PHYTOCHEMICAL CONTENT AND ANTI-OXIDANT ACTIVITY OF HYLOCEREUS UNDATUS AND STUDY OF TOXICITY AND THE ABILITY OF WOUND TREATMENT

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Abstract

In this work, the study of Dragon (Hylocereus undatus) properties was done by studying phytochemical screening, trace element and antioxidant activity. The analyzed of fruit extract by using Gas Chromatography-Mass Spectroscopy (GC-MS) gave many compound. The antibacterial activities of the Dragon extract agonist different types of bacteria (Escherichia coli, Kebsiella sp, Staphylococcus epidermidis and Staphylococcus aureus) were done by using well diffusion method. The toxicity of the extract was examined against the mice by giving all of the groups (extract group and control group) orally dose 50mg/b.w and the results of pathological changes were diagnosed. After that, the extract was converted to a cream and used as wound healing cream. The results show that the cream has higher activity as a wound healing than the extract only. The final result appeared that extract had a good bioactive compounds and antioxidant ability, with the acceptable role in wound healing, which can be applied for many antibacterial applications, anti-inflammatory and could be helpful in preparation of pharmacologically drugs.

Key words : Hylocereus undatus, antioxidant, antibacterial, toxicity, wound healing, medicinal plants.

Introduction

The Hylocereus Pitaya (Dragon) fruit is a perennial, epiphytic, climbing cactus with a triangular beefy, jointed stalk which belongs to the family Cactaceae and of genus Hylocereous (Ruzlan et al., 2010). There are three different species of Pitaya fruit which include Hylocereus undatus or white-pulp with red fruit extract Pitaya fruit. Selenicereus megalathus or white pulp with yellow fruit extract Pitaya fruit and Hylocereus polyrhizus or red- pulp with red fruit extract Pitaya fruit (Ortiz-Hernández et al., 2012).

The fruit of Hylocereus undatus is large in size, oval shape, weighing about 280-5060 grams, 33-36 cm diameter and 14-16 cm long. The fruit has delicate and sweet flesh with intense white color of the flesh and red-purple color of fruit extract. It has a lot of tiny black seeds, which are rich in essential fatty acids (Ariffin et al., 2009). The Hylocereus undatus fruit are rich in fiber, vitamins, calcium, phosphorus, magnesium (Elmarzugi), phytochemicals and antioxidants revealed that Hylocereus undatus fruit is also known to possess medicinal and pharmaceutical properties that could prevent diabetes, cancer and neutralizes toxins in body. It is even helpful in reducing blood sugar levels in Type 2 diabetic patients (Stintzing et al., 2003).

In the past period interest on the topic of antibacterial property of plant extract, which can be attributed to their bioactive compounds has been growing and researchers attributed this to the presence of several bioactive compounds. Phytochemicals such as polyphenols, carotenoids and anthocyanin that are abundantly present in vegetables and fruits such as pomegranates, tomatoes, strawberries, and grapes are required lot of interest due to their functional property (Rao and Rao, 2007).

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The study was conducted to checked up the phytochemical contains of *Hylocereus undatus* fruit extract, determine the antioxidant activity, determine the trace elements, determine the antibacterial potential as eradicate protectant and against *Escherichia coli*, *Klebsiella sp*, *Staphylococcus epidermidis* and *Staphylococcus aureus*, other steps contain the study of toxicity of fruit by trying that on animal models and the study of it is activity to work as a wound healing agent.

**Materials and Methods**

**Collection of the fruit samples**

*Hylocereus undatus* fruit (pitaya) was collected from alrasheed market for vegetables. The fruit was transported to the laboratory of Biochemistry, College of Science, Al-Mustansiryih University. Washed in water, cleaned well to remove all traces of insects, dust and other kinds of pollution. after that we turn it in to juice by using blinder, centrifuged and filtered by a filter paper and putted it in the freezer at 2-4°C to use it in the next experiments.

**Chemical detection of the plant components**

**Qualitative Phytochemical analysis**

According to the standard AOAC (1990) method, the chemical components of the prepared fruit extract were detected using different tests as shown in Table 1. They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, flavonoids, proteins, steroids.

**Determination of trace element**

Ten ml of fruit extract of *Hylocereus undatus* were taken. And mixed with 8 drops of concentrated nitric acid in a conical flask, the mixture was kept for 24 hrs covered by a watch glass. And the trace elements were specified by (Shimadzu AA-670, Flame Atomic Absorption Spectrophotometer) (Mohammed and Abbas, 2016).

