Anticancer activity of plant-derived proteins against human tumor cell lines

Sara A. Abozaid, Hany N. Baraka, Ahmed S. Ibrahim, Ahmed A. Gohar, Farid A. Badria*

Departments of Pharmacognosy and Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt

Received 02 January 2014; Accepted 18 January 2014

ABSTRACT
Cancer today represents a significant public health problem worldwide and the challenge is to produce cost-effective drugs. Recently, a surge of progress was achieved in the area of cytotoxic proteins. Eleven crude protein extracts, from different plants, were tested for anticancer activities against four cell lines; HepG2, Caco-2, HEP-2, and HeLa cells using MTT assay. Out of the tested extracts, Momordica charantia showed the highest anticancer potency of all tested protein fractions that is superior than that of 5-FU. Furthermore, its anticancer activity was characterised by the selective growth inhibitory effect on epithelial derived cell lines, HeLa, Caco-2 and HEP-2, but not in HepG2. On the other hand, Lactuca sativa protein fraction exhibited a potent and a selective anticancer activity against HepG2 that could be used as a lead for further investigations in the area of liver cancer therapy. In conclusion, although these findings are very promising and may open an avenue towards an economic plant-based selective anticancer drug, the exact chemical nature of active fractions remains to be fully explored and elucidated.

Key words: Plant-derived protein; anticancer; HeLa; Caco-2; HEP-2; HepG2

1. INTRODUCTION:
Cancer, a term comprising over 100 types of malignancy, is one of the major burdens of chronic disease in the world. It is very difficult to cure this disease and to clarify its pathogenesis due to its multi-factorial etiology. Early in the 20th century, only cancers small and localized enough to be completely removed by surgery were curable. Later, radiation was used after surgery to control small tumor growths that were not surgically removed. Finally, chemotherapy was added to destroy small tumor growths that had spread beyond the reach of the surgeon and radiotherapist (1-2). As such, chemotherapy is the last chance for many cancer survivors and perhaps the only alternative for patients who have had multiple resections and maximum irradiation. After four decades of cancer chemotherapy era, research has achieved a great success, and a large number of chemotherapeutic agents have been discovered since that time. However, only a few have earned a solid position in the list of useful drugs due to the associated side effects to varying degrees, and the emergence of drug resistance (2). Therefore, developing new anticancer drugs with a higher potency and specificity against cancer cells has become the hotspot of global media attention.

Plant based chemoprevention and therapy have emerged as accessible and promising approaches to cancer control and management (3-6). Of the recent natural products displaying a substantial anti-carcinogenic activity, are plant-derived proteins and peptides (7-9). They offer numerous advantages such as resistant to digestion by the human gut enzymes, bind to gastrointestinal cells and enter the circulation intact. Accordingly, they maintain full biological activity that would be useful in cancer treatment (9-10). Plants that are enriched in such kind of proteins and peptides are belonging to families of e.g. Solanaceae, Fabaceae, Brassicaceae, Cucurbitaceae and Asteraceae (7-13). Thus, screening of anticancer activity within these families would provide an important preliminary data helping in the selection of those with the highest potential antitumor activity for future pre-clinical and clinical studies.

Accordingly, a panel of four cell lines derived from the most common human malignancies: Liver, HEPG-2; Colorectal, Caco-2; Cervical, Hela cell lines; Larynx, HEP-2; has been used in the current study for such preliminary screening. This panel was selected because it is considered as a classic prototype of a cell with high malignancy that includes human xenografts. Additionally, the selection of plants to represent the aforementioned families was made on the basis of their reputation as folk medicines in the treatment of tumors and other related diseases. Table 1 shows the ethnobotanical data of the investigated plant species, including botanical names,
local names, ethnomedical uses, as well as the plant parts employed in this study.

