Cytotoxic effect of an aqueous extract of *Lepidium sativum* L. seeds on human breast cancer cells

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Easily available alternative and natural remedies for breast cancer are sought to reduce or eliminate the disadvantages of conventional treatments. Garden cress seeds (*L. sativum* L.) are known to contain several chemopreventive and chemotherapeutic constituents. The effect of *L. sativum* plant extract on cancers is generally attributed to the constituent isothiocyanates, specifically benzyl isothiocyanate, which are also present in the seeds. The current work examines, for the first time, the cytotoxic effect of the aqueous extract of *L. sativum* seeds on MCF-7 breast cancer cells, using the trypan blue dye exclusion and sulforhodamine B assays, compared to its effect on normal human HFS cells. The MCF-7 cell culture morphology, after exposure to the extract, was studied using light microscopy. Finally, HPLC was used to ascertain whether benzyl isothiocyanate is contained in the seeds extract. The results showed that the *L. sativum* seeds aqueous extract had a significant cytotoxic effect on MCF-7 cells, by causing significant time- and dose-dependent decreases in their viability. In contrast, the HFS cells were significantly more resistant to the cytotoxic effect of the extract. Therefore, the current study provides strong evidence of the *L. sativum* seeds extract’s ability to inhibit growth of breast cancer cells.

**Keywords:** Cancer chemotherapy, Chemoprevention, Breast cancer, *Lepidium sativum*, Garden cress

**IPC Int. Cl.**: A61K 36/00, A01D 4/04, A01D 12/15, A01D 4/34

Breast cancer is the most commonly diagnosed cancer in women worldwide, and the second leading cause of cancer-related deaths among women. Conventional treatments of breast cancer are costly, have serious side effects, and may cause new cancers to occur. Thus, it is of the utmost importance to find effective chemopreventive or chemotherapeutic agents that have minor or no side effects and that are easily and economically available. It is well known that some herbs have strong and beneficial effects when consumed on a regular basis. Some are used as easily available natural remedies to prevent or treat cancers, especially in poor developing countries, but without sufficient scientific proof.

Cruciferous vegetables (Family: Brassicaceae) and their seeds are used in alternative and traditional systems of medicine and healing in many countries. An important member of this group of vegetables is the garden cress (*L. sativum* L.) plant and its seeds, which are used as nutritional constituents and common ingredients in folk remedies used mainly in Arabian and Asian countries. *L. sativum* seeds are recommended for the treatment of many ailments and they have many therapeutic effects.

The seeds of the *L. sativum* plant contain many phytochemicals and their secondary metabolites, such as alkaloids, tannins, flavonoids, and isothiocyanates, which have a wide range of biological activities and known healing effects. Phenolic compounds are present in *L. sativum* seeds, and they have been shown to have diverse functions, such as anti-inflammatory activities that protect the human body from oxidative stress that may lead to cancer.

The oil of the *L. sativum* seeds is rich in alpha linolenic acid, a ω-3 fatty acid, which has been shown to have chemopreventive and chemotherapeutic effects on different types of cancers, including breast, in both animals and human cell line models.

The major secondary compounds of *L. sativum* seeds are the glucosinolates. Glucotropaeolin, the main glucosinolate in *L. sativum*, is degraded to produce benzyl thiocyanate and benzyl isothiocyanate. Benzyl isothiocyanate (BITC) is the most abundant and important isothiocyanate found in the seeds. Isothiocyanates have been shown to have chemical and biological properties that may have potential use in cancer prevention and treatment.
Cells were cultivated in Abdulaziz University Hospital, Jeddah, Saudi Arabia. Normal human skin fibroblasts (HFS) were acquired from the epithelial invasive breast cancer cell line that is hormone-sensitive. This cell line is an epithelial invasive breast cancer cell line, although the mechanism of cell death is not fully understood.

Many studies, as detailed above, have shown that certain constituents of the *L. sativum* plant, and its extracts have chemopreventive and chemotherapeutic effects, but no studies have been done on the effects of any extracts of *L. sativum* seeds on the viability and growth of cancer cells. In fact, and after extensive literature search, we are the only researchers to study the effects of the aqueous extract of *L. sativum* seeds on human breast cancer cells. Therefore, in this work the potential of the aqueous extract of *L. sativum* seeds to induce death of breast cancer cells in tissue culture was investigated in the hope of finding a natural, cheap, and easily available treatment for human breast cancer. This is the first study of the cytotoxic effects of the *L. sativum* seeds extract on cancer cells, and specifically breast cancer.