**Table 1 : The phytochemical screening in Dragon fruit.**

<table>
<thead>
<tr>
<th>Components</th>
<th>Reagent</th>
<th>Note</th>
<th>Results of fruit extract tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Biuret test</td>
<td>Purple blue</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>Liebermann Burchard test</td>
<td>Yellow ppt</td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molish test</td>
<td>Violet ring</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkoloids</td>
<td>Mayer’s reagent Wagner reagent</td>
<td>White ppt Orange ppt</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>Fwrric chloride test</td>
<td>Green ppt</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins and Flavonoids</td>
<td>Lead acetate</td>
<td>Yellow white ppt</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Fast stirring</td>
<td>Dense foam for long time</td>
<td>+ve</td>
</tr>
</tbody>
</table>
FTIR analysis

The fruit extracts powder of *Hylocereus undatus* fruit was washed with distilled water a lot of times to get rid of dust and polluters, then dry it with 45°C in the oven. For comparison, the dried fruit extracts powder of *Hylocereus undatus* were analyzed by FTIR-shimadzu-8400S spectrophotometer, the spectrum was listed in the range of 500-4000 cm⁻¹ (Al-Alwani et al., 2015).

Qualitative determination of free radical scavenging activity (TLC method)

According to Kannan et al. (2010), antioxidant ingredient were analyzed by Thin Layer Chromatography (TLC) followed by DPPH (2, 2-Diphenyl-1-picrylhydrazyl). About 100 µg of extract of Standard Gallic acid and *Hylocereus undatus* fruit was loaded on TLC plates (Merck, 10 × 10 cm²). The plates were air dried and observed under visible and UV-Vis light (240 and 300 nm). Different separated spots were founded as their Rf values. After this examination, 0.05% of DPPH solution in methanol was splashed on the face of TLC plates and incubated for 30 min at room temperature. The active antioxidant pitaya and ingredient were detected as yellowish spots the strength of activity for compounds selected by studying the change in color.

Quantitative determination of Free radical scavenging activity assay (DPPH method)

Free radical scavenging activity was specified according to the method of Braca et al. (2001). With little modification, fruit extract (0.5 mL) was added to 1 mL DPPH solution (2 mL of 0.013 g/L DPPH) in methanol. The reduction of DPPH was measured at 517 nm versus a blank assay at 30 min. The percentage of residual radical in medium is calculated as the absorbance of the sample split by that of DPPH control at the same time multiplied by 100. The amount of sample necessary to decrease the initial DPPH concentration by 50%, EC50, was calculated graphically.

Anti-bacterial studies

Agar well diffusion method for bactericidal susceptibility was carried out according to standard method to assess the presence of antibacterial activity for Aqueous Dragon fruit extract using *Escherichia coli*, *Klebsiella* sp, *Staphylococcus epidermidis*, *Staphylococcus aureus* and one fungi (*Candida albicans*). The Dragon fruit was juiced with blinder then the crude were taken directly to use, the remaining juice then dried by using an oven with 45°C temperature, different solvents were used with this dried extract (95 methanol, 5% DMSO and ethanol). The extractions were accomplished in an Innova 4000 incubator shaker (New Brunswick Scientific, New Jersey, USA) at 40°C for 2 hr prior to filtration by filter paper. The medium was sterilized at 120°C in autoclave. Then the medium was transported into sterilized Petri plates and kept at 37°C for solidification. The bacterial strains were diffusion on the Petri plates using loop. On each plate, a single well of 4 mm diameter was made using a gel pierce. The Dragon extract (5µl) were added into the wells. The plates were incubated at 37°C for 24 hrs. The experiments were accomplished in triplicates and the zone of inhibition was measured (Chakraborthy, 2008).

LD₅₀ examination

In the study we recorded the clinical signs for 24 hr. till 14 day ten adult albino mice (male) were divided at random into two groups containing five mice (25-30g) in every group, all cured groups treated orally by gastric Gavage once daily with different doses of each extract and mice were kept under continued observation for 24 hours after the administration; as following:

Group (1): control group given distilled water.

Group (2): given extract at dose 50 mg/kg b.w orally by gastric lavage one time.

In the end of LD₅₀ analysis all the lab animal (mice) were sacrificed and vital organ (brain and heart) used for histopathological analysis (Soufane et al., 2017).

Histological study

All mice were sacrificed after 24 hours of last treatment. The vital organ (heart and brain) were dissected out and keep in tube contain formalin 10% until sent for Histological examination. The vital organ (heart and brain) were dehydrated in progressively more concentrated alcohols, then embedded in paraffin and cut into sections of 4-5 µm thickness and stained with hematoxylin and eosin (H&E) for microscopical examination. The slides were examined under 40X magnification using an optical microscope (Al et al., 2017).

