Cytotoxic and Antioxidant Activities of the Volatile Constituents of *Brassica tournefortii* Gouan: Growing in Egypt

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Abstract

The volatile constituents of the seeds of *Brassica tournefortii* Gouan, growing in Egypt -prepared by simultaneous solvent extraction (steam distillation using Likens-Nickerson type apparatus (SSD-SE) technique) - were investigated by GC/MS analysis. This resulted in the identification of 14 compounds which amount to 76.1% of the total percentage components detected in essential oil of the seeds. Results revealed that the oil was rich in sulphur compounds represented by di-2-propenyltetrasulfide (19.803%) and 3-(methylthio) propyl isothiocyanate (24.39%). Consequently, the cytotoxic activity against two human cancer cell lines; breast (MCF7) and colon (HCT116) were tested and significant results ($IC_{50} = 1.34$ and $4.5 \mu\text{g mL}^{-1}$ respectively) were obtained. On the other hand, the in vitro anti oxidant activity was also tested exhibiting significant free radical scavenging effects ($IC_{50} = 45.17 \mu\text{g/mL}$) compared to vitamin c ($75.28 \mu\text{g/mL}$) which was used as a reference.

Keywords: *Brassica tournefortii* Gouan; Cytotoxic activity; MCF7; HCT116; Free radical scavenging activity

Introduction

Family Crucifereae (Mustard family, Brassicaceae) [1], is a worldwide distribution. It comprises about 375 genera and 3200 species. *Brassica* is an important genus in the family. The members of the genus are collectively known as cruciferous vegetables as; cabbages, cauliflower, broccoli and brussels sprouts or mustards [2]. Cruciferous vegetables have been extensively studied due to their typical flavor and odor, attributed to volatile sulphur and nitrogen-containing compounds. These vegetables have been reported to lower the risk of developing various cancers [3].

Brassica tournefortii (Gouan) F. Crucifereae is an annual herb. It is also known as Asian mustard, Sahara mustard, Moroccan mustard, African mustard, Mediterranean mustard, Mediterranean turnip, wild turnip, desert mustard, southwestern mustard, Tournefort's mustard, prickly turnip, turnip weed [4]. It grows widely in sandy and loam field, gardens, Mediterranean coastal strip from El-Saloum to Rafah, Sinai proper (El-Tih desert) and Oases of the Libyan Desert [5].

Cancer is a major public health problem in both developed and developing countries. Since 1990, there has been a 22%

increase in cancer incidence and mortality with the four most frequent cancers being lung, breast, colorectal, and stomach [6,7]. Breast cancer is the most frequent malignant tumor in women worldwide. In Egypt, it is the most common cancer among women, representing 18.9% of total cancer cases recorded by the National Cancer Institute in Egypt (NCI) [8]. On the other hand, colorectal cancer is the third most commonly diagnosed and the second leading cause of cancer death in men and women combined in US. In Egypt, colorectal carcinoma is uncommon, but a high proportion of cases occur before age 40 years and mainly in the rectum [9].

Medicinal plants have played important roles in the last five decades in the treatment of cancer and most new clinical applications of plant secondary metabolites and their derivatives have been applied towards combating cancer.

In that respect and since no reports dealt with the chemical composition of the essential oil from *B. tournefortii* Gouan., the aim of this paper is therefore to determine and identify the steam volatiles content of the seeds of *B. tournefortii* Gouan. Furthermore, cytotoxic activity against breast and colon human cancer cell line, antioxidant capacity in scavenging 2,2 diphenyl 1-picrylhydrazyl (DDPH) were also studied.

Material and Methods

Plant materials

Brassica tournefortii Gouan is collected from Cairo-Alex Desert road in March 2007. The plant was kindly authenticated by Prof. Dr. Mohammed El-Gebaly, Senior botanist, El-Orman garden, Giza, Egypt. A voucher specimen (No. BT-2009) is kept in the herbarium museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt.