**Materials and methods**

**Cell lines and culturing of cells**

The human breast cancer cell line MCF-7 (Michigan Cancer Foundation–7) was obtained from the National Cancer Institute, Cairo University, Cairo, Egypt. This cell line is an epithelial invasive breast ductal carcinoma cell line that is hormone-sensitive. Normal human skin fibroblasts (HFS) were acquired from foreskin circumcision operations, King Abdulaziz University Hospital, Jeddah, Saudi Arabia. Cells were cultivated in Dulbecco’s minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (both from GIBCO, Grand Island, NY, USA), 1% L-glutamine, and 1% penicillin/streptomycin and incubated in 5% CO₂, at 37 °C, and 96% relative humidity. All tissue culture work was done at the Tissue Culture Unit, at King Fahd Medical Research Center, Jeddah, Saudi Arabia.

Culture flasks, 6-well cell culture plates, and Petri dishes were purchased from Falcon (Becton Dickinson, Milton Keynes, UK). Polystyrene 24-well and 96-well cell culture plates were purchased from Corning (Corning, NY, USA).

**Aqueous extraction of *L. sativum* seeds**

Dry *L. sativum* seeds were obtained from a local herbalist shop in Jeddah. The type obtained was grown in Al-Qaseem area in Saudi Arabia as authenticated by the herbalist and confirmed by a taxonomist.

Three methods based on traditional ways of extraction and consumption of *L. sativum* seeds were used. The dry seeds were ground, in an electric grinder, to a coarse powder. Using the traditional Moroccan method, 1 gm of powdered seeds was mixed with 100 ml de-ionized water and boiled for 10 minutes, after which the extract was left for 15 minutes at room temperature. For both the second and third methods, 1 gm of powdered seeds was stirred with 99.8 ml of de-ionized water and 0.2 ml of DMSO. For the second method, the mixture was stirred overnight, while for the third method the mixture was boiled for 10 minutes. The extracts were then left at room temperature for 15 minutes. DMSO (0.2%) was added to insure that all ingredients of the seeds were dissolved. The amount of DMSO used was at the allowable tolerable concentration (< 0.2%) for cells.

Each of the three extracts was filtrated first by sterile gauze or any size filter paper, and then by a 9.0 cm filter paper (Whatman Company, NY, USA) to remove the gel-like substances. These resultant filtrates were secondly filtered by a 0.22 µm pore size sterile filter system (Corning Incorporated, Corning, NY, USA). These final stock solutions were frozen at −80 °C and used up to six weeks only.

**Cytotoxic assay of the three extracts on viability of MCF-7 cells**

MCF-7 cells (1×10⁵ cell/ml) suspended in DMEM containing 10% FBS were plated onto 24-well plates at 1 ml per well. After 24 hrs of incubation, the original media was replaced by serum-free media (SFM) that contains one of several concentrations of the extract. As for the controls for each extract, only SFM was used for the first extract, with for the second and third extracts 0.1% DMSO was added in addition to SFM. The cells were harvested by trypsinization and counted by a Vi-CELL XR cell viability analyzer (Beckman Dickinson, Miami, Florida, USA), after 24, 48, and 72 hrs of exposure to the extract.

**Cytotoxic assay of the extract on MCF-7 and HFS cells**

Using the third extract here, and for all subsequent experiments, its cytotoxic effects on the viability of...
MCF-7 and HFS cells were determined as described above. Different concentrations of the extract [extract:SFM (vol/vol); 1:3 (25% extract), 1:1 (50%),
and 3:1 (75%)] were used. The control was SFM containing 0.1% DMSO. The number of living cells was determined following 24, 48, 72, 96 and 120 hrs incubation with the extract.

Proliferative assay

The anti-proliferative effects of the extract on MCF-7 and HFS cells were determined using the sulforhodamine B (SRB) assay\textsuperscript{34}.

