Effects of essential oil extracted from *Citrullus colocynthis* (CCT) seeds on growth of phytopathogenic bacteria

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*Citrullus colocynthis* (CCT) is a non-hardy, herbaceous perennial vine, branched from the base. Originally from Tropical Asia and Africa, it is now widely distributed in the Sistan phytogeographic region of Iran. In a search of alternative ways to control plant disease, essential oil from seeds of CCT was examined for antibacterial properties. The seeds are edible and have a high oil content with a large proportion of linoleic acid (C18:2) which is important for human nutrition and an essential fatty acid also contains only traces of linolenic acid (C18:3). Antibacterial activity of oil separated from the seeds was tested against *Xanthomonas campestris*, *Burkholderia cenocepacia*, *Pseudomonas syringae* and *Agrobacterium tumefaciens*. The agar disc diffusion method was used to assess inhibitory effect by measuring the inhibition zone against the test microorganisms. Antibacterial activity of the seeds oil was confirmed for all bacterial, but with different ranges. This activity was observed to be dose-independent. *X. campestris* was the most sensitive bacterium tested. A weak inhibitory effect was found against *Pseudomonas syringae*. These results offer a scientific basis for the use of *C. colocynthis* seed oil to prevent diseases caused by these bacteria.

Key words: *Citrullus colocynthis*, phytopathogens, agar disc diffusion.

INTRODUCTION

Bacterial pathogens and their control are a serious problem in agriculture practice. Many of the currently available antimicrobial agents for agriculture are highly toxic, non-biodegradable, and cause extended environmental pollution (Vyvyan, 2002). Diseases caused by pathogens including bacteria and fungi significantly contribute to the overall loss in crop yields worldwide (Savary et al., 2006). Despite the existence of plant defense mechanisms, a major difficulty encountered is the lack of effective control agents against some severe plant bacterial diseases. On the other hand, application of chemical derivatives has effectively controlled the plants from bacterial disease but this threatens to contaminate the environment, hindering the management of diseases in crops and agricultural products (Burhan, 2009). The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. Herbal medicine represents one of the most important fields of traditional medicine all over the world (Hamil, 2003). Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities (Mona, 2002). The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan, 2009). Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001). Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavor and cosmetic industries. *Citrullus colocynthis* (Linn.) Schrad., (colocynth, wild-gourd or bitter-apple) is an important medicinal plant belonging to the family Cucurbitaceae. It is a well-recognized plant in the traditional medicine and was used by people in rural areas as purgative, antidiabetic...
and insecticide. Various oils are biocides against a broad range of organisms such as bacteria, fungi, viruses, protozoa, insects and plants (Dung et al., 2008; Gurudeeban et al., 2010). Recent researches are showed that essential oils of many plants possess antimicrobial activities and maybe used for the treatment of different diseases in the near future (Elaisi et al., 2011; Erkan et al., 2012; Vairapan et al., 2012; Sivashothy et al., 2012; Makhloufi et al., 2012; Abdelhady and Aly, 2012). There is vast diversity among aromatic and medicinal plants and different chemotypes of the same species may grow in the same place and produce different oils with different activity (Darokar et al., 1998).

The current work presents an evaluation of antibacterial activity of essential oil from Iranian C. colocynthis and their inhibitory effect against the growth of some phytopathogenic bacteria.

MATERIALS AND METHODS

Oil isolation and extraction

Fresh fruits were collected from south-eastern of Iran during 2010/2011, especially Sistan region in large quantities. The seeds were generally collected after fruit ripening, between September and October. Dried seeds were powder and hydrodistilled for 5 h using a Clevenger apparatus with a water-cooled oil receiver. The oil was dried over anhydrous Na$_2$SO$_4$ and preserved in a sealed vial at 4°C in the dark until further analysis (yield 0.88 %, w/w).

Test microorganisms

The test organisms used in this study (reference strains) were Xanthomonas campestris pv. Campestris (ATCC33913), Pseudomonas syringae pv. Syringae (B728a), Burkholderia cenocepacia (H12424) and Agrobacterium tumefaciens (str. C58). The stock cultures were maintained in nutrient agar (NA) slant at 4°C and sub-cultured monthly. Working cultures were prepared by inoculating a loopful of each test microorganism in 3 ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 24 h. The suspension was diluted with sterile distilled water to obtain approximately 10$^6$ CFU/ml.

Antibacterial testing

The seeds oil was tested for antibacterial activity by the disc agar diffusion method (Murray et al., 1995). Disk diffusion: 5 mm of sterile disks were incorporated in 100 µl of plant extracts (5 mg/disk). The disk (6 mm in diameter, Whatman No. 1) was completely saturated with the extract and allowed to dry. Mueller Hinton (MH) agar plates were swabbed with test bacteria and six extract disks with one of the standard positive control disks (streptomycin) was placed on the MH agar plate. Dimethyl sulfoxide (DMSO) was taken as the negative control (10% DMSO did not show any antibacterial activity). The plates were incubated at 37°C for 24 h and the diameter of the inhibition zones were measured in mm.

