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Assessment of Phytochemial and Antimicrobial Activity of Secondary Metabolites of Plant *Citrullus Colocynthis* L

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ABSTRACT

Low susceptibility to standard antimicrobial drugs and resistance to antibiotics represent a significant challenge to develop effective treatment. Many natural compounds were found to inhibit bacterial and fungal infection. In this study different solvent extracts was prepared from different parts of *Citrullus colocynthis* plant such as pulp, seeds and leaves. The phytochemical constituents of plant were carried out by using methanol and acetone were found tobe such as alkaloids, glycosides, cucurbitacins, flavanoids, saponin and trace of tannin .The raw extracts of both solvents were evaluated for their antimicrobial properties using agar disc diffusion method. The antimicrobial activity of crude ethanolic and acetone extracts were evaluated against the some human and plant pathogens. The result obtained showed wide range of anti-microbial activity. This study offers new opportunities for development new drugs with minimal side effect and high pharmacological efficiency.

Keywords: *Citrullus colocynthis*, antimicrobial effect, resistance, inhibition zone.

INTRODUCTION

Infectious diseases are still a major threat to public health, and resistant to anti-biotic has increased. The multi-drug resistance to chemicals has now become a global concern and this resistance causing failure of treatment, Sharma *et al.*, (2005); Adwan and Mhanna,(2008) ; Hancock, (2005).

The appearance of new microbial strains with low susceptibility to anti-bacterial and anti-fungal drugs are continuously increasing Mahmoud and . Rice.(1999)

Natural products from plants have been exploiting for use in medicine, cosmetics, and dyes, and it can be derived from any parts of plant as leaves, seeds fruits and flowers, etc.

The secondary metabolites in the plant are very good source of phytochemicals which make plant resistant to various environmental stress and the secondary metabolites display a remarkable pharmacological effect as anti-bacterial, anti-fungal, anti-viral and anti-cancer. Many drugs developed from natural products, and the developing of new agents from natural products is long-term process start with acquisition of phytochemical compounds, screening for pharmacological effects, and evaluation of toxicity (Dours and Suffness, 1980).

Scientific efforts are guided toward natural products, the pharmaceutical industries and research centers have been engaged in screening new effective drugs with minimal side effects and adverse reaction.

Sudan one of the largest country in Africa have a wide variation in climatic zones, the variation of climate in Sudan has impact on diversity of vegetation and it was estimated that Sudan encompasses more than 3156, plant species, 125 genra, and 170 families Brown and Massay, (1929) ; Andrewas,(1950,1952,1956) and Elamin, (1990).

Citrullus colocynthis which belong to well-known plant family *cucurbitacea* originated in tropical and sub-tropical region in Sudan Chomicki *et. al* (2019) .

This plant rich in different phytochemical compounds, claimed to exert different therapeutic effects including anti-bacterial, anti-fungal, anti-cancer, anti-inflammatory (Saweya *et al.*, (1986) ;Marzuk *et al.*, (2010) ; Chewech *et al.*, (2017).

According Chawech *et.al*.(2015) the main phytochemical compounds in this plant such as cucurbitacins, alkaloids, flavoniods, glycosides, spaonin and tannin.

The aim of this study was to asses different solvent extracts from Sudanse variety of plant *Citrullus colocynthis* and evaluate their anti-microbial activities on some bacteria and fungi pathogenes

METHODOLOGY

Plant collection

C. colocynthis plant was collected from Wad Medani Town, Gezira State, Sudan. And authenticated by the National Institute for Promotion of Horticultural Exports, University of Gezira by Prof. Mohamed Taha Yosif.

Preparation of plant material

The parts of plant pulp, seeds and leaves were air dried at room temperature (35 ± 2^0C) for one week then they were ground and kept for further use.

Phytochemical screening

The qualitative analysis was carried out according to standard method, flavonoids glycosides, saponin. Tannin were screened according to methods described by Harbone (1998) whereas cucurbitacins screened by the method of Attarad and Scicluna (2001).

