**Citrullus colocynthis** as a bioavailable source of β-sitosterol, antihyperlipidemic effect of oil in rabbits

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**Abstract:** A phytochemical study on the seed and kernel of *Citrullus colocynthis* has been carried out in the present paper. The results revealed the presence of the β-sitosterol in the kernel and seed in different percent (seed contain 16.92% and the kernel contain 6.62%). The lipid lowering active compound (β-sitosterol) has been detected by using TLC, IR, UV and H1-NMR. The present study was designed to reveal the hypocholesterolemic effect of β-sitosterol on the serum total cholesterol and triglyceride in rabbits. Significant drop in serum total cholesterol and triglyceride was observed at 120 h after first administration. These results support the suggestion of *Citrullus colocynthis* oil as a treatment for hyperlipidemia.

**Keywords:** *Citrullus colocynthis*; β-sitosterol; Hyperlipidemia.

**Introduction**

*Citrullus Colocynthis* (Cucurbitaceae), commonly known as bitter apple is a tropical plant that grows abundantly in the Arabian countries and in other parts in Asia. In the traditional medicine, this plant has been used to treat constipation (Alkofahi et al, 2006), diabetes (Gurudeeban, 2010), antimicrobial (Gurudeeban et al, 2011), oedema, fever, jaundice leukaemia, bacterial infections, cancer, hair loss treatment (Dixit, 2007), and as an abortifacient (Madari and Jacobs, 2004). Root of this plant is given in abdominal enlargements and in coughs and as thymic attacks of children (Hakeem and Syed Waseemuddin, 2003). Oils and fats are substances of vegetable or animal origin. The high world demands for oils and fats to meet the multiplex human consumption and the multitudinous industrial needs are the reasons for the increase in the importance of oil seeds and make them play in important role in the national economy of the producing countries (Charly, 1982).

Sitosterols (C29H50O) are widely distributed throughout the plant kingdom, especially of green leaves and they have usual steroid structure sometimes referred to vegetable cholesterol (Fruton, 1962, Eduard and Varro, 1968).

Preliminary pharmacological investigations prove that β-sitisterol has a moderate anti-inflammatory activity (Senatore et al, 1989). On the other hand, β-sitosterol in hypocholesterolemic agent that acts by decrease or blocking the absorption of both exogenous and endogenous cholesterol from the gastrointestional tract, and lowering the blood cholesterol level (Murray et al, 1996; James et al 1975).

The purpose of our researches was the pharmacognostical study of citrullus colocynthis (seed and kernel) cultivated in Iraq-Kurdistan, in order to establish quality criteria of this plant.

**Material and methods**

**Plant material**

*Citrullus colocynthis* fruits were collected from Kurdistan (Northern Iraq) divided in half and the seeds removed by hand from kernel, dried and they were powdered mechanically. The lipid fraction was extracted with petroleum ether (40º-60º) in a soxhlet apparatus. The sol-
vent was evaporated and the lipid fraction was weighted. (Table 1).

Separation of $\beta$-sitosterol

100 ml of alcoholic potassium hydroxide (5% w/v) was added to the oil extract, then refluxed and heated on water bath for 3 hrs. the solution was extracted ,while just warm ,three times with ethyl acetate (100 ml),poured each ethyl acetate extract into another separator containing (40ml) of distilled water. The acetate extracts were combined ,dried Na2SO4 poured in to a weighted flask and evaporated, the pale yellow oily materials was weighted (Butter,1973).Table ( 1)

Standard and reagents

$\beta$ -Sitosterol (purity 98%) was purchased from sigma Aldrich chemie GmbH (Aldrich Division, Steinbeim , Germany).

Characterisation of extracts

Thin layer chromatography was performed on pre coated silica gel-G plates (10×10) (Emerck,Germany) for characterization of the extract. Cyclohexan-acetone-acetic acid 65:33:2 v/v as mobile phase, gave best resolution for petroleum ether extract. The spots were visualized using 20% H2SO4 as derivatising agent ,after that we used IR, UV and H-NMR for identification of $\beta$-sitosterol.

Animal

Male domestic rabbits were used in an experiment in june 2011. the rabbits were adapted for five day before the start of the experiment. The animal were divided in to two groups with approximately the same weight distribution (1.55-1.85Kg) in each group

- Group 1: control group 4 rabbits-non treated $\beta$-sitosterol
- Group 2: tested group 6 rabbits received 80mg/10ml of $\beta$-sitosterol per animal /day

Water suspension of the dried $\beta$-sitosterol was given orally using special stomach tube, at 9.00am and 9.00pm every day for a week.

