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Phytochemical screening, antioxidant and antimicrobial activities of some Sudanese medicinal plants against standard and isolated microorganisms

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Abstract

The three important medicinal plants; *Aristolochia bracteolata*, *Citrullus colocynthis* and *Salvia officinalis* are well known for their use in Sudanese traditional medicine. They are used for the treatment of bacterial and fungal infections mainly that of skin infections. The objective of this study is to screen the ethanolic extracts of these plants, for the presence of different secondary metabolites; and the investigation and comparison of their antioxidant activity and antimicrobial activity against the standard microorganisms including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* and against the isolated microorganisms causing skin infections *Staphylococcus aureus*. The screening for the secondary metabolites was carried out using the standard methods. The antimicrobial activity of the plants extracts against the standard and isolated microorganisms were investigated using disk diffusion method; and the minimum inhibitory concentrations (MIC) were calculated. Their antioxidant activity was evaluated using DDPH radical scavenging assay. The results show that, all three plants contained cardiac glycosides, terpenoids and sterols and none contained anthraquinones, carbohydrates and compound reducing sugars. The highest antimicrobial activity of the extracts was exhibited with *C. colocynthis* extract against *C. albicans* fungus with inhibition zone of 16 mm. and MIC of 25 mg/ml. The antioxidant activity of *A. bracteolata*, *C. colocynthis*, *S. officinalis* extracts was found to be 48.8 ± 0.04 , 11.1 ± 0.16 , 82.9 ± 0.02 respectively.

Keywords: Phytochemical Screening, Antioxidant, Antimicrobial, Sudanese, Medicinal Plants, Isolated, Microorganisms

1. Introduction

Aristolochia bracteolata, *Citrullus colocynthis* and *Salvia officinalis* are the three important plants used in Sudanese traditional medicine [1-3]. *Aristolochia bracteolata* belongs to the Aristolochiaceae family is an evergreen herbaceous perennial with deciduous woody vines [4]. In Sudan; *A. bracteolata* is traditionally used as an analgesic, anti-scorpion, and anti-snake. It is also used in the treatment of tumors, malaria and for fevers [1]. The whole plant was used as a purgative, anti-pyretic and anti-inflammatory. It also possesses a potent anti-allergic activity and has pronounced antibacterial and antifungal activities as well as antioxidant activity [4, 5]. It was reported to contain alkaloids, triterpenoids, steroids and sterols, flavonoids, tannins, phenolic compounds and cardiac glycosides [4].

Citrullus colocynthis (Cucurbitaceae) is a perennial, monoecious, spreading, roughly hispid creeping herb with simple tendrils and pinnate leaves. Flowers are yellow, ovary sparsely hispid and fruits are globose, yellow and smooth with a bitter taste [6]. It is a valuable cucurbit plant, widely distributed in the desert areas of the world. Its fruits are usually recognized for its wide range of medicinal uses as well as pharmaceutical and nutraceutical potential [2]. The plant has been reported to possess a wide range of traditional medicinal uses including in diabetes, leprosy, common cold, cough, asthma, bronchitis, jaundice, joint pain, cancer, toothache, wound, mastitis, and in gastrointestinal disorders such as indigestion, constipation, dysentery, gastroenteritis, colic pain and different microbial infections [2]. The plant contains alkaloids, glycosides, terpenoids, tannins, anthraquinone and carbohydrates [7]. It is also found to contain curcubitacins A, B, C, D, E, I, J, K, and L and Colocynthosides A, and B as well as saponins, cardiac glycosides and sterols [2, 8].