Chemicals

All chemical reagents were of analytical grade and all solvent were of GC and HPLC grade. DDPH and vitamin C standards were purchased from Sigma-Aldrich St. Louis, MO, USA)

The human breast (MCF7) and colon (HCT116) cancer cell line was obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection. The tumor cell lines were

maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

Preparation of volatile constituents

A sample of the seeds (100 g) was crushed and placed in a 1 L flask with 0.5 L of water (to allow enzymatic degradation of glucosinolates to sulphur containing Compounds). The mixture was subjected to simultaneous solvent extraction-steam distillation using a Likens-Nickerson type apparatus for 5 h, using diethyl ether as the extracting solvent to yield yellowish oil (0.2 ml), which was dried over anhydrous sodium sulphate. The extract was stored at low temperature (-4°C) prior to GC/MS analysis

GC/MS analysis

Investigation of the prepared oil was carried out by GC/MS on SHIMADZU GC/MS-QP system. The instrument was equipped with DB₁ column, (30 MX 0.53 i.d., 1.5 μm film thickness); carrier gas, helium, with 1 ml/min., flow rate; detector used, FID; sample size, 0.5 μl ; injector temperature, 25°C ; detector temperature, 280°C ; oven temperature program, 40°C (1 min.)- 150°C (1 min.) at $7.5^{\circ}\text{C}/\text{min}$ - 250°C (2 min.) at $1.2^{\circ}\text{C}/\text{min}$. Ionization mode, EI, mass ionization voltage was 70 ev. The start-stop masses were 30-500 aum.

The constituents of the oils were identified by comparing their retention times, Kovat's indices and mass fragmentation patterns with those of available reference samples, GC/MS database, spectrometric electronic libraries (Wiley138, NIST, NBS) and comparing with the published data [10,11]. Kovat's retention indices of the compounds were calculated relative to the retention times of a series of *n*-paraffin hydrocarbons with a logarithmic scale [12]. Relative percentages were calculated from the Total Ion Chromatograms by the computerized integrator.

Results of the GC/MS analysis were recorded in the Table 1.

Peak no.	R_t (min)	RI	Area percentage	Identified Constituent
1	10.74	-----	24.394	Propane, 1-isothiocyanato-3-(methylthio)
2	12.19	-----	2.732	2-propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)
3	13.95	1430	1.785	Ionone
4	14.59	1441	6.690	Aromadendrene
5	16.25	1451	0.824	2-Pentadecanone,6,10,14-trimethyl
6	17.24	1551	1.864	Elimicin
7	17.43	1585	3.073	8,11-Octadecanoic acid, methyl ester
8	18.94	1671	0.440	α -Bisabolol
9	19.50	1876	2.797	1-Hexadecanol
10	21.53	-----	1.706	Hexadecanoic acid, methyl ester
12	21.22	-----	19.803	di-2-propenyltetrasulfide
13	22.25	2015	1.480	<i>n</i> -Heneicosane
14	22.29	-----	1.536	12-Octadecanoic acid, methyl ester
15	23.41	-----	3.431	<i>n</i> -Octadecanoic acid

M⁺: Molecular ion peak; m/z: mass/charge ratio; R_t : Retention time. RI: Retention index

Table 1: Results of GC/MS analysis of the volatile constituents of the seeds of *Brassica tournefortii* (Gouan).

Assessment of cytotoxic activity

Cells were placed in a 96-multiwell plate (10^4 cells/well) in a fresh medium for 24 hours before treatment with the samples. Different concentrations of the samples under investigation (0, 1, 2.5, 5 and 10 $\mu\text{g}/\text{ml}$) were added to the cells. Triplicate wells were prepared for each individual concentration. The cells were re-incubated with the samples for an additional 48 hours at 37°C in an atmosphere of 5% CO_2 . After 48 hours, cells were fixed with trichloroacetic acid, washed and stained with 0.4% (w/v) Sulphorhodamine-B (SRB) stain. Excess stain was removed by four washes with 1% acetic acid to remove unattached dye. The protein-bound dye was recovered using unbuffered Tris EDTA. Cytotoxicity was assessed using Sulphorhodamine-B (SRB) assay [13]. The optical density (O.D.) of each well was measured spectrophotometrically at 564nm with an ELIZA microplate reader (Meter tech. Σ 960, U.S.A.). The percentage of cell survival was calculated as follows:

Survival fraction = O.D. (treated cells)/ O.D. (control cells).
The IC_{50} values (the dose of the oil which reduces survival to 50%). The experiment was repeated 3 times for each cell line [13].

