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Original Research

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF FRUIT, SEED AND ROOT EXTRACTS OF *CITRULLUS COLOCYNTHIS* PLANT

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Abstract: *Gastrointestinal, skin, pulmonary and cardiovascular problems have been reported all over the world on massive scale. The treatment of these problems has become tough due to genetically modified bacterial strains and fungal infections. The present studied was conducted to evaluate the antimicrobial activities of Citrullus colocynthis plant extract. The ethanolic extract of dried fruit pulp, seed, and root was evaluated with respect to anti-bacterial and anti-fungal properties. The anti-microbial profile studied against four bacterial strains (2 Gram negative and 2 Gram positive) while anti-fungal profile studied against four fungal species. All the bacterial and fungal strains used in the study showed sensitivities against the respective extracts. The zones of inhibition ranged between 7 mm to 23 mm, and 6 mm to 23 mm in against bacterial and fungal strains, respectively. The extract of seed found to be less effective against both the organisms. It was suggested from our study that the extract of Citrullus colocynthis may be used in medicines to cure bacterial diseases.*

Keywords: *Citrullus colocynthis*, antifungal, antibacterial, fruit pulp, seed, root, zones of inhibition

Introduction

Citrullus colocynthis plant belongs to the family Cucurbitaceae. It contains one of the outstanding collections of the genetically diversified organisms in the kingdom of plants. A large number of plants of this family are drought tolerant, susceptible to flooding, cold season and resistant to harshness of barren lands (Dhakad *et al*., 2017; Kapoor *et al*., 2020). *Citrullus colocynthis* plant is an herb which shows perennial mode of life. The herbs are commonly found drawn along the ground or other surface commonly found in desert lands of Punjab and Sindh in Pakistan and central and southern areas of India (Bhasin *et al*., 2020; Yazit *et al*., 2019). Besides, Arabia, West Asia, Mediterranean and Tropical African regions are home for it. Its tendrils are not so complex, 2-3 feet fine with hair like projections on them (Kouadri and Satha, 2018; Riaz *et al*., 2015). Leaves are round and positioned alternatively on the leafstalk. Each leaf is about 4 to 9 centimeters in long and contains nearly 4 to 7 lobes on it with rough and hairy appearance showing bright green color on the upper while pale green on the lower surface of the leaves. Seeds contain a big range of fatty acids like stearic acid, myristic acid, palmitic acid, oleic acid, linoleic acid and linolenic acid (Davidovich-Rikanati *et al*., 2015; Pravin *et al*., 2013). The protein portion is abundant in amino acids like lysine, leucine and methionine. It also contains vitamins B1, B2 and niacin. Minerals like calcium, magnesium, potassium, iron and manganese are also present (Gurudeeban *et al*., 2010; Hussain *et al*., 2014). The aerial part and fruit contains flavonoid glycoside quercetin, flavone-3-glucoside viz iso-vitexin, iso-orentine and iso-orentine-3-methyl ether (Dastmalchi *et al*., 2007; Sharififar *et al*., 2007). The fruit contains a complex amount of Cucurbitane type triterpine glycoside viz colocynthoside A and B. Cucurbitane type triterpene glycoside ciz cucurbitacin E 2-O-beta-D-glycoside its aglycone Cucurbitacin E and 2-O-beta-D-glycopyranosyl-cucrbitacin B and 2,25-di-O-beta-D-glycopyronasyl-cucurbitacin L (Davidovich-Rikanati *et al*., 2015; Song *et al*., 2015).

The importance of *C. colocynthis* plant is disclosed in certain studies as it has various medicinal effects. Anti-inflammatory effect (Onyeji *et al*., 2017), anti-oxidative effect (Bernard and Olayinka, 2010), anticonvulsant effect (Kaushik *et al*., 2015; Mehrzadi *et al*., 2016), anti-alopecia effect (Dhanotia *et al*.,

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2011), anti-fungal effect (Rezai *et al*., 2017; Salehi *et al*., 2019), and anti-diabetic effect (Jayaraman *et al*., 2009). In our study, anti-microbial and anti-fungal activities of ethanolic extracts of *Citrullus colocynthis* plant were studied using the fruit pulp, seeds and roots of matured plant.

Materials and methods

Extract preparation

Citrullus colocynthis fruits and roots were collected from local areas in Lahore, Pakistan. Roots and fruits were dried under sunlight. Seeds were separated from dried fruits. Fruit pulp, seeds and roots were converted into powdered form. About 70 grams of samples were taken into powdered form and were soaked into 250 ml of ethanol as a solvent. The crude preparation was left for a week and filtered. The filtrate thus separated was dried using rotatory using at high pressure and temperature. The crude extracts were weighed, and were dissolved into known volume of dimethyl sulfoxide or DMSO as a solvent to obtain required concentrations of 25 mg, 50 mg, 75 mg and 100 mg/1 ml DMSO.