Preparation of extracts

Each of the plant part of *C. colocynthis* such as seeds, pulp and leaves, were ground to fine particles separately and subjected to cold extraction with organic solvent such as methanol and acetone for ten hours with continuous stirring, after that the mixture was filtrated using filter paper (Whatmann number 1). The filtrates were collected and hence the solvent was evaporated by rotary-evaporator at 45^0C this step was repeated three times.

Preparation of stock solution

A stock solution was prepared from each part of plant seeds pulp and leaves with different solvent such as methanol and acetone at concentration of 5 mg/ml and evaluated for antimicrobial activity at dose of 25 µL/disc.

Anti-microbial bioassay was performed according to agar-disc diffusion method where four bacterial strains (*Stapholoccus aureus*, *Samonella typhi*), as Gram negative and (*Klebsiella peneumonia* and *Esherachia coli* as Gram positive. Two yeast strains (*Candia korusi* and *Candida albican*) .In addition , three pathogenic plant fungi strains such as *Fusarium spp., Sphacelotheca sorghi and Phytosphra spp.*were also testeds. Standard antibiotic (Gentemycin 80 mg/ml) and antifungal (Nystatin 200 mg/ml.)
were used as as positive control.

Assessment of antimicrobial activity of extracts

This was carried out as described by Heatley (1944). Once the agar medium (Mueller-Hinton Agar and potato dextrose agar) solidified ,they seeded with the microorganisms to make an even lawn thereafter discs containing 25µL/ from each stock solution of the extract were loaded on surface of solid media and incubated at 37^0C for 48 hours. The activity of different solvent extract was determined by measuring diameter of clear zone around the disc in millimeter in mm. All tests were performed in duplicate with standard drug as positive control.

RESULTS

Phytochemical analysis

Phytochemical analysis was carried out to screen the constituents of plant *C. colocynthis* according to method of analysis.

The tests were conducted to screen specific parts of plant *C. colocynthis* (pulp, seeds and leaves). The results showed high presence of alkaloids, flavonids, cucurbitacins, glycosides and saponin in all tested samples.Moderate presence of saponin and trace or absent of tannin in some tested samples .Results indicated

Table 1. Qualitative analysis different part of *C.Colocynthis*

Plant part	Solvents	Alkloids	Flavanoids	Cucurbitacins	Glycosides	Saponins	Tannins
Pulp	Methanol	+++	++	+++	+++	+++	±
	Acetone	+++	+++	+++	+++	+++	±
Seeds	Methanol	+++	++	+++	+++	++	±
	Acetone	+++	++	+++	++	++	-
Leaves	Methanol	++	++	++	++	+	-
	Acetone	+	+	+	++	±	-

some variation in amount of phytochemical compound in different solvents and it could be attributed to solubility in solvents. The more bioactive compounds were found in pulp and seeds tested samples of *C. colocynthis* (Table 1).

Table 2. Antibacterial effect of different solvent extract from different parts of *C.colocynthis*

Organ of plant	Solvents	*S.paratyhphi*	*Staphylococus auerus*	*Klebsiella pneumonia*	*E.coli*
Pulp	Methanol	12 mm	0 mm	18 mm	15 mm
	Acetone	9 mm	0 mm	0 mm	0 mm
Seeds	Methanol	9 mm	0 mm	6 mm	0 mm
	Acetone	0 mm	23 mm	0 mm	0 mm
Leaves	Methanol	11mm	0 mm	10 mm	11 mm
	Acetone	13 mm	0 mm	0 mm	0 mm
Ref. drugs	Gentamicyin 80 mg/ml	20 mm	24 mm	20 mm	20 mm

Assessment of antibacterial activity

Four bacterial strains were tested against different solvents of different parts of *C. colocynthis* two strains were gram negative and the other two strains were positive.