MCF-7 and HFS cells were separately cultivated in 96-well microtiter plates, at \(1 \times 10^5\) cell/well in DMEM containing 10% FBS. After 24 hrs, 100 \(\mu\)l of SFM that contains a concentration (20%, 30%, 40%, 50%, 60%, 70%, or 80%) of extract was added. The treated cells were allowed to incubate for 48 hrs. Adherent cells were then fixed by the addition of 50 \(\mu\)l of cold 40% (w/v) trichloroacetic acid in distilled water, and subsequently incubated for 60 min at 4°C. Using a plate washer, the plates were then washed 5 times with deionized water. Next, the cells were stained with 100 \(\mu\)l of 1% acetic acid in distilled water, and subsequently incubated for 10 min at room temperature. Unbound stain was removed and the plate was washed with 200 \(\mu\)l of 1% acetic acid, and then incubated for 5 min at room temperature. Subsequently, the plates were air-dried and 200 \(\mu\)l of Tris base (10 mM, pH 10.5) were added to each well. Finally, the plates were shaken gently for 5 minutes, after which the absorbance (OD) of each well was read on an ELx800 ELISA reader (BioTek Instruments Inc., Winooski, Vermont, USA) at 490 nm.

Evaluation of cell culture morphology

The effects of the extract on cell culture morphology of both MCF-7 and HFS cells were determined by light microscopy, after staining the cells with Coomassie blue. MCF-7 (\(1 \times 10^5\) cell/ml) and HFS cells (\(1.5 \times 10^5\) cell/ml) were each suspended in DMEM containing 10% FBS and then cultivated, at 5 ml per well, onto 6-well plates. After the formation of one layer of cells, the original DMEM was replaced with 5 ml of SFM containing one of the different concentrations of the extract (25%, 50%, or 75%). After 48 hrs, adherent cells were washed twice with 3 ml of PBS (Sigma-Aldrich, St. Louis, MO, USA). Subsequently, 2 ml of 4% formaldehyde were added for 5 min to fix the cells. After discarding the formaldehyde, 2 ml of Coomassie blue were added for 5-10 min to stain the fixed (non-living) cells. The plates were then washed several times with tap water, left to dry, and finally observed and photographed under an Eclipse TS100 inverted microscope (Nikon, Tokyo, Japan) equipped with DS-Fil digital camera (Nikon, Tokyo, Japan).

Detection of benzyl isothiocyanate by paired-ion HPLC

To determine whether benzyl isothiocyanate is a constituent of the seeds extract, it was subjected to paired-ion HPLC as described previously\textsuperscript{35}. BITC was monitored at 250 nm. The two solvents used in the gradient were 6 mM tetraethylammonium bromide in water and acetonitrile. Using the above conditions, Zhang\textsuperscript{35} reported a retention time of 14.9 minutes for BITC.

Statistical analysis

The data are represented as mean ± standard deviation (SD). Statistical comparisons were performed using the three-way ANOVA followed by the Dunnett’s multiple comparisons test to determine the statistical significances of the effects of different incubation times and extract concentrations on the viability of cells. A statistically significant difference was 0.01 \(\leq P < 0.05\), 0.001 \(\leq P < 0.01\) was a statistically high significant difference, while 0.05 \(\leq P < 0.10\) was considered tending toward being statistically significant.

Results

Cytotoxic effects of the three extracts on the viability of MCF-7 cells

Using the three-way ANOVA test (Fig. 1), the number of living cells treated with each extract was not significantly affected with increasing concentrations of the extracts compared with the respective controls, except for the highest concentration (1:1) for each extract where the number of living cells decreased highly significantly (\(P = 0.001\)) compared to the controls. The comparison between the three extracts for each incubation time gave highly significant P values equal to 0.00, 0.001, and 0.00 after 24, 48, and 72 hrs of exposure, respectively. This means that there were highly significant differences between the effects of the extracts at each incubation period.

The number of living MCF-7 cells that were treated with the extracts increased highly significantly after 72 hrs of exposure to the first (\(P = 0.001\)), second (\(P = 0.002\)), and third (\(P = 0.008\)) extracts, and significantly after 48 hrs (\(P = 0.042\)) of exposure to
the second extract compared with the number of cells after 24 hrs of exposure for each extract (Fig. 1).

As a whole, the effects of the three extracts on MCF-7 viability were close to each other. The third extract was the extract of choice for all subsequent experiments because of the slightly, but not significantly, more depressing effect of the highest concentration on the cell viability compared to the other two extracts. The number of living MCF-7 cells treated with the highest concentration (1:1) of the third extract compared to the control showed a trend toward a significant decrease (P = 0.053), while cells treated with the same concentration of the first and second extracts (P = 0.257 and 0.104, respectively) did not show any differences from the controls. Additionally, the third extract contains DMSO, which is important to ensure that the active components of the seeds were extracted. Thus, the highest concentration (1:1; 50%) of the third extract with two new additional concentrations (1:3; 25% and 3:1; 75% extract) were used to treat both MCF-7 and HFS cells in all subsequent experiments.

