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 study was conducted to evaluate the antimicrobial activities of Citrullus colocynthis plant extract. The ethanol 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 (Koudadi 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 isovitexin, 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 triterpene glycoside viz colocynthiside A and B. Cucurbitane type triterpene glycoside cis cucurbitacin E 2-O-beta-D-glycoside its aglycone Cucurbitacin E and 2-O-beta-D-glycopyranosyl-cucurbitatin B and 2,25-di-O-beta-D-glycopyranosyl-cucurbitatin 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-aloepecia effect (Dhanotia et al., 2020).
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

| microorganisms         | Concentration (mg/ml) and zone of inhibition (mm) |
|------------------------|--------------------------------------------------|
|                        | 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                |
| Klebsiella pneumonia    | 15      | 17      | 15      | 19      | 28               | 0                |

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

| microorganisms         | Concentration (mg/ml) and zone of inhibition (mm) |
|------------------------|--------------------------------------------------|
|                        | 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

| microorganisms         | Concentration (mg/ml) and zone of inhibition (mm) |
|------------------------|--------------------------------------------------|
|                        | 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                |
| Klebsiella pneumonia    | 13      | 15      | 18      | 21      | 20               | 0                |

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

| microorganisms         | Concentration (mg/ml) and zone of inhibition (mm) |
|------------------------|--------------------------------------------------|
|                        | 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

| microorganisms         | Concentration (mg/ml) and zone of inhibition (mm) |
|------------------------|--------------------------------------------------|
|                        | 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                |
| Klebsiella pneumonia    | 11      | 13      | 15      | 20      | 21               | 0                |

Table 6. Antifungal activity of seed extract of Citrullus colocynthis

| microorganisms         | Concentration (mg/ml) and zone of inhibition (mm) |
|------------------------|--------------------------------------------------|
|                        | 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 antifungal 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.
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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