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Invitro Evaluation of Antimicrobial Activity and Phytochemical Investigation of Crude Extract from Selected Sudanese Medicinal Plants

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Abstract

In this study antimicrobial activity of ethanolic extract of *Citrullus colocynthis, Ekebergia capensis* and *Echium rauwolfii* used in Sudanese folk medicine was detected against five standards bacterial strain viz *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 53657), using well-diffusion agar diffusion method. The drude extract of *C. collosynthis* showed good activity against most organisms (25-12) in different concentrations. ethanolic extract of *E. capensis* showed high activity in most microbial (28-11) in different concentrations but no activity against *E. coli* and *K. pneumoniae* in concentration (6.2). The crude extract of *E. rauwolfii* showed good activity against all microorganisms (26-12) in all concentrations used, except in concentration (6.2) there was no activity against *E. coli.* Phytochemical investigation of secondary metabolic compounds such as alkaloids, tannins, glycosides, saponins, turbines, flavonoids, is found in these plants.

Keywords: *Invitro;* Antimicrobial activity; *Citrullus colocynthi; Ekebergia capensis; Echium rauwolfii*

Introduction

Antimicrobial drugs have caused a dramatic change not only of the treatment of infectious diseases but of a fate of mankind [1]. Infections due to variety of bacterial etiologic agents such as pathogenic *Escherichia coli*, *Salmonela spp.*, *Staphylococcus aureus* are most common. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world [2]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases, one approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important resource to combat serious diseases in the world. According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents [3]. *Citrullus colocynthis* (L.) is a member of family *Cucurbitaceae*, is annual or perennial (in wild), herbaceous, bearing monoecious type flowers, pepo fruit and numerous seed. Its fruits are used as robust laxative. *C. colocynthis* fruits are known for pain relieving, cathartic, anti-inflammatory, antioxidative, anti-diabetic effects. *Ekebergia capensis* Sparrm (Meliaceae) is a semi-deciduous or evergreen, large to medium-sized tree growing up to 100cm in diameter and 35m in height. The bole is usually straight or sometimes crooked, branchless for up to 12cm, stem may be swollen, buttressed and fluted in forests or short and unfluted in open woodland [4]. The bark of *E. capensis* is boil in water and use for the control of gonorrhea, tuberculosis as well as diarrhea, the leaves or roots are used as an emetic, vermifuge, abscesses, acne, acute gastritis, boils, chronic cough, dysentery, headache, heartburn and scabies [5]. *Echium rauwolfii* Del. is an erect annual herb with narrow leaves and a 12-15 mm long, white- or flesh-coloured corolla [6]. *E. rauwolfii* seeds contain specialty oil; it has many potentials uses in the pharmaceutical industry for treatment of eczema, acne, and other skin disorders and in the cosmetic and personal care products industry [7]. The present study was conducted to investigate the new antimicrobial and phytochemical screening of three Sudanese medicinal plants (*Citrullus colocynthis*, *Ekebergia capensis*, *Echium rauwolfii*).

Materials and Methods

Plant materials

The aerial part of *Citrullus colocynthis*, *Ekebergia capensis* and *Echium rauwolfii* were collected from Sabalogah waterfall, River Nile State, Sudan between February 2017 and April 2016. The plant was identified and authenticated by the taxonomists of biology department, faculty of pure and applied sciences, International University of Africa. All plant parts were air-dried, under

the shadow with good ventilation and then ground finely in a mill until their uses for extracts preparation.

Preparation of crude extracts

Extractions from aerial part of *Citrullus colocynthis*, *Ekebergia capensis* and *Echium rauwolfii* were carried out by simple maceration process. The plants were taken and ground in ethanol 80%. This mixture was kept for 72 hours at room temperature (25°C) in extraction bottle. After 72 hours, mixture was filtered by Whatman-41 filter paper. Ethanol was then completely evaporated by rotary evaporator to obtain pure extracts.