Salvia officinalis is a member of the Lamiaceae family popularly known as salvia or sage, is an aromatic plant widely distributed in the world. Common sage, since ancient times, has been an ingredient in perfumes, a flavoring in a variety of food preparations, and a medicinal plant used in the healthy Mediterranean diet [3, 9]. It has been traditionally used for the pain relief, protecting the body against oxidative stress, free radical damages, angiogenesis, inflammation, bacterial and virus infection, etc. Sage tea has been traditionally used for the treatment of

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digestive and circulation disturbances, bronchitis, cough, asthma, angina, mouth and throat inflammations, depression, excessive sweating, skin diseases, and many other diseases [3]. Sage is stated to possess carminative, antispasmodic, antiseptic, antioxidant and astringent properties [3]. The predominant medicinally valuable metabolites are terpenes, cardiac glycosides, bitter principles, tannins, sterols and phenolic compounds e.g rosmarinic acid [10]. Recent studies have identified flavonoids and phenolic glycosides isolated from the plant [11].

2. Materials and Methods

1. Plant Material Collection and Preparation

The plant materials were purchased from the herbal store (Atarat Al-Tyman) market at Omdurman, Sudan in October 2015. They are authenticated by taxonomist at the National Centre for Research/ Sudan. The voucher specimens were deposited at Pharmacognosy Department, Faculty of pharmacy, University of Medical Science and Technology (UMST). The collected specimens were preserved for further laboratory investigation.

The extracts were prepared by 96% ethanol and their chemical constituents, antioxidant and antimicrobial activity were evaluated.

2. Phytochemical Screening Test

The phytochemical constituents of the extracts were detected using standard procedures as described by Trease and Evans [12] and Sofowora [13].

Detection of Flavonoids

Two ml of the extract was mixed with diluted NaOH to produce a yellow discoloration. A change in color from yellow to colorless upon addition of dil. HCl is an indication of the presence of flavonoids.

Detection of Tannins

Two ml of the extract were stirred with about ten ml of distilled water and then filtered. Few drops of 10% ferric chloride solution were added to two ml of the filtrate. Occurrence of a black, black and green, blue – green indicates the presence of tannins.

Detection of Saponins

Two ml of the extract was boiled with five ml of distilled water, filtered; about three ml of distilled water was further added to the filtrate and shaken vigorously for about 50 minutes. Frothing which persisted for about thirty minutes indicates the presence of saponins.

Detection of Cardiac Glycosides

• Keller Killiani test

Two ml solution was evaporated to dryness, the residue was then dissolved in two ml of (3.5%) ferric chloride in glacial acetic acid, transferred to a clean dry test tube, and two ml of conc. H₂SO₄ was poured carefully on the wall of the test tube. Development of reddish brown ring at the junction between the two layers indicates the presence of cardiac glycosides.

Detection of sterols

Two ml of extract was evaporated to dryness, the residue was dissolved in two ml of chloroform and transferred to a clean dry test tube, two ml of acetic anhydride was added followed by addition of conc. H₂SO₄ carefully to the wall of

the tube. Color development from violet to blue or green indicates the presence of the steroidal moiety.

Detection of Alkaloids

Two ml of the extract was acidified with 1% HCl, few drops of Mayer's reagent was added, appearance of turbidity indicates the presence of alkaloids.

Detection of Terpenoids (Salkowski Test)

Two ml of the extract was mixed with two ml of chloroform. Three ml of conc. H₂SO₄ was added carefully to form a layer; formation of a reddish brown color at the interface indicates the presence of terpenoids.

Detection of Carbohydrates

The alcoholic extract was dissolved in five ml of distilled water and filtered. The filtrate was treated with two drops of alcoholic α -naphthol. The formation of a violet ring at the junction indicates the presence of carbohydrates.

Detection of Reducing Sugars

Two ml of the extract was heated with equal volumes of Fehling solution A and B. Appearance of a precipitate indicates the presence of reducing sugars.

Detection of Compound Reducing Sugars

Two ml of the extract was hydrolyzed by boiling with five ml diluted HCl; the resulting solution was neutralized with NaOH solution. A few drops of Fehling solution were added, then it was heated on a water bath for two minutes. Appearance of a reddish-brown precipitate indicates the presence of compound reducing sugars.