Results demonstrated that the maximum inhibition zone was recoeded for acetone seeds extract comparable to control positive. The methanolic extract of pulp showed higher inhibition for than other parts as 12, 15, and 18 mm for ***S.paratyhphi***, ***E.coli*** and ***Klebsiella pneumonia*** respectively, these finding were higher than that obtained by leaves and seeds that were extracted by methanol, noticeably aecton extract of seed gave 23 mm diameter which was very similar to that gave by gentamice .

From the results was found the crude extract from pulp seeds and leaves in different solvent were more potent against tested pathogen. The results revealed the methanolic and acetone extracts from plant *C. colocynthis* have potent effects against gram negative and gram positive bacterial strains similar result observed by Phate *et al.* (2011) (Table 2) showed inhibition zone in millimeter (mm) .

Assessment of fungal activity

The results demonstrated large size of inhibition zone for crude methanolic extract from pulp against candida strains, whereas less activity was observed for extract from seeds against *candida* strains relative to positive control. It was observed that extract from leaves did not show any fungicidal activity against candida strains (Table 3) showed inhibition zone in millimeter (mm).

Table 3. Antifungal effect of different solvent extract from different parts of *C.colocynthis*

		Candida krusei	***Candida albicans***
Pulp	Methanol	17mm	22mm
	Acetone	8mm	0mm
Seeds	Methanol	6mm	0mm
	Acetone	0mm	0mm
Leaves	Methanol	0mm	0mm
	Acetone	0mm	0mm
Ref. drugs	Nystatin 200 mg/ml	20mm	24mm

Assessment of plant pathogenic fungi

The present study was conducted against three selected strains of plant fungi and the result showed that only methanolic extract from seeds gave positive results against *Sphacelotheca sorphi*, whereas other extracts from different parts of *C. colocynthis* did not show any activity against plant pathogen (Table 4)

Table 4. Antifungal plant pathogen effect of different solvent extract from different parts of *C.colocynthis*

Plant part	***Solvents***	***Phytosphra spp***	***Sphacelotheca sorghi***	***Fusarium spp***
Pulp	Methanol	0 mm	0 mm	0 mm
	Acetone	0 mm	0 mm	0 mm
Seeds	Methanol	0 mm	16 mm	0 mm
	Acetone	0 mm	0 mm	0 mm
Leaves	Methanol	0 mm	0 mm	0 mm
	Acetone	0 mm	0 mm	0 mm
Control	Nystatin 200 mg/ml	18 mm	20 mm	18 mm

DISCUSSION

Different solvents extract from vartious parts of plant *Citrullus colocynthis* were examined for their antimicrobial activity against selected bacteria and fungi species relative to positive control.

The outcomes in this study propose the potent activity of the extracts against selected pathogen. And the study showed the different in the results of acting variety microorganism and variation of results could be due to phytochemical solubility in solvents which can inhibit enzymatic effect in the cytoplasm of microorganism membrane (Greulach, 1973).

The different in results from different studies may be due to type of solvent which has play a significant role in the process of extraction reported (Bakhit 2011), (Al-zabiydi *et al.* 2009). The results demonstrated that plant *C. colocynthis* containing different phytochemical constituents such as cucurbitacins which could be act as antibacterial and antifungal at the same time (Elawad, 1981).

The results revealed the presence of different phytochemical compounds in the most solvent extracts and the study proposed the combination of all these phytochemical compounds has synergistic pharmacological effect against different tested samples.

CONCLUSION

From the results obtained it could be concluded that extracts from different parts of plant *C. colocynthis* with different solvents displayed wide range of pharmacological effect against all selected microorganism and may be act as a good potent antimicrobial and it can be drug or precursors of drug. Furthermore studies need to determine the pharmacological effect of phytochemical compounds and their toxicity. This study offer new opportunities for development new effective antimicrobial treatment from natural compounds with minimal side effect.

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