In–Vitro Cytotoxic Active Compounds Isolated from Isatis microcarpa J. Gay Ex Boiss
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ABSTRACT
This is the first study on the cytotoxic activity of Isatis microcarpa J.Gay ex Boiss, a plant growing widely in north Sinai, Egypt. The in vitro cytotoxic activity of the total extract, petroleum ether, dichloromethane, ethyl acetate and methanol fractions were studied on hepatic (HEPG2) and breast carcinoma (T47D) cell lines. It could be concluded that, the most active fraction was the dichloromethane. Three compounds were isolated and identified from this fraction, two indigo dyes and one phenolic acid. They were identified as Tryptanthrin, Indirubin and gallic acid by using different spectroscopic methods.

Keywords: Isatis microcarpa, cytotoxic activity, indigo dyes, phenolic acids, tryptanthrin, indirubin, gallic acid.

INTRODUCTION
Cancer is a life threatening disease, and according to the WHO (2008) about 75 million people die due to it representing 13% of the total death this year. It is supposed that naturally active agents isolated from wild plants are used as alternative curative and more safe than other chemically synthesized drugs. Therefore the aim of the present study was to search for these agents among the Egyptian desert wild plants. Family Cruciferae is one of the important plant families due to the medicinal importance of its members, the family contains 372 genera and 4060 species, one of them is Isatis to which the plant under investigation belongs. Isatis microcarpa J.Gay ex Boiss. is an annual plant grows on the Mediterranean coastal region, north and middle Sinai and middle Asia. Preliminary phytochemical screening of Isatis microcarpa indicated the presence of glucosinolates, phenolic acids, flavonoid and anthraquinones. The isolated and purified glucosinolate compounds were identified as 4-methylthio-3-butenyl glucosinolate, 6-methyl sulfonyl hexyl glucosinolate and 6-methyl sulfonyl-6-hydroxyhexyl glucosinolate. Also phenolic acids identified as, ferulic acid, gallic acid and caffeic acid. The isolated and purified anthraquinines compounds were chrysophanol, physcion and emodin. In the present study, the cytotoxic activity of the different plant fractions has been studied and the compounds responsible for these activities were isolated, purified and identified.

MATERIAL AND METHODS
Plant materials
Aerial parts of Isatis microcarpa was collected from El-Arish, North Sinai during 2012. The plant was identified in the Herbarium of the Desert Research Centre. The plant was cleaned, dried in shade, ground to fine powder, and then used in the following investigations.
Preparation of the extracts
Air dried plant powder (500 g) was extracted using methanol in Soxhlet apparatus. The methanolic extract was evaporated in vacuum at 45°C. Fractionation of the dried plant powder (500 g) using different polar solvents (petroleum ether (40-60°C), dichloromethane, ethyl acetate and methanol) was carried out. These fractions were kept for cytotoxic activity investigation and isolation of the compounds.
In vitro assay of cytotoxic activity
Two kinds of human cancerous cell lines were used: human liver carcinoma (HEPG2) and human breast carcinoma (T47D). Cytotoxicity of the different extracts and IC50 were obtained using the method described by Skehan et al. Leading to four fractions, fraction 3 subjected to sephadex column using methanol as eluent. This column lead to compound (1). Subfraction (B) subjected to crystallization leading to compound (2). Subfraction (C) subjected to sephadex column using methanol as eluent. This lead to compound (3).
Identification of the isolated compounds
The isolated compounds were subjected to H-NMR and 13C-NMR spectroscopic methods.

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RESULTS

**In-vitro cytotoxic activity**

The total extract, petroleum ether, dichloromethane, ethyl acetate and methanol fractions were tested against two cancer cell lines [Human liver (HEPG2) and Breast (T47D)] and the percentage inhibition of the vital cells was represented in table (1) and figure (1), all fractions lead to decrease the number of vital cancer cells. From the IC\textsubscript{50} of all fractions against the two cell lines [table (2) and figure (2)] it was found that, the dichloromethane fraction showed the lowest IC\textsubscript{50} (thus the most effective). The effect was better on the breast carcinoma cells (IC\textsubscript{50} = 20.8 µg/ml) than on liver carcinoma cell line (IC\textsubscript{50} = 34.3 µg/ml). Thus the active compounds of this fraction were isolated.

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**Table 1: Cytotoxic Activity of Total Extract and Different Fractions of *Isatis microcarpa* J.Gay ex Boiss. on Human Liver (HEPG2) and breast (T47D) Carcinoma Cell Lines.**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Total extract</th>
<th>Petroleum ether fraction</th>
<th>Dichloromethane fraction</th>
<th>Ethyl acetate fraction</th>
<th>Methanol fraction</th>
<th>Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HEPG2 T47D</td>
<td>HEPG2 T47D</td>
<td>HEPG2 T47D</td>
<td>HEPG2 T47D</td>
<td>HEPG2 T47D</td>
<td>HEPG2 T47D</td>
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<tr>
<td>0.0</td>
<td>100 100</td>
<td>100 100</td>
<td>100 100</td>
<td>100 100</td>
<td>100 100</td>
<td>100 100</td>
</tr>
<tr>
<td>5.0</td>
<td>59.5 95.2</td>
<td>88.6 87.4</td>
<td>95.2 74.1</td>
<td>94.8 77.2</td>
<td>92.2 53.7</td>
<td>21.4</td>
</tr>
<tr>
<td>12.5</td>
<td>61.9 81.1</td>
<td>76.2 71.1</td>
<td>85.7 64.1</td>
<td>75.7 77.8</td>
<td>62.5 43.3</td>
<td>20.1</td>
</tr>
<tr>
<td>25.0</td>
<td>57.1 47.4</td>
<td>65.2 51.9</td>
<td>63.8 43.0</td>
<td>61.4 52.2</td>
<td>57.1 37.8</td>
<td>18.2</td>
</tr>
<tr>
<td>50.0</td>
<td>42.9 41.5</td>
<td>34.0 35.9</td>
<td>26.7 43.3</td>
<td>41.4 48.1</td>
<td>47.1 43.7</td>
<td>34.1 25.2</td>
</tr>
</tbody>
</table>