Wound healing for mice

The mice were placed in cages with appropriate conditions and to ensure that there is no injury or symptom is prevented the examination process. The dorsal area of the mice circled and the area was localized with lidocaine sprayer at a concentration of 10% to create the wound in a radius of (1.0) cm² by surgical scalpel, leaving the wound open until redness is indicative of acute inflammation, the mice were treating on a daily basis and record the observations that occur during the treatment process Monitor the behavioral changes that the animal exhibits during it (Abood et al., 2015).
Results and Discussion

Qualitative phytochemical analysis

Phytochemical screening for Dragon fruit shows that Juice extract contains various kinds of compounds and the result were positive for proteins, carbohydrates, saponins, alkaloids, phenolic compounds, tannins and flavonoids. That act as primary antioxidants and mainly responsible for the reducing property of the extract, wishagreed with the study of Sushant et al. (20120).

Determination of trace element

For the determination of trace element atomic absorption were used and the flame method has makes sure that Hylocereus undatus contains (Zn, Cu, Mg, Ca, Pb, Se, Fe and Cd) the result were shown in table 2. And this result has agreed with a slight different in the concentration with Tsdale et al. (2015). The appearance of some metals like Selenium, Iron and Copper helps to evaluate the ability of working as anti-oxidant.

FTIR analysis

The FTIR analysis was used to identify the reactive groups in the fruit extract. The spectra of fruit extract of Hylocereus undatus showed in fig. 1. The peaks near 686 and 669 cm\(^{-1}\) is Designation to CH out of plane bending vibrations of replaced ethylene systems – CH=CH, the peak at 1058 to 1174 cm\(^{-1}\) represents C-O stretching vibration. The peak of 1435 to 1484 cm\(^{-1}\) corresponds to aromatic group contained C-C stretching. The peak at 1637 cm\(^{-1}\) 1 corresponds to the C=O stretching vibrations which represented the carbonyl group for ketone structure. The peak at 2852 and 2926 cm\(^{-1}\) corresponds to the O-H of carboxyl group (Coates, 2000). The peak at 3031 cm\(^{-1}\) also contains O-H group which is the hydroxyl group in dragon fruit dye Broad peaks between 3288-3417 and 3230-3416 cm\(^{-1}\) corresponds to -NH stretching in amide, most of the active components such as C=O (which are attributed to carbonyl) and O-H (corresponds to the hydroxyl group) usually in carboxylic acid.

Table 2: The concentration of trace element in Hylocereus undatus.

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Symbol</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>Zn</td>
<td>140</td>
</tr>
<tr>
<td>Copper</td>
<td>Cu</td>
<td>34</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mg</td>
<td>24</td>
</tr>
<tr>
<td>Calcium</td>
<td>Ca</td>
<td>13</td>
</tr>
<tr>
<td>Lead</td>
<td>Pb</td>
<td>13</td>
</tr>
<tr>
<td>Selenium</td>
<td>Se</td>
<td>8.5</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe</td>
<td>2.16</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Cd</td>
<td>0.0133</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Co</td>
<td>Nil</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Al</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Table 3: The scavenging activity for 1-standard Gallic acid. 2-Dragon fruit.

<table>
<thead>
<tr>
<th>Concentration of compound by µg/ml</th>
<th>Standard Gallic acid %</th>
<th>Hylocereus undatus fruit %</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>82.9</td>
<td>65.7</td>
</tr>
<tr>
<td>50</td>
<td>69.3</td>
<td>60.8</td>
</tr>
<tr>
<td>25</td>
<td>53.3</td>
<td>50.4</td>
</tr>
<tr>
<td>15</td>
<td>46.1</td>
<td>44.6</td>
</tr>
<tr>
<td>10</td>
<td>43.98</td>
<td>34.39</td>
</tr>
</tbody>
</table>
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In TLC method the spots on layer determine the activity of Dragon fruit as compared with standard Gallic acid, the figure shows that the fruit have a good activity against DPPH compound; this is depending on the high anti-oxidant content in the fruit extract.

Quantitative determination of Free radical scavenging activity assay (DPPH method)

The DPPH method to determine the scavenging activity for fruit extract show that it have a potent scavenging activity and IC$_{50}$, but the results show that the scavenging activity for the fruit extract is 29µg/ml (Fidrianny and Sahar, 2014).

Anti-bacterial studies

In the present work, four pathogenic bacteria (Escherichia coli, Klebsiella sp, Staphylococcus epidermidis and Staphylococcus aureus) and one fungi (Candida albicans) were tested for their sensitivity to different solvents of Hylocereus undatus peel using well diffusion method. The diameters of inhibition zone exhibited by each extract towards the selected bacteria. The four extracts were; ethanol extract (REE), Dmso extract (RDE), aqueous extract (RAE), methanol extract (RME) and crude extract (RCE), which exhibited inhibition zones of about 7-11 mm against certain bacteria, indicating a broad spectrum activity against both gram positive and gram negative bacteria (Nurmahani et al., 2012).