Cytotoxic effects of the extract on the viability of MCF-7 and HFS cells

Using the three-way ANOVA test (Fig. 2), the viability of MCF-7 cells decreased with increasing concentrations of the extract and exposure time (Fig. 2 A), thus showing time- and dose-dependency. The viability of MCF-7 cells was decreased by the three concentrations (25%, 50%, and 75%) of the extract, compared with the respective controls, after 24, 48, 72, 96, and 120 hrs. The decrease in the viability was highly significant for all concentrations and incubation times, except for the 25% extract concentration at 24 hrs of incubation, which showed a significant decrease.

The Dunnett’s two-sided test was used to compare the number of living cells for each extract concentration and incubation time with the cells’ viability at its respective 24 hrs incubation (Table 1). The number of living control MCF-7 cells was highly significantly increased after incubating with the extract for 48, 72, and 120 hrs, while it was significantly increased after 96 hrs of incubation. When MCF-7 cells were incubated with 25% extract, there were no significant changes in the number of living cells with increasing incubation time. On the other hand, cells treated with 50% extract led to a highly significant decrease in the number of living cells after 72, 96, and 120 hrs, but no difference
resulted after 48 hrs. Finally, at 75% extract, there was a significant decrease in the number of living cells after 96 hrs and a highly significant decrease after 120 hrs, while the 48 and 72 hrs of incubation showed no differences. Each of these experiments was performed in triplicates.

As for the viability of HFS cells, the Dunnett’s two-sided test was used for the analysis (Fig. 2 B) of the differences between each respective control and the different concentrations of extract and incubation times, as done for MCF-7 cells above. After 24 hrs of incubation, the three concentrations (25%, 50%, and 75%) of the extract caused a highly significant decrease in the number of living HFS cells compared to the control. Highly significant decreases were also observed for the 50% and 75% concentrations after 72 hrs and for the 75% after 96 and 120 hrs, comparing each incubation time to its control. Significant decreases were observed for the 50% and 75% concentrations after 48 hrs of incubation, for the 25% after 72 hrs, and for the 25% and 50% concentrations after 120 hrs of incubation, comparing each to its respective control.

To compare each extract concentration at each incubation time with the 24 hrs incubation, the Dunnett’s two-sided test was used (Table 2), as done above. The numbers of living HFS cells that were treated with the 25% and 50% concentrations of \textit{L. sativum} seeds extract, in addition to the control, were not significantly different from the number of cells after 24 hrs. The number of living HFS cells treated with 75% of extract did not show any significant differences, except for the 96 and 120 hrs of incubation where the viable HFS cells decreased significantly. Thus, the effects of the extracts on HFS cells were not time-or dose-dependent.

### Antiproliferative effects of the extract

Using the SRB assay, the three-way ANOVA demonstrated (Fig. 3) that the normal HFS cells were found to be relatively insensitive to growth inhibition by the extract, compared with MCF-7 cells, since the number of HFS cells continued increasing with the increased concentration of the extract. After 48 hrs of exposure, the proliferation of MCF-7 cells was highly significantly increased with increasing concentrations of the extract compared with the control. On the other hand, the HFS cells proliferation increased significantly with increasing extract concentrations for all concentrations, except for 80%.

### The effects of the extract on cell culture morphology

The sheet like growth of cells (cell density) of both MCF-7 and HFS cells (Fig. 4) was lost with the appearance of signs of apoptosis. However, the effect

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**Table 1**— Effects of incubation times for extract concentrations on MCF-7 cells viability. Results are compared with viability after 24 hrs (control). P values were determined using the Dunnett’s two-sided test.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Control</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 hrs</td>
<td>0.000 HS</td>
<td>0.469 NS</td>
<td>0.052 NS</td>
<td>0.927 NS</td>
</tr>
<tr>
<td>72 hrs</td>
<td>0.000 HS</td>
<td>0.239 NS</td>
<td>0.006 HS</td>
<td>1 NS</td>
</tr>
<tr>
<td>96 hrs</td>
<td>0.013 S</td>
<td>0.234 NS</td>
<td>0.000 HS</td>
<td>0.002 S</td>
</tr>
<tr>
<td>120 hrs</td>
<td>0.009 HS</td>
<td>0.397 NS</td>
<td>0.000 HS</td>
<td>0.001 HS</td>
</tr>
</tbody>
</table>