Test microorganisms

The crude extracts of *C. collosynthis*, *E. capensis* and *E. rauwolfii* was tested against four standard bacteria and one fungi, that is: One Gram positive bacteria *Staphylococcus aureus* (ATCC25923) and three Gram negative bacteria (Pseudomonas aeruginosa (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 70063) and one fungi *Candida albecans* (ATCC 7596) using the cup-plate agar diffusion method. The study was conducted in the microbiology lab, Department of Microbiology, Faculty of Pure and Applied Sciences, International University of Africa, Khartoum, Sudan.

Determination of antimicrobial activity of the extracts by well diffusion method

Agar well diffusion technique as described by Cheesbrough M [8] was used to determine the antimicrobial activity of the extract. An 18ml of Nutrient agar plates that has been checked for sterility were seeded with 100µl of an overnight broth culture of each bacterial isolate in sterile petridish a standard to cut uniform well on the surface of the agar, the well filled with 2ml of each extract were the add a sterile syringe one of the well in each Nutrient agar plate is left unfilled as control. All the plates were incubated at 37 °c for 24 hours and observed zones of inhibition. A significance zone of inhibition has been observed around each well and the diameter of such zone was measured in millimeters.

Phytochemical screening test

The phytochemical constituents of the extracts were detected using standard procedures as described by and Hatil et al. [10].

Detection of alkaloids

Mayer's test: One 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.

Wagner test: One ml of the extract, 2ml of Wagner's was added, and the reaction mixture was observed for the formation of reddish-brown precipitate indicates the presence of alkaloids.

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 glycosides

1ml of solvent extract was dissolved in 2ml of glacial acetate acid containing one drop of $FeCl_3$ solution. This was then under laid with 1.0ml of concentrated H_2So_4. A brown ring obtained at the interface indicated the presence of glycosides.

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 terpendoids (Salkowski test)

Two ml of the extract was mixed with two ml of chloroform. Three ml of conc. H_2So_4 was added carefully to form a layer; formation of a reddish-brown color at the interface indicates the presence of terpenoids.

Detection of flavonoids

Lead acetate test: 1ml extract was added to 1ml of 10% lead acetate. It was gently shaken. A muddy brownish precipitate indicates the presence of flavonoids.

Ferric chloride: 1ml extract was added to 10% $FeCl_3$. The mixture was shaken. A wooly brownish precipitate will indicate the presence of flavonoid.

Results and Discussion

Yields (%) of ethanolic extracts of medicinal plants

The yield% of ethanolic extract of aerial part for *C. collosynthis*, *E. capensis* and *Echium rauwolfii* were 2.5%, 3.8%, and 2.7% respectively Table 1. The ethanolic extracts investigated antimicrobial and phytochemical screening.

Table 1: Yields Percentage of ethanolic extracts of Plants.

Plants	Family	Part Used	Weight of Samples (g)	Yield (%)
C. collosynthis	*Cucurbitaceae*	Aerial parts	200	2.5
E. capensis	*Meliaceae*			3.8
E. rauwolfii	*Boraginaceae*			2.7

Antimicrobial activity

The results of crude extracts of *C. collosynthis*, *E. capensis* and *Echium rauwolfii* (aerial part) exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (28 to 12mm) in length. Ethanolic extracts of plants at different concentrations (100, 50, 25, 12.5 and 6.2) showed high activity for most organisms (Table 2) and (Figure 1-3). Therefore, these results showed that the extracts tested inhibited the growth of all organisms though the sensitivities of microbial varied. The results of antimicrobial tests were interpreted as active (>18 mm), moderately active (14-18mm) and inactive (<14mm) [11]. The study conducted by Marzouk B, et al. [12] revealed that the ethanolic ex-

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tract of roots, leaves and fruits for *C. collosynthis* showed activity against gram-positive bacteria such as (*Bacilus pumilus*, *Staphylococcus aureus*), where the crude extract of the root and fruits were more active for the negative bacterial bacteria such as (*Bacillus subtilis*), while it did not appear effective against Some Gram-negative bacteria (*Escherichia coli*, Pseudomonas aeruginosa). The research concluded that active response against the various strains of bacteria may be because of flavonoid, carbohydrates, tannin and glycosides that are reported. The study conducted by Kimutai N, et al. [5] revealed that the aqueous extract of *E. capensis* is the most active antimicrobial.