Test microorganisms

Four species of bacteria and four species of fungi were acquired to be used in the study from microbiology lab of the University of Lahore. Two species were Gram positive while other two species were Gram negative. Bacterial species were included gram positive *Staphylococcus aureus* and *Bacillus subtilis*, Gram negative *Pseudomonas aeruginosa* and *Klebseilla pneumonia*. Fungal species were included *Fuserium oxysporum*, *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus niger*.

Antibacterial assay

For antibacterial activity, well diffusion method and disc diffusion method were followed (Bauer *et al*., 1966). For inoculation, bacterial stock cultures prepared in glycerol were used. Muller Hinton agar plates were seeded with bacterial cultures. Wells were cut for applying well diffusion method and 10 µl of plant extracts (fruit pulp, seed and root extracts with different concentrations) were poured. For applying disc diffusion method, discs soaked with extracts and positive and negative control were put on the culture plates and all the plates were incubated at 37˚ Celsius for 22-24 hours. After the incubation period, diameter of the zones of inhibition was measured. Ciprofloxacin was used as a positive control.

Antibacterial assay

For antifungal assay, cultures of fungus were made by growing the fungal strains in Sabouraud's dextrose agar plates. Plates were seeded with fungal strains and then discs of extracts (fruit pulp, seed and root with different concentrations) and negative control and positive control (fungi zone discs 15 µg per disc) were placed on the plates by using sterilized forceps. All the plates were incubated at 25˚ Celsius for 24 hours. Results were noted and zones of inhibition were measured.

Result

The results showed that all bacterial and fungal strains were sensitive against the extracts of fruit pulp, seed and root of *Citrullus colocynthis* (Figure 1, Tables 1-6). The results indicated that ethanolic extract of fruit pulp of *C. colocynthis* show activity against Gram-positive bacteria significantly than Gram-negative bacteria. Table 1 shows maximum activity of *S. aureus* from 11 mm at 25 mg/1 ml of DMSO to 22 mm at concentration of 100 mg/1 ml DMSO. The results from table 2 showed highest zone of inhibition against *A. fumigatus* from 10 mm (at 25 mg/1 ml DMSO) to 19 mm (at 100 mg/1 ml DMSO). The table 3 shows highest zone of inhibition of root extract of *C. colocynthis* against *B. subtilis* 11 mm (at 25 mg/1ml DMSO) and 23 mm (at 100 mg/1ml DMSO). The results form table 4 shows highest zone of inhibition against *A. fumigatus* 11 mm (at 25 mg/1ml DMSO) and 21 mm (at 100 mg/1ml DMSO). The table 5 shows highest zone of inhibition of seed extract of *C. colocynthis* against *B. subtilis* 11 mm (at 25 mg/1 ml DMSO) and 22 mm (at 100 mg/1ml DMSO). The table 6 shows highest zone of inhibition against *F. oxysporum* 15 mm (at 25 mg/1ml DMSO) and 21 mm (at 100 mg/1ml DMSO). Both the bacterial and fungal strains showed greater zones of inhibition by increasing the concentrations of the extracts.

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Table 1. Anti-bacterial activity of *Citrullus colocynthis* fruit extract against bacterial species

	Concentration (mg/ml) and zone of inhibition (mm)					
microorganisms	**25**	**50**	**75**	**100**	**Positive control**	**Negative control**
Staphylococcus aureus	11	16	19	22	24	0
Bacillus subtilis	13	15	16	21	24	0
Pseudomonas aeruginosa	12	13	18	19	25	0
Klebseilla pneumonia	15	17	15	19	28	0

Table 2. Antifungal activity of *Citrullus colocynthis* fruit against fungal species

	Concentration (mg/ml) and zone of inhibition (mm)					
microorganisms	**25**	**50**	**75**	**100**	**Positive control**	**Negative control**
Candida albicans	9	13	15	18	21	0
Aspergillus fumigatus	10	16	15	19	23	0
Aspergillus niger	12	15	22	23	22	0
Fuserium oxysporum	10	13	14	15	22	0

Table 3. Antibacterial activity of root extract of *Citrullus colocynthis*

	Concentration (mg/ml) and zone of inhibition (mm)					
microorganisms	**25**	**50**	**75**	**100**	**Positive control**	**Negative control**
Staphylococcus aureus	14	20	20	22	28	0
Bacillus subtilis	11	15	21	23	26	0
Pseudomonas aeruginosa	7	12	20	20	20	0
Klebseilla pneumonia	13	15	18	21	20	0

Table 4. Antifungal activity of fungal species against root extract of *Citrullus colocynthis*

	Concentration (mg/ml) and zone of inhibition (mm)					
microorganisms	**25**	**50**	**75**	**100**	**Positive control**	**Negative control**
Candida albicans	9	14	17	19	19	0
Aspergillus fumigatus	11	15	18	21	21	0
Aspergillus niger	7	11	19	20	20	0
Fuserium oxysporum	18	20	18	22	21	0

Table 5. Antibacterial activity of seed extract of *Citrullus colocynthis*

	Concentration (mg/ml) and zone of inhibition (mm)					
microorganisms	**25**	**50**	**75**	**100**	**Positive control**	**Negative control**
Staphylococcus aureus	10	11	14	15	20	0
Bacillus subtilis	11	8	20	22	20	0
Pseudomonas aeruginosa	9	13	14	17	7	0
Klebseilla pneumonia	11	13	15	20	21	0