3. Antimicrobial Bioassay

Antimicrobial testing was carried out using agar diffusion method with some minor modification [14]. Nutrient agar media was used as a growth medium for the bacterial organisms. Another growth medium composed of sabouraud dextrose powder was prepared for promoting fungal growth. The media was placed in an autoclave using the following conditions 37 °C and 15 lbs pressure for 15 minutes for sterilization. The media (20ml) was then poured in a petri dish under aseptic conditions and (0.2ml) of the intended microorganism was introduced into prepared media. After the media had solidified, two discs containing the prepared extracts were added in each plate.

4. Antioxidant Activity Test

DDPH Radical Scavenging Assay

In the free radical scavenging experiment Shimada *et al.* (1992) [15], 10 μ l from the extracts (5mg/ml) were added to 90 μ l of 300 μ M DPPH solution placed in a 96-well microtiter plate. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. The mixture was incubated in the dark at room temperature for 30 min. After incubation, the absorbance of the remaining DPPH was read against a blank at 517nm using multiplate reader spectrophotometer. Propylgallate was used as the positive control and DMSO as the negative control. All tests and analyses were carried out in triplicate. The inhibition of free-radical DPPH in percent (%) or the capacity to scavenging the DPPH radical (radical scavenging activity) was expressed as EC₅₀ value (mg ml⁻¹).

3. Results and Discussions

Phytochemical screening results of *A. bracteolate*, *C.*

colocynthis and *S. officinalis* are shown in table (1). The results for *A. bracteolata* revealed the presence of alkaloids, tannins, cardiac glycosides, sterols, terpenoids and reducing sugars which was compatible with chemical constituents previously reported in the plant [4]. Results obtained for *C. colocynthis* revealed the presence of flavonoids, saponins, cardiac glycosides, sterols, terpenoids as stated in previous study [2, 7, 8]. *S. officinalis* screening results revealed the presence of tannins, bitter principles, saponins, cardiac glycosides, sterols, terpenoids which complied with chemical of the plant [10, 11]. There are slightly difference shown in the secondary metabolite content and that previously reported in

the plants this could be due to many factors such as genetic factors, habitat, the season of plant collection and the techniques used in evaluation [16]. The secondary metabolites that have antimicrobial activities include flavanoids, tannins, terpenoids and alkaloids [17] are existent in the different three extracts with different percentage. Therefore, based on the chemical constituents present the antimicrobial activities of the three plants extracts are found to be different. According to the difference in the secondary metabolites that had been found in the three extracts the antioxidant activity was also found to be differ.

Table 1: Phytochemical Screening Results of *A. bracteolata*, *C. colocynthis* and *S. officinalis* extracts

Screening Test	<i>A. bracteolata</i>	<i>C. colocynthis</i>	<i>S. officinalis</i>
Flavonoids	Negative	Positive	Negative
Tannins	Positive	Negative	Positive
Saponins	Negative	Positive	Positive
Cardiac Glycosides	Positive	Positive	Positive
Sterols	Positive	Positive	Positive
Alkaloids	Positive	Negative	Negative
Terpenoids	Positive	Positive	Positive
Carbohydrates	Negative	Negative	Negative
Reducing Sugars	Positive	Negative	Negative
Compound Reducing Sugars	Negative	Negative	Negative

The results of antimicrobial activity of the three plants extracts against five standard microorganisms; *B. subtilis*, *P. aeruginosa*, *E. coli*, *S. aureus* bacterial strain and *C. albicans* fungal strain as well *S. aureus* clinical isolate are shown in table (2) and figure (1). The results revealed that *C. colocynthis* had the highest antimicrobial activity against all tested microorganisms with diameter zone of inhibition ranging from 14-16 mm, *A. bracteolata* had a diameter zone of inhibition ranging from 11-12 mm while *S. officinalis* had the weakest activity with diameter zone of inhibition ranging

from 10-11 mm against *B. subtilis*, *S. aureus*, *C. albicans* and was inactive against *E. coli* and *P. aeruginosa*. The antimicrobial activity could be attributed to some of the detected compounds in these plant extracts such as tannins, saponins, alkaloids, flavonoids and terpenoids [17, 18]. The results justifies the traditional use of these plants as antimicrobial agents against the most of these organisms and for skin diseases [19] whereas, the microorganisms such as *S. aureus*, *P. aeruginosa*, *C. albicans* were linked to various skin diseases [20].

