EVALUATION OF ANTIMICROBIAL ACTIVITY OF FLAVONOIDS EXTRACT FROM CUCURBITA PEPO LEAVES

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ABSTRACT

Investigation of antibacterial activity of flavonoids extract of Cucurbita pepo was carried out in this study on Gram positive and Gram negative pathogenic bacteria. The results exhibited variable susceptibilities of microorganism for different concentrations of flavonoids extract. The activity of this extract was associated with high concentration. Using plate methods, flavonoids extract of Cucurbita pepo had the highest effect and wide diameter of growth inhibition zones against serratia and Escherichia coli and it has no effect on growth of Bulkholderia pseudomallia. Staphylococcus aurus and Streptoccocus sp.

INTRODUCTION

Bacterial infection contribute largely in general health problems of man and have been reported to be responsible for over 50% of death recorded in developing countries, antimicrobial drugs have failed in their activity against the pathogen due to the development of drug resistance [1]. This increasing resistance to antibiotics has therefore resulted in the search for leads new organic molecules from plants with antimicrobial properties [2]. Plants have been valuable source of natural products for maintaining human health especially in the last decades with more intensive studies for natural therapies and the use has gradually increased according to the world health organization [3].

Cucurbita pepo (pumpkin) is a member of the family curcurbitacea, its an annual plant with yellow flowers it has aclimbing stem up to 12 Cm. long and a fruit with ovoid shape with a curved green shell inside the shell is a flat, round yellow and white seed enclosed in a husk [4].
Cucurbita pepo leaves have been used in traditional medicine as antihelminthic agent and there are for supportive treatment in functional disorders of the bladder and for difficulties in urination [5] as well as childhood enuresis nocturnal and irritable bladder have been treated successfully with pumpkin, it has been also used to eradicate tape worm [6]. Other researchers showed that it is possessed anti-inflammatory antibacterial, antiviral, analgesic effects and cardioprotective activity [7] in addition it has been widely applied in the treatment of benign prostatic hyperplasia in men, obesity, skin problems [8, 9].

Cucurbita pepo have been used in our research because of their antimicrobial material, which are due to compound synthesized in the secondary metabolism of this plant so these products are known by their active substances such as flavonids compounds [10].

**MATERIAL AND METHOD**

**Plant material and extraction:**

_Cucurbita pepo_ leaves were purchase from the local market of Basra. The leaves of _Cucurbita pepo_ were sun dried and powdered. Powder (50gm) was extracted by mixing with 250ml ethanol (70%) for 24 hrs. using reflex extraction. The extract was filtered through wattmann No. 31 for removal particles then filtrate was mixed with 1% lead acetate and filtered with wattmann No. 31. residue was dissolved with 25ml acetone and 30ml HCl conc. and left to dry to evaporation the solvent and then obtained black solid residue about 5 gm [6].

**Microorganisms Test:**

The level of sensitivity of seven types of pathogenic bacteria were previously isolated and identify by other worker these bacteria was determined by using the disk diffusion method (Kirby Bauer) as described by [11]. Circular paper disc measuring 7.0 mm was cut from Whatman No.1 filter paper.

Each group of 2 plates was inoculated with each of the test organism which was fully spread on the Muller-Hinton agar medium. Finally the discs impregnated with extracts (6.25, 12.5, 25, 50, 100 mg/kg.) which contain carefully placed into the culture plates and allowed to stand for a few minutes before being incubated for 24hrs. at 37°C. After this period it was possible to observe inhibition zone overall, cultured bacteria with halos equal to or greater than 7mm. were considered susceptible to the tested extract. They were then examined for growth and signs of inhibition. The zone of inhibition were determined by measuring diameter of clearance a cross the disc with a ruler.
RESULTS

Results of disc diffusion test (table 1) which illustrated by photographs which are listed in figure 1 (A-F) and figure 2 (A-H). showed that the flavonids extract of *Cucurbita pepo*, if properly processed, could be used to treat some stubborn *Serratia, E.coli* and *Bacillus* infections. This agreement with result of [16] which reported the antimicrobial properties of compounds obtained from *Parthenium argentatum* which inhibited the growth of *Bacillus subtilis, E.coli* and *P.aeruginosa*.

It was concluded that the flavonoids extract (ethanolic extract) 70% were toxic to many types of bacteria and showed have antimicrobial activity. The antimicrobial activity from this extract was tested against seven genera of bacteria, which showed it had activity against *Klebselia, Bacillus, E.coli* and *Serratia* [16].