Table 6. Antifungal activity of seed extract of *Citrullus colocynthis*

	Concentration (mg/ml) and zone of inhibition (mm)					
microorganisms	**25**	**50**	**75**	**100**	**Positive control**	**Negative control**
Candida albicans	11	12	14	19	19	0
Aspergillus fumigatus	12	15	16	20	20	0
Aspergillus niger	14	17	17	22	21	0
Fuserium oxysporum	15	19	19	21	21	0

Discussion

The effect of *Citrullus colocynthis* plant extracts using fruit pulp, seeds and roots at different concentrations 25 mg/1 ml DMSO, 50 mg/1 ml DMSO, 75 mg/1 ml DMSO, and 100 mg/1 ml DMSO against the bacterial and fungal strains (Marzouk *et al*., 2009; Marzouk *et al*., 2010). All the organisms showed sensitivity against the extracts but the zones of inhibition were smaller as compared to other ones in the seed extracts of the plant. The zones of inhibition were greater in the gram-positive bacteria (*S. aureus* and *B. subtilis*) as compared to the gram-negative bacteria and zones of inhibition were also increased by increasing the concentrations of the extracts (Jayaraman *et al*., 2009; Mehrzadi *et al*., 2016). Gram-negative bacteria *Pseudomonas aeruginosa* showed sensitivity against the ethanolic extract of *C. colocynthis* contrary (Salehi *et al*., 2019) who studied soil bacteria at the concentration of 10%. As much as antifungal results are concerned, all the

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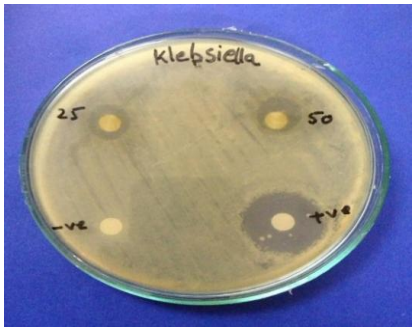
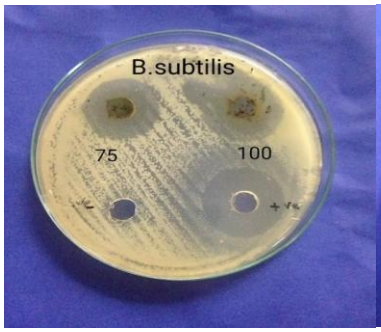
extracts showed good results against all the fungal strains especially anticandidal activity (*C. albicans*), the efficiency of extracts activity was increased by increasing the concentration of the extracts. The potential of activity of each organ is dependent on plant extract and which is a crude mixture composed of compounds having activity and non-activity (Sharififar *et al*., 2007; Yazit *et al*., 2019). The plant organ and the nature of solvent play an important role in the activity of the extract in given organisms. The polarity and non-polarity factor in the solvents, used in the preparation of extracts affects the efficiency of extracts at greater extent (Dastmalchi *et al*., 2007; Song *et al*., 2015).

Conclusion

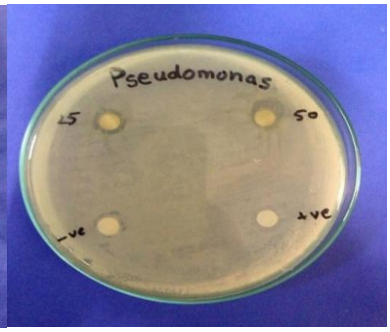
The study concluded that *Citrullus colocynthis* plant has a good efficiency against common human pathogens when prepared in ethanol and its activity also increased by increasing the concentrations of the extracts. This study is based on plant extract from three different organs (fruit pulp, seed and root), on four bacterial and four fungal species and including Gram positive and Gram negative bacteria also Candidal species (*C. albicans*). The results presented in the study showed that natural products examined can be a better source for the creation of modern ways to treat infections in gastrointestinal, skin and pulmonary infections.



C. colocynthis fruit pulp (*K. pneumonia*) (Disc diffusion method)



C. colocynthis root (*B.subtilis*) (Well diffusion method)



C. colocynthis seed (*P.aeruginosa*) (Disc diffusion method)

C. colocynthis root (*S.aureus*) (Disc diffusion method)

C. colocynthis fruit pulp (*A. niger*)

C. colocynthis fruit pulp (*F. oxysporum*)

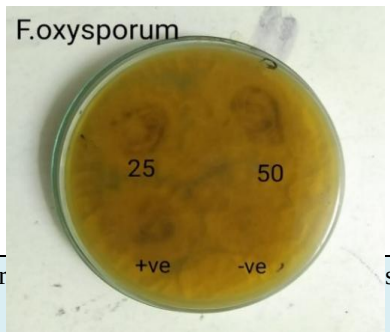


Figure 1. Antibacterial and antifungal activities of C. colocynthis plant extracts

Conflict of interest

The authors declared absence of conflict of interest.

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