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Research Article

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 Cruciferea 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 costal region, north and middle Sinai and middle Asia[1]. 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[2]. 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 cytoxic 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 IC_{50} were obtained using the method described by Skehan *et al*.[3]

Isolation of active compounds

The dichloromethane fraction (the most active cytotoxic fraction) was applied to column chromatography using silica gel as stationary phase and dichloromethane as mobile phase and increasing the polarity gradually using ethyl acetate then methanol. This column led to three subfractions (A, B and C).

Subfraction (A) was subjected to column chromatography using silica gel stationary phase and chloroform eluent. 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[1] H-NMR and[13]

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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.

Concentration (µg/ml)	*Percentage of Survival Cells*											
	Total extract		Petroleum ether fraction		Dichloromethane fraction		Ethyl acetate fraction		Methanol fraction		*Doxorubicin*	
	HEPG2	T47D	HEPG2	T47D	HEPG2	T47D	HEPG2	T47D	HEPG2	T47D	HEPG2	*T47D*
0.0	100	100	100	100	100	100	100	100	100	100	100	*100*
5.0	59.5	95.2	88.6	87.4	95.2	74.1	71.4	94.8	77.2	92.2	53.7	*21.4*
12.5	61.9	81.1	76.2	71.1	85.7	64.1	75.7	77.8	62.5	64.1	43.3	*20.1*
25.0	57.1	47.4	65.2	51.9	63.8	43.0	61.4	52.2	57.1	57.4	37.8	*18.2*
50.0	42.9	41.5	34.0	35.9	26.7	33.3	41.4	48.1	47.1	43.7	34.1	*25.2*

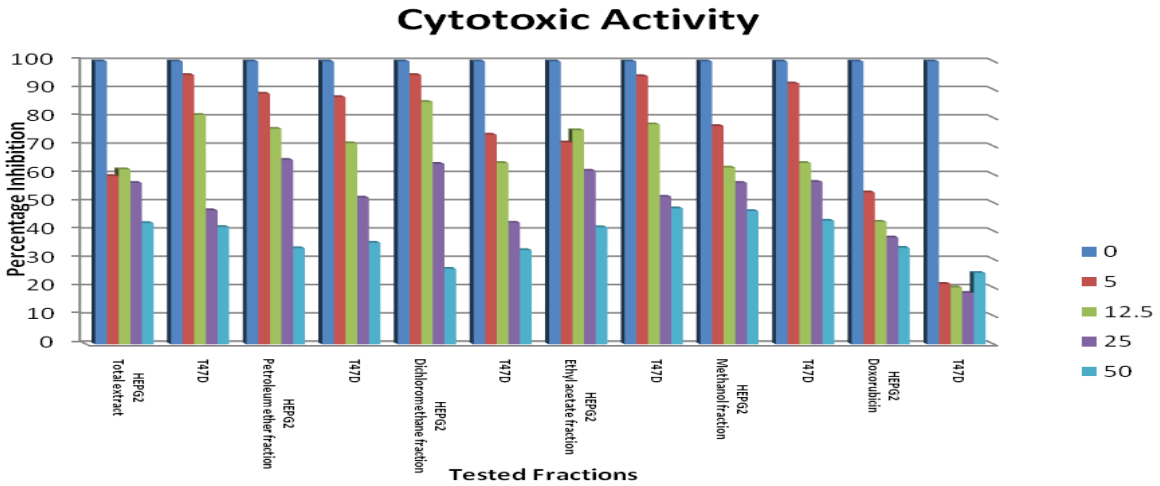


Figure 1: Cytotoxic Activity of Total Extract and Different Fractions of *Isatis microcarpa* J.Gay ex Boiss. on Human Liver (HEPG2) and Breast (T47D) Carcinoma Cell Lines