HS: Highly Significant (0.001 ≤ P < 0.01)
S: Significant (0.01 ≤ P < 0.05)
NS: Not Significant

**Table 2**— Effects of incubation times with extract on HFS cells viability. Results are compared with viability after 24 hrs (control). P values were determined using the Dunnett’s two-sided test.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Control</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 hrs</td>
<td>0.53</td>
<td>1</td>
<td>0.774</td>
<td>0.998</td>
</tr>
<tr>
<td>72 hrs</td>
<td>0.736</td>
<td>0.742</td>
<td>0.822</td>
<td>0.829</td>
</tr>
<tr>
<td>96 hrs</td>
<td>0.218</td>
<td>0.348</td>
<td>0.97</td>
<td>0.039 S</td>
</tr>
<tr>
<td>120 hrs</td>
<td>0.874</td>
<td>0.869</td>
<td>0.979</td>
<td>0.049 S</td>
</tr>
</tbody>
</table>

S: Significant (0.01 ≤ P < 0.05)
was more marked on MCF-7 cells compared with HFS. It was also observed that the effect was more evident with increasing concentrations of the extract as the amount of apoptotic cells increased. There was a more significant effect of the 75% concentration on both cells, but the effect was more pronounced on MCF-7 cells where more apoptotic MCF-7 cells were observed compared to apoptotic HFS cells.

Fig. 4—Effects of *L. sativum* seeds extract on cell density after 48 hours of treatment. The cells were examined under TS100-F inverted microscope using a 10× objective lens. (A1-A4) represents the effects of extract on MCF-7 cells (control, extract at 25%, 50%, and 75% respectively). (B1-B4) represents the effects of the extract on HFS cells (control, extract at 25%, 50%, and 75% respectively).
Paired-ion HPLC separation of the extract

Benzy1 isothiocyanate was not detected in the extract by using HPLC, as shown in Fig. 5, since there were no peaks at a retention time of 14.9 minutes.

Discussion

Despite its great medicinal value and many beneficial effects, garden cress has not received the attention it deserves. To our knowledge, other than the current study here and our previous study, there are no other in-vitro or in-vivo studies on the anti-cancer effects of garden cress seeds extract. A number of studies have been done on benzyl isothiocyanate, which is contained in garden cress seeds, and on extracts of other cruciferous vegetables.

In this study, three traditional extraction methods of L. sativum seeds were used. An aqueous extract was chosen rather than an alcoholic one to better emulate the natural route of use of the seeds for therapeutic treatments. It is well known that an alcoholic extract is superior to an aqueous one since more of the active substances would be extracted and solubilized in the extract. The three extracts were tested for their growth depression, or cytotoxic effect, on MCF-7 breast cancer cells. The number of MCF-7 cells treated with the three extracts at different concentrations (1:10, 1:100, 1:1000, or 1:10000) and for different incubation times (24, 48, and 72 hrs) showed no significant differences compared to the control. It was expected that the viability of MCF-7 cells would decrease with increasing concentration of extract. This lack of decreased viability may be due to the presence of nutritional constituents in the extract or insufficient amounts of cytotoxic components at these low concentrations. Only the highest concentration of all three extracts, at all incubation periods, showed significant decreases ($P = 0.001$) in the number of living MCF-7 cells compared with the respective controls. The effects for the third extract were similar to the other two extracts, but it had a higher cytotoxic effect tending toward significance ($P = 0.053$) at the highest concentration, and thus it was used to carry out the rest of experimental work. An added component of this extract was DMSO, which was used to ensure the extraction of most ingredients of the seeds.

Using the trypan blue dye exclusion assay, the extract had a non-selective cytotoxic effect on the viability of MCF-7 and HFS cells in a time and dose-dependent manner. The extract showed strong cytotoxic effects on the viability of MCF-7 cells with an IC50 ~25% compared with HFS that had an IC50 ~50% after 24 hrs of extract exposure. The higher concentration of extract (75%) decreased the viability by ~72% and ~87% for MCF-7 cells and ~60% and ~50% for HFS cells, compared with the control, after 24 and 48 hrs of extract exposure, respectively. Therefore, the HFS cell line was relatively less sensitive to the cytotoxicity of the extract compared with MCF-7 cells.