Table (1): Diameter of inhibition zone with different concentration of extract effect on bacteria used:

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Concentration of flavonids extraction (mg/ml)</th>
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<tr>
<td></td>
<td>6.25 DIZ(mm )</td>
</tr>
<tr>
<td>Klebsiella pneumoniae 10^{-1}</td>
<td>10</td>
</tr>
<tr>
<td>Klebsiella pneumoniae 10^{-2}</td>
<td>14</td>
</tr>
<tr>
<td>Bacillus subtilis 10^{-1}</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus subtilis 10^{-2}</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus aureus 10^{-1}</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus 10^{-2}</td>
<td>0</td>
</tr>
<tr>
<td>Bulkholderia pseudomallie 10^{-1}</td>
<td>0</td>
</tr>
<tr>
<td>Bulkholderia pseudomallie 10^{-2}</td>
<td>0</td>
</tr>
<tr>
<td>E.coli 10^{-1}</td>
<td>15</td>
</tr>
<tr>
<td>E.coli 10^{-2}</td>
<td>10</td>
</tr>
<tr>
<td>Serratia 10^{-1}</td>
<td>15</td>
</tr>
<tr>
<td>Serratia 10^{-2}</td>
<td>10</td>
</tr>
<tr>
<td>Streptoccocus sp. 10^{-1}</td>
<td>0</td>
</tr>
<tr>
<td>Streptoccocus sp. 10^{-2}</td>
<td>0</td>
</tr>
</tbody>
</table>

DIZ = Diameter of inhibition zone measured in millimeter.

*10^{-1}, 10^{-2} = dilution of bacterial broth.
Figure (1): Inhibition zones induced by flavonoids extract on some positive bacteria

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<table>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>E</td>
<td>F</td>
</tr>
</tbody>
</table>

A: *Bacillus subtilis* $10^{-1}$, B: *Bacillus subtilis* $10^{-2}$
C: *Staphylococcus aureus* $10^{-1}$, D: *Staphylococcus aureus* $10^{-2}$
E: *Streptoccocus sp.* $10^{-1}$, F: *Streptoccocus sp.* $10^{-2}$
Figure (2): Inhibition zones induced by flavonoids extract on some Negative bacteria
A: *Klebsiella pneumoniae* $10^1$, B: *Klebsiella pneumoniae* $10^2$
C: *Burkholderia pseudomallie* $10^{-1}$, D: *Burkholderia pseudomallie* $10^{-2}$
E: *E.coli* $10^1$, F: *E.coli* $10^2$
G: Serratia $10^1$, H: Serratia $10^2$
DISCUSSION

The flavonoids extraction of *Cucurbita pepo* in present study revealed the medical importance of this plant through the antimicrobial activity. A variable susceptibility of bacteria against different concentration of the flavonoids extraction of *Cucurbita pepo*.

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the concentration of antibiotic resistant bacteria. The objective of this study was to evaluate the potential of the flavonoids extraction of *Cucurbita pepo* on standard bacteria strains. The flavonoids compounds have effective against bacteria[12,13] because of the site (s) and number of hydroxyl groups on flavonoids group are thought to be related to their relative toxicity to the bacteria that increased hydroxylation results increased toxicity [14 &15], the antimicrobial properties of plant have been investigated by a number of researchers world wide, it was documented that among the compound extracted from these plants, inhibited the growth of *Staph. aureus*, *E.coli* and *Klebsiella pneumoniae* and also the effects of *Cucurbita pepo* may relate to an influence of plant compounds on immunocompetent cells.

*Cucurbita pepo* possess antibacterial qualities, will surely enhance their application among other uses, as alternative to antibiotics for effective treatment of bacterial infections, *Cucurbita pepo* and also have medicinal uses including antihelminthic as well as natural laxative. In addition it has been widely applied in treatment of benign prostatic hyperplasia in men, obesity, skin problems and irritable bladder (enuresis) in children[16].

تقدير الفعالية المضادة للميكروباث للمستخلص الفلافونيندي المعزول من أوراق نبات *Cucurbita pepo*

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الخلاصة

فقد تم دراسة الفعالية المضادة للميكروباث للمستخلص الفلافونيندي لوراق نبات الب揸تي. وقد اخترعت بعض أنواع البكتيريا الموجبة والسالبة لصبغة كرام. بنيت الدراسة أن هذه الميكروباث لها حساسية مختلفة اتجاه المستخلص وحسب نوع الميكروب والتركيز المستخدم وقد لوحظ أن التأثير الأكبر للمستخلص كان على جرثومة *Serratia*، *Bulkholderia* و *Streptococcus sp.* و *Staphylococcus aureus* أما الجرافيم *Escherichia coli* فلم تتأثر بالمستخلص *pseudomallie*. 
REFERENCES


