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ANTIBACTERIAL SCREENING OF *CITRULLUS COLOCYNTHIS*

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ABSTRACT

Crude ethanolic extracts of fruits, leaves, stems and roots of *Citrulus colocynthis* Schrad were examined for their antibacterial potentialities against Gram positive and Gram negative bacilli. Ethanolic extracts of fruits, leaves, stems and roots were found to be active against Gram positive bacilli, viz., *Bacilus pumilus* and *Staphylococcus aureus,* while fruit and root extracts in double strength gave positive results against Gram positive bacillus *(Bacillus subtilis)*. The Gram negative bacilli viz., *Escherichia coli* and *Pseudomonas aeruginosa* showed no response.

INTRODUCTION

Citrullus colocynthis belongs to the family Cucurbitaceae. Members of this family are generally dioecious herbs which may be prostate or climbing by means of tenderils. Fruit is fleshy and many fruits are used as vegetable or as edible fruits. *Citrullus colocynthis* is a small scarbid perennial creeping herb with prostate or climbing Stem, bearing smooth spherical fruits which are mottled green when young and some what yellow when ripe (Shah & Qadry, 1985). Colocynth was well known to Greeks and Romans, both Dioskurides and Pliny being familiar with it. The drug was equally known to the Arabian Physicians and was produced in Cyprus and Spain during the ages. It is mentioned in Anglosaxon herbal of eleven century (Trease, 1976). It is native of warmer parts of Asia, Syria, Egypt and Martine region of the Mediterranean. It is cultivated to some extent in Spain, Sicily and Morocco for purpose of export. It occurs through the sub-continent and is seen growing wild in the warm and arid sandy tract of northwest, central and south India and on the sea shore of coromandal coast (Anonymous, 1970).

In moderate doses a drastic hydrogogue, cathartic and diuretic; in large doses emetic and gastro-intestinal irritant; in small doses it is expectorant and alterative. Physicians use this drug extensively as a drastic purgative in ascites and jaundice and in various uterine conditions, especially in amenhorroea. Colocynth in the form of the solid extract enters in to many of the purgative pills of modern pharmacy. It is useful in biliousness, fever, intestinal parasites, constipation, hepatic and abdominal, visceral and cerebral congestions, dropsy, etc. Juice of the fruit mixed with sugar is a house-hold remedy in dropsy (Anonymous, 1970).

Root is useful in jaundice, in jaundice the root is given with Gur, ascites, urinary diseases, reheumatism, etc. In reheumatism 180 grams of a mixture of equal parts of the roots, long pepper and gur are taken daily. Root is given in abdominal enlargements and in coughs and asthamatic attacks of children. For intestinal inflammation, tumours, etc. a powder of root is given for three days in doses of 45 grains well mixed with caster oil. A poultice of root is useful in inflammation of the breast of nursing mothers (Dastur, 1962).

The main chemical constituents of *C. colocynthis* reported in the literature are docosan-1-ol acetate, 0, 13-dimethyl-pentadec-13-en-1-al, 11, 14-dimethyl hexadecane, 14-ol 2-one, 10, 14-dimethyl hexadecane 14, ol, 2-one, linoleic acid, oleic acid, carbohydrate, amino acid, organic acid, lipid, sterols and phenols (Ayub and Yankov 1981; Basalah *et al*., 1985; Habs *et al*., 1984 and Navot and Zamir 1986).

MATERIALS AND METHODS

Different parts of the plant *Citrullus colocynthis* i.e., roots stems, leaves and fruits were collected from Dadu district, Sindh during the month of January, 2000.

The fruits, stems, leaves and roots of *Citrullus colocynthis* were carefully separated from weeds, soil particles, added adulterants and other extraneous matter, and were dried at room temperature for one month except fruit which took three months to dry. The fruits, stems, leaves and roots were crushed to coarse power separately. 50 grams of the powder of fruits, leaves, stems and roots were homogenized in 100 ml of ethyl alcohol (90%) separately in homogeniser and operated for 5 minutes. The process was repeated for three to four times with the same quantity of alcohol. The extract was filtered through filter paper under vacuum. The alcohol extract was evaporated through rotary evaporator under reduced pressure at 40^{o}C.