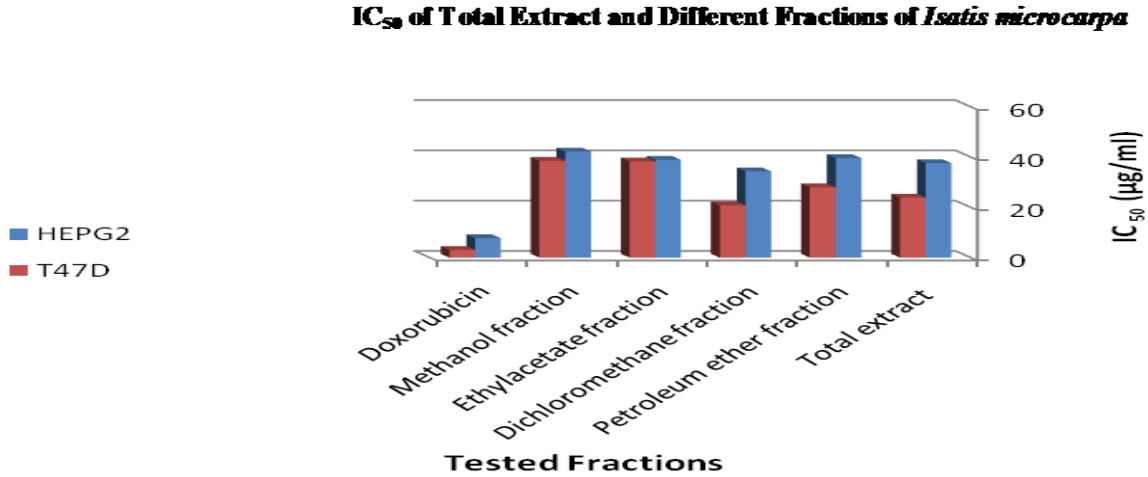


Figure 2: IC_{50} of Total Extract and Different Fractions of *Isatis microcarpa* J.Gay ex Boiss. on Human liver (HEPG2) and Breast (T47D) Carcinoma Cell Lines.

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)

RESULTS

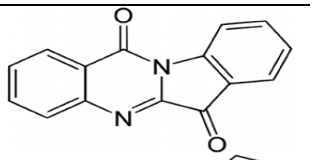
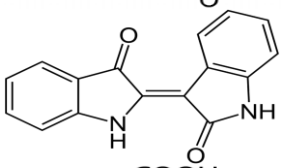
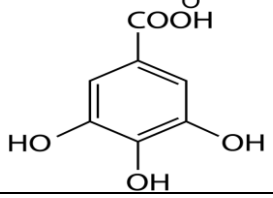
In-vitro cytotoxic activity

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_{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_{50} (thus the most effective). The effect was better on the breast carcinoma cells (IC_{50} = 20.8 µg/ml) than on liver carcinoma cell line (IC_{50} = 34.3 µg/ml). Thus the active compounds of this fraction were isolated.

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.

	IC 50 (µg/ml)	
	HEPG2	*T47D*
Total extract	37.6	*23.9*
Petroleum ether fraction	39.6	*28.0*
Dichloromethane fraction	34.3	*20.8*
Ethyl acetate fraction	38.9	*38.3*
Methanol fraction	42.2	*38.5*
Doxorubicin	7.73	*3.08*

Table 3: Name and structure of isolated compounds

Comp. number	Name	Structure
1	*Tryptanthrin* (Indolo [2,1-b]quinazoline-6,12-dione)	
2	*Indirubin*	
3	Gallic acid	

Identification of the isolated compounds

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

Yellow solid; ^{1}H NMR (300 MHz $CDCl_3$) δ= 8.57 (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, 0.56 Hz); ^{13}C NMR (75 MHz $CDCl_3$) 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 ($CDCl_3$) 7.19 (2H, s, H-2,6), ^{13}C-NMR ($CDCl_3$): 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[2].

DISCUSSION

Three compounds were isolated from the dichloromethane fraction of the aerial parts of *Isatis microcarpa* J.Gay ex Boiss.; two indigo compounds and one phenolic acid. These compounds have been previously studied for their anticancer activity and showed a potent activity. Compound 1 (Trypanthrin) showed anti-microbial, antiinflammatory, immunomodulatory as well as its anti-tumor effect on the murine myelomonocytic leukemia WEHI-3B JCS cells by causing cell cycle arrest and by triggering cell differentiation[4]. Compound 2 (Indirubin) used in treatment of chronic myelogenous leukemia. Indirubin inhibits cyclin-dependent kinases (CDKs) and induces cell cycle arrest and apoptosis in cancer cells[5]. Compound 3 (Gallic acid) besides having antineoplastic and bacteriostatic activities, gallic acid possesses antimelanogenic and antioxidant properties[6]. A phenolic fraction from evening primrose (*Oenothera biennis*) containing gallic acid showed anti-tumour activity[7]. Gallic acid has shown anticancer properties in prostate carcinoma cells[8,9]. It is believed that, gallic acid is partially responsible for the antiangiogenic activities of sweet leaf tea (*Rubus suavissimus*) extract[10]. Gallic acid has been proposed to be a candidate for treatment of brain tumours as it suppresses cell viability, proliferation, invasion, and angiogenesis in human glioma cells[11]. Gallic acid induced HeLa cervical cancer cells death via apoptosis and/or necrosis[12].

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.

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