Table 2: Antimicrobial activity of different concentration of the plant extracts against standard organisms.

Plant name	Conc./ mg/ml.	MIDZ				
		E. c	S. a	Ps. a	Kl. p	C. a
C. collosynthis	100	25	18	20	18	22
	50	22	15	18	17	20
	25	20	14	16	16	18
	12.5	19	13	15	15	17
	6.2	15	12	13	12	15
E. capensis	100	25	25	27	26	28
	50	20	21	25	21	25
	25	17	20	20	17	20
	12.5	15	15	18	15	17
	6.2	-	11	15	-	15
E. rauwolfii	100	20	25	26	24	25
	50	15	20	22	20	22
	25	13	17	17	19	20
	12.5	12	15	16	17	17
	6.2	-	13	15	15	16

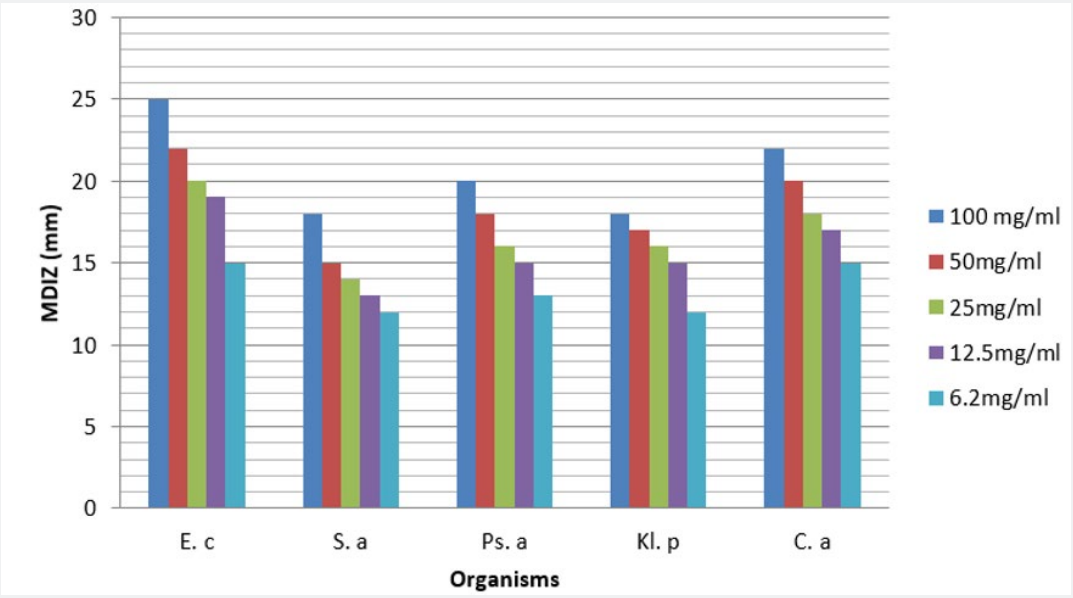

Figure 1: Antimicrobial activity of different concentration of C. collosynthis crude extract against standard organisms.

Phytochemical screening of crude extracts

Phytochemical verification of ethanolic extracts of *C. collosynthis*, *E. capensis* and *E. rauwolfii* for detection of secondary metabolites such as alkaloids, tannins, glycosides, saponins, Terpenoid and flavenoids (Table 3). Alkaloids, terpenoids and flavonoids appeared in all raw extracts of *C. collosynthis*, *E. carpensis* and *E. rauwolfii*. While tannin compounds were seen in *E. carpensis* and *E. rauwolfii*, glycosides compounds appeared in *C. collosynthis* and *E. rauwolfii*, Saponins also appeared in *E. carpensis* only.