The weights of residue obtained from 50 grams powders of fruits (2.37 g), leaves (8.27 g), stems (2.24 g) and roots (5.00 g) were obtained.

Growth Media

All the bacteria *Bacillus pumilis, Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were cultivated on nutrient agar medium.

Nutrient agar medium

Beaf extract	3.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar	15-20 g
Distilled water	1000 ml
pH	7.4

All the ingredients were mixed and dissolved in distilled water then sterilized by autoclaving at 121^{o}C (151 lb/sq. inch), for fifteen minutes (Cruickshank *et al*., 1975).

Preparation of Inoculum

The test organism was maintained by bi-weekly transfer on agar slants of nutrient agar medium. Growth was washed from slants with sterilized 3 ml of normal saline. This suspension was used to inoculate in wide based flask containing 200 ml of the same medium supplemented with 10 g of agar per litre. The flask was incubated for 24 hours at 37^{o}C. Growth was harvested by washing with 15 ml of normal sterilized saline. Usually 0.08 to 0.1 ml of the concentrated suspension was used to inoculate 100 ml of agar medium. The suspension was frozen (Ayoub *et al.,* 1981a, 1981b and 1981c).

Antibacterial Screening

The extracts obtained from fruits, leaves, stems, and roots were studied for antibacterial activity. The antimicrobial activity of extract from fruits, leaves, stems and roots against various Gram-positive and Gram-negative bacteria were observed. The organisms given below were collected from Central Drugs Testing Laboratory, Karachi and Sandoz Pharmaceuticals, Jamshoro.

Gram-positive

1. *Bacillus pumilis*
2. *Bacillus subtilis*
3. *Staphylococcus aureus*

Gram-negative

1. *Escherichia coli*
2. *Pseudomonas aeruginosa*

EXPERIMENTAL

Preparation of Dilution:

All the glass apparatus used were sterilized in an oven at 200^{o}C, for thirty minutes. A series of four dilutions were prepared of each extract and marked n_1, n_2, n_3 and n_4. The dilutions were prepared as follows:

i. First dilution (n_1) was prepared by dissolving one gram of dry extract in 5 ml of ethyl alcohol (200 mg/ml).
ii. Second dilution (n_2) was prepared by taking one ml of dilution n_1 and one ml of ethyl alcohol (100 mg/ml).
iii. Third dilution (n_3) was prepared by taking one ml of dilution n_2 and one ml of ethyl alcohol (50 mg/ml).
iv. Fourth dilution (n_4) was prepared by taking one ml dilution n_3 and one ml of ethyl alcohol (25 mg/ml).

Preparation of Assay Plates:

Petri dishes were sterilized in an Autoclave at 121^{o}C (15 lb pressure/sq in for 15-20 minutes then labeled with the name of bacterias whose inocculum were prepared. Each Petri dish was divided into four equal parts and each part was marked accordingly. One part was marked as control, while other three parts used for each sterilized in conical flask (500 ml) by autoclaving at 121^{o}C (15 lb pressure/sq. in.) for 15-20 minutes. Some quantity of media was prepared for each part of the plant and for different inoculums. 0.1 ml of bacterial inoculum was transferred in (100 ml) sterilized melted nutrient agar medium for bacterial inoculation at temperature not more than 45^{o}C and was gently shaked to mix the inoculum. In each sterilized Petri dish 20 ml of inoculum media was poured carefully at sterilized environment to avoid contamination and then allowed to solidify at room temperature. Now small uniform and superficial holes were made by the help of sterilized borer in the center of each part of Petri dish then each hole was sealed with one drop of same melted media. One drop of extract dilution of n_1, n_2, n_3 and n_4 were dropped with the help of pipette very carefully in three holes of individual Petri dishes, respectively with the help of pipette. The fourth one hole in each Petri dish was left as control and one drop of ethyl alcohol was dropped in it.