2. MATERIALS AND METHODS:

2.1. Plant material:
Six plants seeds were bought from local market in Egypt (Lupinus termis, Trigonella foenumgraecum, Lens culinaris, Raphanus sativus, Linum usitatissimum and Lactuca sativa) while Momordica charantia, and Datura stramonium were collected from college of pharmacy farm, Mansoura university between May and July 2009. Plants were identified by staff members of the Pharmacognosy Department, faculty of Pharmacy, Mansoura University. Voucher specimens were deposited at the herbarium of the Department of Pharmacognosy, faculty of pharmacy, Mansoura University. The seeds of Momordica charantia were separated from its fruits in two stages fully ripe and moderately ripe. Plant materials were dried shade and then grounded.

2.2 Protein extraction:
Plants proteins were prepared from powdered plant material by extracted overnight with normal saline buffer (PBS), pH 7.2 at 4ºC. The extracts were centrifuged at 10,000 rpm at 4ºC for 10 min. as described in protocol 17624685 with some modification. The supernatant was freeze dried and kept at -20ºC prior to assay. Protein concentration was estimated spectrophotometrically by Bradford method using Coomassie dye procedure (14) against BSA stock solution at 595 nm.

2.3 In vitro assay for cytotoxic activity (MTT assay):
The prepared crude protein extracts (eleven extracts) were tested for their in vitro cytotoxic activity against four human cell lines, namely, epithelial colorectal adenocarcinoma cell line (Caco-2), hepatocellular carcinoma cell line (HepG2), cervix cancer cell line (HeLa), and human epidermoid carcinoma of Larynx (HEP-2), using MTT assay (15) with some modifications. The cell lines were obtained from culture department, VACSERA, Egypt. All cell lines were maintained in a fresh RPMI-1640 and Minimum essential medium (MEM-E) supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin in a humidified atmosphere of 5% CO2 at 37 ºC. 96-well microplates were seeded with 100 µl medium containing 10^4-10^5 cells in suspension. After 24 h incubation and attachment of the cells, the cells were incubated with the appropriate dilution of test materials in quadruplicate for a specified period. Thereafter, the supernatant was removed and cells were washed 2 times with PBS then 20 µl of MTT (5 mg/ml) solution was added to each microwell and incubated for 3h. The supernatant was removed and 50 µl DMSO were added to each well for 15 min with shaking. The optical density (OD) of each well was measured spectrophotometrically at 570 nm using microplate reader (Dynatech- USA). The effect of protein extract on the proliferation of cancer cells was expressed as the % cytoviability, using the following formula: % cytoviability = (A_s of treated cells / A_s of control cells) 100%.

3. RESULT AND DISCUSSION:
The anticancer activities of the isolated protein fractions from 8 medicinal plants representing 6 different families were evaluated in cell-based assays using a panel of four cell lines derived from the most common human malignancies. Thereafter their anticancer potencies compared to that of 5-FU, a drug extensively used in adjuvant and palliative chemotherapy for cancer. Anticancer activity was expressed as the concentration that caused 50% loss of the cell monolayer (IC_50). Using this approach, dose response and time course cytotoxicity of the standard agent, 5-FU, was initially carried out. The dose-response effect of 5-FU was more evident after 72 hours of incubation than at 48 hours, whereas at 24 hours an IC_50 was not reached with 5-FU at any concentration tested (1.5-100 µg/mL, data not shown). Therefore, 72 hours has been chosen as the incubation period for the dose-viability response of all tested compounds.