The antiproliferative effects of the extract on both MCF-7 and HFS cells were also determined by using the SRB assay. The extract had a significant antiproliferative effect on MCF-7 cells after treatment for 48 hrs with an IC50 ~40%-50% while the extract had a proliferative effect on HFS that significantly increased with increasing concentrations, except for the 80% concentration which tended toward a significant increase compared with the control.

The differences in the IC50 between the trypan blue dye exclusion assay and the SRB assay may be due to the fact that in the SRB assay the experimental media that contains the different concentrations of the extract (20%, 30%, 40%, 50%, 60%, 70%, or 80%) was added without first discarding the original media (as instructed in the method). This led to the dilution of each concentration to the half, making the actual used concentrations to become 10%, 15%, 20%, 25%, 30%, 35%, and 40%. Thus, the IC50~25% obtained for MCF-7 cells using the trypan blue dye exclusion assay is nearly the same as the IC50 ~40%-50% obtained by using the SRB assay, which should be adjusted to IC50 ~20-25%.

From this experiment it may be concluded that the 70% concentration is the desired therapeutic dose, since it inhibited the growth of MCF-7 by 64.03% but
increased the growth of HFS by 133.33%. The number of MCF-7 cells was higher in the SRB assay compared with the trypan blue dye exclusion assay at the same dose and time, which may be due to the presence of dead cells that were not detached, yet the ODs of their adhesion proteins were read as living cells, and due to the added dilution of the extract as indicated above.

It has been shown that\textsuperscript{38} the ethanolic \textit{L. sativum} leaves extract had no anti-proliferative activity on MCF-7. Other researchers have shown\textsuperscript{5} that the hydroalcoholic extract of \textit{L. sativum} leaves inhibits division and growth of MCF-7 cells by 2.68% and amelanotic melanoma cell line C32 by 4.32%, but it does not have antiproliferative activity on a prostate cancer or renal adenocarcinoma cell lines. The leaves extracts are similar to the seeds extracts in composition, but they differ in the amounts of different components\textsuperscript{5}.

In the current study, morphological changes occurred in MCF-7 and HFS cells, compared to the control, upon exposure to 25%, 50%, and 75% \textit{L. sativum} seeds extract for 48 hrs. Marked decreases in the densities of Coomassie blue stained cells were observed with increasing concentration of the extract. Loss of cell-to-cell contact was observed markedly in cultures of both types of cells at 75% extract concentration.

HPLC of the extract showed that BITC was not present. This finding was expected since BITC is polar and thus would be extracted more easily using alcohols. Therefore, it is evident that the cytotoxic effects of the extract are not attributed to the presence of BITC, which is known to possess anticancer activities. Thus, other ingredients present in the extract must be responsible for the observed activities.

The aqueous extract was equally, and in some experiments more, effective against MCF-7 cells compared to HFS cells. In general, the highest (75%) dose of extract was cytotoxic for both MCF-7 and HFS cells in most assays. The resulting effect of garden cress seeds extract on the cancer cells is especially important since the MCF-7 cells are known to be strongly resistant to chemotherapeutic drugs.

Significance of the study and some constructive recommendations

Many herbs used as nonconventional treatments are easily prepared and administered without the need of medical personnel with the goal that they provide a way of self treatment or cure. The low or no toxic effects of these treatments make them more amenable for use. Thus, the main advantages of such treatments are that they may be prepared and administered at home by the patient herself or a family member. This leads to such treatments being easily used, not require a specially trained health practitioner or hospitals, and possibly increase patient compliance. This is especially important and essential in developing countries where a majority of the populations are poor, have limited resources, and live in remote rural areas that are far from hospitals making expensive treatments at hospitals or health centers inaccessible, impractical, and of low priority. These factors make herbal medicines even more important to developing countries.

Further study of the individual components of the aqueous \textit{L. sativum} seeds extract is recommended to try to determine the ingredient(s) that may be responsible for its antineoplastic effects, which may be used as natural and low cost drugs to fight cancer with minimal or no side effects. It is also recommended to test the cytotoxic effects of the extract on other types of cancers in general and breast cancers in particular. In addition, it is recommended to test higher and lower concentrations of the extract and to use it in combination with other promising nonconventional treatments or chemical chemotherapy to enhance its effects.

Conclusion

The findings of this study highlight the usefulness of using local and easily available plant products and constituents in treating or preventing diseases. The findings are encouraging and warrant further work on the aqueous extract of \textit{L. sativum} seeds and its effects on breast cancer and possibly even on other types of cancers.

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References