specimen were deposited in the herbarium.

Extraction of plant material
Thirty grams from the required powdered plant’s parts were separately macerated in 300 ml of methanol, chloroform and distilled water for 24 hours in a conical flask at room temperature with intermittent shaking then each extract was filtered, prepared (20% solution) and kept in a refrigerator until use.

Preliminary phytochemical screening of different extracts of the plants
As described by Mosa et al., (2012) [9] 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 flavonoids.
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 flavonoids.
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 tartrate 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.

2.2, Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH) radical scavenging assay
The DPPH radical scavenging was determined according to the method of Shimada et al. (1992) [10], with some modifications. In 96-wells plate, the test samples were allowed to react with 2.2, Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300 μM). The test samples were dissolved in dimethyl sulfoxide (DMSO) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiple reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicates.
III. RESULT AND DISCUSSION

The healing power of medicinal plants may be due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and sterols. Thus, preliminary phytochemical screening qualitatively estimates the presence of pharmacologically active chemical compounds [11] as shown in Table 1.

Table 1. Preliminary phytochemical screening of plants

<table>
<thead>
<tr>
<th>Test</th>
<th>Combreteum hartamannianum (leaves)</th>
<th>Hydnora abyssinica (rhizome)</th>
<th>Striga hermonthica (whole plant)</th>
<th>Ficus vasta (leaves)</th>
<th>Guiera senegalensis (leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayer’s reagent</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Wagner’s reagent</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dragendorff’s reagent</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shinoda’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amyl alcohol (Flavonoids glycosides)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KOH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

C. hartamannianum leaves extract exhibits absence of sterols and saponins. Hydnora abyssinica rhizomes extract showed presence of tannins, cardiac glycosides, terpenes, flavanoids, flavonoidal glycosides, and reducing sugars. Striga hermonthica whole plant extract reveals presence of tannins, alkaloids, cardiac glycosides, sterols, flavanoids, carbohydrates and reducing sugars. To some extent these results are consistent with the study carried out by Koua et al., (2011) [11], although in this study the methanolic extract of Striga hermonthica whole plant showed absence of saponins. Ficus vasta leaves extract showed absence of tannins, sterols, flavanoids glycosides and anthraquinone glycosides. Guiera senegalensis leaves extract showed the presence of tannins, saponins, terpenes, flavonoids and carbohydrates. Although Sterols and triterpenes are commonly distributed in plants [12], in this study they appear only in Striga hermonthica leaves extract. As presented in Table 2, the ethanolic extracts of all studied plants elicit adequate radical scavenging activity (RSA). The ethanolic extract of Guiera senegalensis leaves reveal the highest RSA % (90%), while Striga hermonthica whole plant extract showed the lowest one (29%). The iron chelating effect of the ethanolic extract of the all plants is insignificant. The high RSA percentage exhibited by the plants may be due to the presence of phenolic compounds (tannins, flavonoids, and anthraquinone glycosides) and terpenes [1].

Table 2. Free radical scavenging activity (RSA) & iron chelating effects

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>%RSA ± SD</th>
<th>%Iron chelating ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guiera senegalensis (leaves)</td>
<td>90 ± 0.014</td>
<td>05 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>Hydnora abyssinica (rhizome)</td>
<td>77 ± 0.01</td>
<td>02 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>Combreteum hartamannianum (leaves)</td>
<td>86 ± 0.03</td>
<td>11 ± 011</td>
</tr>
<tr>
<td>4</td>
<td>Ficus vasta (leaves)</td>
<td>88 ± 0.02</td>
<td>03 ± 0.11</td>
</tr>
<tr>
<td>5</td>
<td>Striga hermonthica (whole plant)</td>
<td>29 ± 0.06</td>
<td>23 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>PG/EDTA</td>
<td>95 ± 0.04</td>
<td>96 ± 0.02</td>
</tr>
</tbody>
</table>

IV. CONCLUSION

We conclude and recommend that, the studied plants reveal promising antioxidant activity that require further studies to make them useful for treatment of many diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes mellitus and cancer disease.

REFERENCES

hartmannianum and Guiera senegalensis on the oxidative stability of sunflower oil. Emirates Journal of Food and Agriculture sciences. 18(2):20-28