Minimum inhibitory and minimum bactericidal concentrations

Micro-dilution susceptibility assay was performed using the NCCLS and CLSI methods for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (NCCLS, 1993; CLSI, 2009). Bacteria were cultured overnight at 30°C. The test samples of oil were dissolved in 5% DMSO. Dilutions were prepared in a 96-well microtiter plates to get final concentrations ranging from 0 to 4 µg/ml. Finally, 20 µl of inoculum (10$^{-6}$ - 10$^{-7}$ CFU/ml) was inoculated onto the microplates and the tests were performed in a volume of 200 µl. Plates were incubated at 30°C for 24 h. The standard reference drug, ampicillin, was used as a positive control for the tested plant pathogenic bacteria. The lowest concentrations of tested samples, which did not show any visual growth after macroscopic evaluation, were determined as MICs, which were expressed in µg/ml. Using the results of the MIC assay, the concentrations showing complete absence of visual growth of bacteria were identified and 50 µl of each culture broth was transferred onto the agar plates and incubated for the specified time and temperature as mentioned above. The complete absence of growth on the agar surface in the lowest concentration of sample was defined as the MBC. Each assay in this experiment was replicated three times.

Statistical analysis

The data obtained for antibacterial activity of essential oil and various extracts were statistically analyzed and mean values were calculated. A Student’s t test was computed for the statistical significance of the results at p<0.05. All experiments were performed at least, three times (unless indicated otherwise) and were highly reproducible.

RESULTS AND DISCUSSION

The oil obtained from the seeds of CCT revealed relatively potential antibacterial effects at the concentrations utilized against all selected plant pathogenic bacterial (Table 1). Xanthomonas campestris pv. Campestris (ATCC33913) was found most sus-ceptible pathogenic bacteria to the oil of CCT seeds. The diameter of the inhibition zones of the oil against the tested strains of Xanthomonas were in the range of 14-20 mm. On the other hand, standard streptomycin showed both lower antibacterial (Xanthomonas) and comparable (Burkholderia) effect as compared to the seeds oil dependent of bacterial species (Table 1). The minimum inhibitory concentration of the extracts varied between 35.0 - 81.86 µg/ml while the minimum bactericidal concentration was between 59.0-123.0 µg/ml (Table 1). As shown in Table 1, the minimum concentrations of seeds oil were found more susceptible to the tested plant pathogenic bacteria of X. campestris pv. Campestris (ATCC33913) as compared to the other bacteria. The seeds oil had a detrimental effect on Xanthomonas. The seeds oil displayed remarkable antibacterial activity against tested strains such as X. campestris pv. Campestris (ATCC33913), Burkholderia cenocepacia (H12424) and Agrobacterium tumefaciens (str. C58) with MIC and MBC values of 35.26-70.1, 62.5-250 and 62.5-250 µg/ml, respectively. On the other hand, the seeds oil displayed better antibacterial effect against the tested bacterial pathogens as MIC values as compared to standard streptomycin (MIC: 300-500 µg/ml). However, in some cases, the extracts had a higher antibacterial effect.
Table 1. Antibacterial activity, Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of seeds oil of CCT against selected plant pathogenic bacterial.

<table>
<thead>
<tr>
<th>Bacterial pathogen</th>
<th>Seeds oil  a</th>
<th>Standard  b</th>
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<tbody>
<tr>
<td></td>
<td>IZ c</td>
<td>MIC</td>
</tr>
<tr>
<td>Xanthomonas campestris pv. Campestris (ATCC33913)</td>
<td>15.0±0.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Pseudomonas syringae pv. Syringae (B728a)</td>
<td>8.0±0.0</td>
<td>65.2</td>
</tr>
<tr>
<td>Burkholderia cenoceopa (H2424)</td>
<td>14.0±0.0</td>
<td>37.0</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens (str. C58)</td>
<td>12.0±0.0</td>
<td>81.86</td>
</tr>
</tbody>
</table>

*a oil used at 1.000 µg/disc; b Standard: streptomycin (20 µg/ml, all values are in µg/ml); *p<0.05 significant, *Data are expressed as the diameter of inhibition zones (IZ) in mm; Values are given as an average of triplicate experiments.

as compared to the standard antibiotic which might be due to the presence of highly bioactive compounds in seeds oil. The increased awareness of the environmental problems associated with conventional non-biodegradable agrochemicals has led to the search for non-conventional chemicals of biological origin for the management of post-harvest disease in fruits and vegetables (Abhay et al., 2012). Bioactive compounds are naturally produced in the plants and among of them essential oil are important for the physiology of plants contributing properties confer resistance against microorganisms, other organisms and even antibacterial activities (Abhay et al., 2012; Cantore et al., 2009; Kotan et al., 2010). The observed antibacterial properties of CCT essential oil show its potential for the practical use of the essential oil towards plant pathogenic bacteria as a natural bactericide and it was similar with previous studies (Marzouk et al., 2010). The preliminary qualitative phytochemical screening of CCT was reported in previous paper (Najafi et al., 2010; Gurudeeban et al., 2010). Analysis of CCT fatty acid methyl esters showed the presence of palmitic, stearic, oleic, linoleic and linolenic acids in appreciable quantities (Kulkarni et al., 2012). The obtained results suggest that the use of CCT oil as antibacterial agent may be judiciously applied to prevent the decay of fruits and vegetables due to bacteria. The isolation and purification of the phytochemical followed by a detailed study might result in identification of lead compound and thus a potential cure for the diseases caused by this bacteria.

REFERENCES


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