Conclusion

The antibacterial activity of the plants under the study (*Citrullus colocynthis*, *Ekebergia capensis* and *Echium rauwolfii*) have

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shown high and low activity the antibacterial screening proved the significance of these plants and the frequent use by the healers as traditional medicines and indicated a good guiding for further research in these plants.

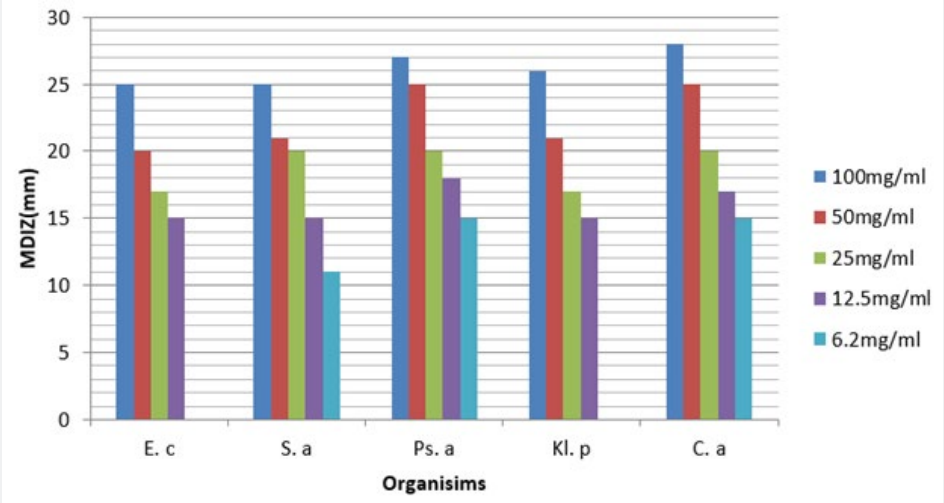

Figure 2: Antimicrobial activity of different concentration of C. collosynthis crude extract against standard organisms.

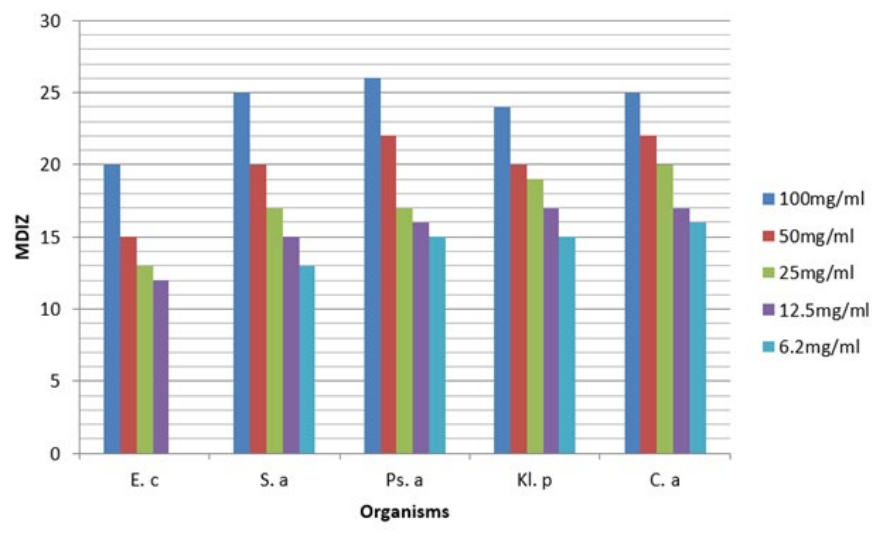

Figure 3: Antimicrobial activity of different concentration of E. rauwolfii crude extract against standard organisms.

Table 3: Phytochemical screening of the Plants extract.

Tested	PLANTS		
	C. collosynthis	*E. carpensis*	*E. rauwolfii*
Alkaloid	+	+	+
-Wagner's test -Mayer's test	+	+	+
Tannins	-	+	+
Glycoside	+	-	+
Saponnins	-	+	-
Terpenoid	+	+	+
Flavonoid	+	+	+
- lead acetate			
- Ferric chloride	+	+	+

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