This process was repeated for each part of the plant and also for individual microorganism. All the Petri dishes with bacterial inoculation were incubated at 37^{o}C for 24 to 48 hours. After

incubation period the zones of inhibitions were measured results were noted. Another set of Petri dishes were prepared in the same way in which different commercially available antibiotics were used and applied in the same procedure carefully. The purpose of the preparation of this set was to compare the antibacterial activity of the crude extract with the antibiotic. The antibiotics used were Erythromycin stearate (Tablet) and oxytetracycline HCl (capsule).

The double concentration of fruit dry extract (1g/2.5 ml) is comparable which is equal to 0.2 µg/ml of erythromucin stearate and Oxytetracycline HCL (Ayoub & Yankov 1981, Basalah *et al*. 1985 and Betty *et al*., 1991).

RESULTS AND DISCUSSION

The fruits, leaves, stems and roots of the *Citrullus colocynthis* Schard were extracted with ethyl alcohol by using homogenizer for extraction and rotary evaporator used to evaporate ethylalcohol under high vacuum.

The results obtained from the parts of *Citrullus colocynthis* Schrad, i.e. fruits, leaves, stems and roots are shown in the Table 1, 2 and 3.

Table 1
Average zone of inhibition for *Staphyloccocus aureus*

S. No.	Dilution	Zone of Inhibition (cm)			
		Fruit extract	Leaves extract	Stem extract	Root extract
1.	First	1.30	1.31	1.27	1.32
2.	Second	1.22	1.12	1.24	1.14
3.	Third	1.19	1.10	1.21	1.12
4.	Fourth	1.18	1.06	1.10	1.09

Table 2
Average zone of inhibition for *Bacillus pumilus*

S. No.	Dilution	Zone of Inhibition (cm)			
		Fruit extract	Leaves extract	Stem extract	Root extract
1.	First	1.27	1.23	1.23	1.21
2.	Second	1.26	1.16	1.20	1.17
3.	Third	1.203	1.163	1.19	1.11
4.	Fourth	1.173	1.083	1.10	1.06

Table 3
Average zone of inhibition for *Bacillus subtilis*

S. No.	Dilution	Zone of Inhibition (cm)			
		Fruit extract	Leaves extract	Stem extract	Root extract
1.	First	1.02 (1.20 with double conc.)	-ve	-ve	1.05
2.	Second	-ve	-ve	-ve	1.02
3.	Third	-ve	-ve	-ve	-ve
4.	Fourth	-ve	-ve	-ve	-ve

A sufficient time period (24 hours) was given for thorough and complete extraction but same result and quantity was obtained when extraction was made in homogenizer with ethyl alcohol (400 ml as menstrum) for 15 minutes and evaporated the ethyl alcohol under reduced pressure using Rotary evaporator and drying the extract at 40^{o}C in oven. Another change was made in the extraction process by using normal saline as menstrum, and soaked over night instead of ethyl alcohol. Then same series of dilutions were prepared but results were negative. Changes were also made in dilution series after first dilution of ethyl alcohol, then further dilution were prepared with sterilized water and results were negative. For experimental work the bacterial inoculum was prepared in normal saline as mentioned in method and the results were compared by changed method, i.e., inoculum was prepared in sterilized water and phosphate buffer, positive results were obtained in the changed method. The same results were obtained by prolonging the incubation period from 24 hours to 48 to 72 hours. The same zone of inhibition was obtained by changing the size of borer one number less.

During performing the experiment for checking the antibacterial activity of crude extracts extreme care was taken to prevent the contamination. All the apparatus and growth media were sterilized for this purpose using oven and autoclave. A sterilized environment was made for inoculum. However, the crude extract was significantly active which shows the prominent antibacterial activity. The crude extract also shows appreciable inhibition of the growth of *Staphylococcus aureus, Bacillus pumilus* and *Bacillus subtilis*. The antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* were negative.

The conclusion which drown from the above discussion is that crude extracts showed active response against the some strains of bacteria may be due to carbohydrates, flavonoids, glycosides and tannin which are reported in the literature.

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