Antioxidant activity

Antioxidant activity was assayed using a modified quantitative 2,2-diphenyl-1-picrylhydrazyl DDPH assay [14]. The solution of DDPH was prepared using HPLC grade methanol and DDPH (sigma-aldrich) at a concentration of 0.004%. Lyophilized extract was dissolved in water at concentration of 1–100 mg mL^{-1} with 5 μL of each tested solution added to 100 ml of DDPH solution. A blank sample was run using HPLC grade methanol. After 30 minute incubation period at room temperature, the absorbance was read against a blank at 550 nm. Vitamin C (sigma-aldrich) was used as positive control at a concentration of 1–100 mg mL^{-1} . Antioxidant activity is expressed as the concentration of extract

inhibiting DDPH formation by 50% relative to methanol (IC_{50} value). Inhibition of the DDPH free radical in percent (I %) was calculated according to this formula:

$$I\% = \left[(A_{blank} - A_{sample}) / A_{blank} \right] \times 100$$

A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the extract. Values are expressed as IC_{50} . Each measurement was performed in duplicate.

Results and Discussion

The steam volatile constituents collected by simultaneous solvent extraction–steam distillation using a Likens–Nickerson type apparatus from *B. tournefortii* Gouan. was obtained as yellow oil with disagreeable odor, in a yield of 0.04%. GC/MS analysis of the oils (Table 1) showed the presence of 14 compounds, representing 72.55% of the total constituents of the essential oil. The oil was dominated by sulphur compounds amounting to 52.9% which could be responsible for the odor of the oil. They were represented as 3-(methylthio) propyl isothiocyanate 24.394% (this compound was obtained from the hydrolysis of Glucoiberberin glucoside) [15] and di-2-propenyltetrasulfide (19.803%) from thermal degradation of S- methyl cysteine sulfoxide [16-19].

Other detected constituents were ionone 1.7%, elemicin 1.864% and aromadendrene 6.69%.

In recent years, cancer prevention by natural products has received considerable attention. The potential protective role of cruciferous vegetables and active components present in these vegetables, such as isothiocyanates and indole-3-carbinol, has been extensively studied. Results have consistently shown that the chemopreventive agents derived from this class of vegetables of the Cruciferae family influence carcinogenesis during initiation and promotion phases of cancer development [20]. Other studies support that the anticarcinogenic mechanisms that have been revealed for isothiocyanates is the inhibition of cell proliferation [21]. The cytotoxic activity of the volatile extract of the seeds of *B. tournefortii* Gouan. was tested against breast (MCF7) and colon (HCT116) human cancer cell line. The volatile extracts showed pronounced cytotoxic activity against breast (MCF7) (Figure 1) and colon (HCT116) (Figure 2) human cell line with IC_{50} 1.34 and 4.5 $\mu\text{g mL}^{-1}$ respectively. This activity could be attributed to the isothiocyanates (ITCs) which represent an important and promising chemopreventive compound [22-27]. In recent years, numerous investigations have shown that sulfur-containing compounds induce apoptosis in multiple cell lines (including breast and colorectal cell line) and experimental animals [28].

Oxidative stress may initiate molecular events in the cancer process, and reduction of oxidative stress may protect against carcinogenesis [29]. Crucifers contain numerous antioxidant substances that potentially induce oxidant enzymes, and combination of theses may protect against carcinogenesis [30]. The *B. tournefortii* Gouan. volatile extract was assessed for its capacity to scavenge DDPH free radical along with vitamin C as positive control. The oil reduced the concentration of DPPH with an IC_{50} value of 45.17 $\mu\text{g/mL}$, which was lower than that of

Figure 1: The relation between the surviving fraction of Breast human cell line and the concentrations of the volatile extracts of the seeds of the *B. tournefortii*.

Figure 2: The relation between the surviving fraction of colon human cell line and the concentrations of the volatile extracts of the seeds of the *B. tournefortii*.

vitamin-C, with an IC_{50} of 75.28 $\mu\text{g/mL}$ this showed that the oil has moderate antioxidant activity related to vitamin-C. These findings support the cytotoxic activity of the volatile constituents obtained from *Brassica tournefortii* Gouan might also be attributed to the antioxidant activity revealed by oxygenated and sulphur constituents of the oil. These effects together with the previous reported mechanism of action of sulphur compounds suggest the use of *Brassica tournefortii* as a chemopreventive natural drug. This is the first report of isolation and cytotoxic activity of the oil from *Brassica tournefortii* Gouan.

Conclusion

GC-MS study of volatile extracts from *Brassic tournefortii* Gouan. seeds led to the identification of 14 compounds. The Formation of the identified isothiocyanate derivative (3-(methylthio) propyl isothiocyanate) can be explained by the hydrolysis of Glucoiberberin glucoside Results evidenced the correlation between crucifer vegetables and potent cancer-preventive therefore, introducing *B. touernefortii* as a new herbal

chemopreventive candidate for growth inhibition of the two studied types of cell lines.

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