LD$_{50}$ examination

The acute toxicity test of the dragon fruit extract compared with the control group shows that after the
Phytochemical Content and Anti-oxidant Activity of *H. undatus*

A number of clinical signs like Tack cardiac, internal respiration, sedation have been shown which continue for a period of 1-2 hrs, but after 24 hrs all sign disappeared and all animal return to normal state this may be due to the nature of the plant extract and its abundance with active ingredients that may show their effects immediately after giving. Extract have no mortality or morbidity after the extract dose, but appearance of different signs, which revealed that the extract was not toxic with this dose or concentration used (Hsu et al., 2011).

Histological study

Histological examination of the collected groups with the plant extract noted in the section of the heart tissue, that there was a degeneration in some heart cells with emergence the case of apoptotic for others as shown in fig. 6 (Tsai et al., 2011), while the brain section (fig. 7) shows the emergence of Oedema, degenerative changes in the brain tissue with the advent of Mishra et al. (2013), apoptotic cells for some brain cells and this indicates that the plant extract contains some active substances that have a simple toxic action, which effect on the animal.

### Table 4: The inhibition zone for fruit extracts.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Symbol</th>
<th>REE</th>
<th>RDE</th>
<th>RAE</th>
<th>RME</th>
<th>RCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td>9mm</td>
<td>7mm</td>
<td>11mm</td>
<td>10mm</td>
<td>11mm</td>
</tr>
<tr>
<td><em>Klebsiella</em> sp</td>
<td></td>
<td>11mm</td>
<td>11mm</td>
<td>9mm</td>
<td>11mm</td>
<td>10mm</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td></td>
<td>10mm</td>
<td>7mm</td>
<td>11mm</td>
<td>9mm</td>
<td>11mm</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td>10mm</td>
<td>9mm</td>
<td>11mm</td>
<td>8mm</td>
<td>11mm</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td></td>
<td>9mm</td>
<td>8mm</td>
<td>10mm</td>
<td>9mm</td>
<td>11mm</td>
</tr>
</tbody>
</table>

Ethanol extract (REE), Dmso extract (RDE), Aqueous extract (RAE), Methanol extract (RME), Crude extract (RCE).

### Table 5: shows the LD50 for Dragon fruit.

<table>
<thead>
<tr>
<th>No.</th>
<th>Dose of extract</th>
<th>No. of mice/group</th>
<th>No. of dead/No. of animal</th>
<th>Sign of animal treated with extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50mg/kg b.w</td>
<td>2</td>
<td>0.2</td>
<td>Irregular heartbeat (tachycardia), simple tremor, stiffness or stiffened hair which takes few minute then disappeared.</td>
</tr>
</tbody>
</table>

Fig. 5: Shows the inhibition zone for (1- RME 2- REE 3- RDE 4-RME 5- RCE).
Immediately after administration, this is confirmed by its concentration in the cardiac and cerebral tissues, so resulting pathological changes (Saleh et al., 2013).

Wound healing for mice

By examining ointment in the treatment of wounds developed in white mice the ability of ointment was
observed by made the healing faster than the water extract as seen in the figures, where the ointment speeds up the formation process of the connective tissue in the outer skin areas (Perez et al., 2005). This due to the plant contains a lot of active substances that interact with the components of ointment give the ointment this effective therapeutic role, ointment works on The composition of the outer envelope surrounds the open wound so that it can be removed from external influences. The ability of the ointment penetrates into the skin tissue which increases the tensile strength in the skin tissue and increases the production height the epithelial layer and collagen composition around the wound area thus enhance the healing process and return the mouse skin to its normal state (Villegas et al., 1997). In the comparison with the ficudin that use as a standard, the appearance of hair in near to be equal to the cream of dragon, while the cream shows better ability than control group in the healing ability and hair appearance. No side effects or behavioral changes were observed by the laboratory animal (mice) length treatment period (Tahir et al., 2017).

**Conclusion**

The present works explained the phytochemical, antioxidant activity of Dragon (*Hylocereus undatus*) fruit, study of their bioactivity versus pathogenic bacteria and study the toxicity effect on mice. The results showed that the Dragon was a good source for antioxidant ability observed by chemical DPPH method. The Dragon fruit was rich in phenolic compounds, antioxidants, unsaturated fatty acids, terpenes, many of trace elements and others. Likewise, the extract shows effective agent against both gram-negative, gram-positive bacteria. The surgical and histological study show that fruit extract had a very little toxic effect. Also, it had ability of wound healing by using the extract cream, which shown an amazing ability to heal the wounds and had a higher activity than the water extract alone. This may be useful in a variety of applications in pharmaceutical, biomedical fields, industrial appliances.

**Acknowledgements**

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