Table 2: Antimicrobial Activity Results of *A. bracteolata*, *C. colocynthis* and *S. officinalis* extracts

Plant Extract	Inhibition Zone (mm) of Tested Organisms					
	Standard Organisms					Isolated Organism
	Bacteria				Fungi	<i>S. aureus</i>
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	
<i>A. bracteolata</i>	11	12	12	11	11	11
<i>C. colocynthis</i>	14	14	15	15	16	15
<i>S. officinalis</i>	10	11			11	12

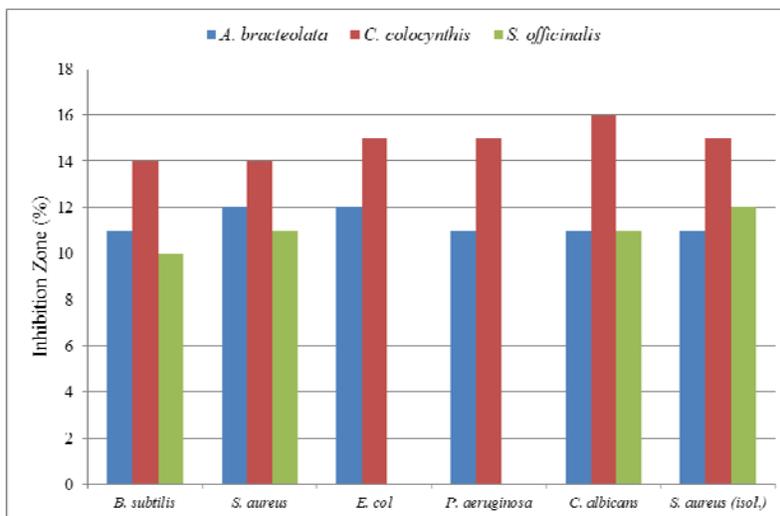


Fig 1: Antimicrobial Activity Results of *A. bracteolata*, *C. colocynthis* and *S. officinalis* extracts

The antioxidant activity results of *A. bracteolata*, *C. colocynthis* and *S. officinalis* extracts are shown in table (3). The results showed that, *S. officinalis* had highest antioxidant activity of 82.9 ± 0.02 in compared to the standard propylgallate of 89.7 ± 0.01 , whereas, *A. bracteolata* showed the moderate activity (48.8 ± 0.04), and the *C. colocynthis* was found to be of the weak antioxidant activity (11.1 ± 0.16). The antioxidant activity may attributed to terpenoid content^[5], as well as phenolic compounds (tannins)^[19] and flavonoids^[16, 19] that are found to be present in the extracts. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides^[5]. This antioxidant activity could be useful in prevention of atherosclerotic and heart diseases.

Table 3: Antioxidant Activity Results of *A. bracteolata*, *C. colocynthis* and *S. officinalis* extracts

Sample	RSA \pm SD (%)
<i>A. bracteolata</i>	48.8 \pm 0.04
<i>C. colocynthis</i>	11.1 \pm 0.16
<i>S. officinalis</i>	82.9 \pm 0.02
Propylgallate	89.7 \pm 0.01

Conclusion

In conclusion, the study has revealed that the ethanolic extracts of *A. bracteolata*, *C. colocynthis* and *S. officinalis* plants possessed both antimicrobial and antioxidant activities with different degree which justified their use as antimicrobial agents in Sudanese traditional medicine.

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