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**Figure 1:** Cytotoxic Activity of Total Extract and Different Fractions of *Isatis microcarpa* J.Gay ex Boiss. on Human Liver (HEPG2) and Breast (T47D) Carcinoma Cell Lines

**Figure 2:** IC\textsubscript{50} of Total Extract and Different Fractions of *Isatis microcarpa* J.Gay ex Boiss. on Human liver (HEPG2) and Breast (T47D) Carcinoma Cell Lines.

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C-NMR analysis on Bruker High Performance Digital FT-NMR Spectrometer Advanced III 400 MHz (270 MHz for $^1$H-NMR and 67.5 MHz for $^{13}$C-NMR)

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**RESULTS**

C-NMR analysis on Bruker High Performance Digital FT-NMR Spectrometer Advanced III 400 MHz (270 MHz for $^1$H-NMR and 67.5 MHz for $^{13}$C-NMR)
Table 2: IC_{50} of Total Extract and Different Fractions of *Isatis microcarpa* J.Gay ex Boiss. on Human liver (HEPG2) and Breast (T47D) Carcinoma Cell Lines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HEPG2 (μg/ml)</th>
<th>T47D (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total extract</td>
<td>37.6</td>
<td>23.9</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>39.6</td>
<td>28.0</td>
</tr>
<tr>
<td>Dichloromethane fraction</td>
<td>34.3</td>
<td>20.8</td>
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<tr>
<td>Ethyl acetate fraction</td>
<td>38.9</td>
<td>38.5</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>42.2</td>
<td>38.5</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>7.73</td>
<td>3.08</td>
</tr>
</tbody>
</table>

Table 3: Name and structure of isolated compounds

<table>
<thead>
<tr>
<th>Comp. number</th>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trypanthrin (Indolo [2,1-b]quinazoline-6,12-dione)</td>
<td><img src="image" alt="Structure of Trypanthrin" /></td>
</tr>
<tr>
<td>2</td>
<td>Indirubin</td>
<td><img src="image" alt="Structure of Indirubin" /></td>
</tr>
<tr>
<td>3</td>
<td>Gallic acid</td>
<td><img src="image" alt="Structure of Gallic acid" /></td>
</tr>
</tbody>
</table>

Identification of the isolated compounds

**Compound 1: Trypanthrin** (Indolo [2,1-b]quinazoline-6,12-dione)

Yellow solid; ^1^H NMR (300 MHz CDCl₃) δ = 8.87 (d, 1H, J = 8.12 Hz), 8.39 (d, 1H, J = 1.24 Hz), 7.97 (d, 1H, J = 7.64 Hz), 7.86-7.70 (m, 3H), 7.61 (t, 1H, J = 7.1 Hz), 7.36 (t, 1H, J = 7.05 Hz); ^1^C NMR (75 MHz CDCl₃) δ = 117.5, 120.6, 125.4, 126.3, 127.1, 129.7, 130.0, 133.2, 134.6, 145.3, 146.6, 160.4, 183.8.

**Compound 2: Indirubin**

Blue purple solid; ^1^H NMR (DMSO) δ, 10.88 (s) and 10.63 (s; NH and N'H), 8.74 (d, 1H, J = 7.52; H-4), 7.62 (d, 1H, J = 7.44; H-4'), 7.5 (m, 1H; H-6'), 7.30 (d, 1H, J = 7.96; H-7'), 7.21 (m, 1H; H-6), 6.98 (m, 2H; H-5 and H-5'), 6.87 (d, 1H, J = 7.6; H-7).

**Compound 3: Gallic acid**

Offwhite amorphous powder; ^1^H-NMR (CDCl₃) 7.19 (2H, s, H-2,6), ^1^C-NMR (CDCl₃): 121.0 (C-1), 109.0 (C-2 & C-6), 145.9 (C-3 & C-5), 138.3 (C-4), and 168.0 (C-7).

Gallic acid was previously identified and isolated from this species.

**CONCLUSION**

This is the first study on the cytotoxic activity of *Isatis microcarpa* against liver and breast cancer. From the previous results it could be concluded that, the plant extracts possess cytotoxic activity against both cell lines and the most active fraction was dichloromethane (lowest IC_{50}). It is supposed that the cytotoxic activity of the dichloromethane fraction is mainly due to the three isolated compounds (Trypanthrin, Indirubin and gallic acid). It is recommended to study the mechanism of action of these compounds. This plant needs more investigations about its side effects on different body organs and to introduce this plant into clinical trials. Further investigation should be carried out to increase the active ingredients using tissue culture techniques to facilitate its applied industrialization.

**REFERENCES**

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