Serum total cholesterol and triglyceride were analyzed by an enzymatic CHOD-POP method using the test kit of bio Me’rieuxsa (69280.Marcyl/France).

Statistical analysis

All value expressed as mean ±standard error (SEM). Independent student’s test was applied to analyze the significance of differences between mean values and critical p-value were considered to be significant.

Results and discussion

The results indicated that Citrullus colocynthis seed and kernel contain $\beta$-sitosterol (Table 1), to examine the $\beta$-sitosterol in oil, a simple TLC has been performed, and the solution of standard of $\beta$-sitosterol has been used as markers (Table 2). The infrared spectrum of $\beta$-sitosterol showed in (Figure 1), while the nuclear magnetic resonance spectrum (H1-NMR) of $\beta$-sitosterol showed in (Figure 2). In comparison between TLC, IR,H-NMR, and UV spectrum , we found that the compound appeared to be $\beta$-sitosterol which isolated from Citrullus colocynthis.

After that, the present study was designed to reveal the hypercholesterolemic effect of $\beta$-sitosterol of Citrullus colocynthis ,on the serum total cholesterol and triglycerides in rabbits.

Significant drop (P<0.01) in serum total cholesterol and triglyceride was observed, at 120hrs.after first administration (Table 3 and 4). Quantification of $\beta$-sitosterol in petroleum ether extract, show that the sample of Citrullus colocynthis seed (16.95%), contain more $\beta$-sitosterol than the kernel of Citrullus colocynthis (6.62%).

The results of this study are generally in agreement with those of (Bhadra et al., 1991) and (Sato et al, 1995) in that of the $\beta$-sitosterol accumulation caused a significant reduction in cholesterol content of cell.

The present experiments lead us to the conclusion Citrullus colocynthis at it antihyperlipidemic effect, and these properties due to the presence of $\beta$-sitosterol. And further studied are in progress in our laboratory to iso-
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Table 1: Oil content in the plant.

<table>
<thead>
<tr>
<th>Wt of plant</th>
<th>Oil gm</th>
<th>β-sitosterol gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 gm</td>
<td>10.49</td>
<td>6.62%</td>
</tr>
<tr>
<td>Plant kernel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 gm</td>
<td>27.48</td>
<td>16.95%</td>
</tr>
<tr>
<td>Plant seed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Property of β-sitosterol TLC.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Solvent</th>
<th>Rf</th>
<th>λ max in CH3OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>Cyclohexan-aceton-acetic acid (65:33:2)</td>
<td>8.7</td>
<td>278.3</td>
</tr>
<tr>
<td>Kernel</td>
<td>Cyclohexan-aceton-acetic acid (65:33:2)</td>
<td>8.7</td>
<td>278.3</td>
</tr>
</tbody>
</table>

Figure 1: I.R. spectrum of compound β-sitosterol (B)

Table 3: Statistical analysis of the effect of β-sitosterol on total cholesterol

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Mean µg/dl</th>
<th>Calculated ±t</th>
<th>Tabulated ±t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T1</td>
<td>83.4</td>
<td>0.471</td>
<td>3.13</td>
</tr>
<tr>
<td>T2</td>
<td>72</td>
<td>2.35</td>
<td>3.13</td>
</tr>
<tr>
<td>T3</td>
<td>63</td>
<td>3.12</td>
<td>3.13</td>
</tr>
</tbody>
</table>

Figure 2: 1H-N.M.R. spectrum on compound β-sitosterol (B).

Table 4: Statistical analysis of the effect of β-sitosterol on triglyceride

<table>
<thead>
<tr>
<th>TG</th>
<th>Mean Mg/dl</th>
<th>Calculated ±t</th>
<th>Tabulated ±t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T1</td>
<td>68.01</td>
<td>1.07</td>
<td>3.13</td>
</tr>
<tr>
<td>T2</td>
<td>44.6</td>
<td>1.81</td>
<td>3.13</td>
</tr>
<tr>
<td>T3</td>
<td>37.5</td>
<td>7.25</td>
<td>3.13</td>
</tr>
</tbody>
</table>

T1=48 hrs. after administration (2days)
T2=96 hrs. after administration (4days)
T3=120 hrs. after administration (6days)

References


Butter Worth and Copublisher (1973) Pearson, Laboratory Techniques in food analysis, London


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