The results of our preliminary screening indicated that proteins derived from Momordica charantia fully and moderately ripen fruits had the highest anticancer potencies of all tested medicinal plants against colorectal carcinoma with IC_50 values of 0.14 and 1.6 µg/mL, respectively (Figures 1A and B), followed by proteins derived from Trigonella foenumgraecum seeds, Momordica charantia moderately as well as fully ripen seeds, and Datura stramonium seeds, with IC_50 values of 5.8, 9.5, 16.5, and 28.8 µg/mL, respectively (Figures 1C, D, E and F). These anticancer effects are superior than that observed for 5-FU, the classic reference cytotoxic agent (Figure 1G). The American National Cancer Institute had assigned a promising anticancer agent for future bio-guided studies if it exerts an IC_50 value ≤ 30 µg/ml (10; 16). Consequently, the aforementioned protein fractions would be potential anticancer candidates for future preclinical studies in treating colorectal cancer, as their IC_50 values are extremely low to avoid any possible unspecific effects. However, the precise molecular mechanisms underlying the strong inhibitory action of these isolated protein fractions remain to be fully clarified. Next in anti-cancer potency against Caco-2 are
proteins derived from *Lupinus termis* seeds with IC\textsubscript{50} value of 118.5 µg/mL (Figure 1H). On the other hand, an IC\textsubscript{50} was not reached at any tested concentration (1.5-100 µg/mL) for protein fractions isolated from seeds of *Lens culinaris*, *Linum usitatissimum*, *Raphanus sativus*, and *Lactuca sativa* (Figures 1 I, J, K and L, respectively).

On contrast to what has been demonstrated in Caco-2 cell line, the protein fractions isolated from *Trigonella foenumgraecum* as well as *Lactuca sativa* seeds showed the strongest anticancer activities against HepG2 cell line in a dose-dependent manner with IC\textsubscript{50} values of 6.1 and 7.7 µg/ml, respectively (Figure 2A and B). Obviously, these anticancer activities are more prominent than that of 5-FU, (Figure 2D). Following this further, the protein fraction isolated from *Lens culinaris* exhibited an anticancer activity against HepG2 that is comparable to that of 5-FU with IC\textsubscript{50} value of 15 µg/ml (Figure 2C). Protein fractions isolated from *Raphanus sativus* seeds and from *Linum usitatissimum* seeds came next in their HepG-2 anticancer efficiency with IC\textsubscript{50} values of 35.2 and 53.7 µg/ml, respectively (Figures 2E and F). Interestingly, plant-derived proteins from *Momordica charantia* fruits as well as seeds, that were shown to possess potent anticancer activities against colorectal carcinoma showed very weak cytotoxic activities against HepG-2 in which an IC\textsubscript{50} was not reached at any tested concentration (Figures 2G, H, I, and J). Likewise, the observation that significant cytotoxic activities of protein fractions isolated from both *Lupinus termis* and *Datura stramonium* seeds on Caco-2 cell line was not detected in HepG-2 (Figures 2 K and L), implies a differential selectivity of isolated anticancer proteins toward tumor cell lines.

In light of selective plant-derived proteins’ anticancer properties, interest in their differential activities was investigated further using other epithelial cell lines such as (HeLa and its derivative, Hep-2), the continuous cancer cell lines that have been a mainstay of cancer research ever since their isolation. Generally, among the selected plants proteins under investigation, *Momordica charantia* fruits moderately ripen (Figure 3A), and fully ripen (Figures 3B), showed the highest anticancer potencies of all tested protein fractions that were 40 and 20 times, respectively, higher than that of 5-FU (Figure 3F). Whereas, *Momordica charantia* seeds fully or moderately ripen showed anticancer potencies that were 8.5 or 3.5 times, respectively, higher than that of 5-FU (Figures 3C and D). Next in anti-cancer potency against HeLa, are proteins derived from *Lens culinaris* seeds followed by those extracted from *Lupinus termis* seeds with IC\textsubscript{50} values of 24.8, and 129.11 µg/mL, respectively, (Figures 3E, and G). On the other hand, an IC\textsubscript{50} was not achieved at any concentration tested in the range of (1.5-100 µg/mL) for protein fractions isolated from seeds of *Trigonella foenumgraecum* (Figure 3H), *Datura stramonium* (Figure 3I), *Linum usitatissimum* (Figure 3J), *Raphanus sativus* (Figure 3K), and *Lactuca sativa* (Figures 3L).

Moving to the next cell line (Hep-2), we have obtained closely related results to those previously reported for Hela cell line in which Momordica charantia fruits moderately ripen (Figure 4A) and fully ripen (Figures 4B) showed the highest anticancer potencies of all tested protein fractions followed by *Momordica charantia* fully ripen seeds (Figure 4C). Additionally, *Momordica charantia* moderately ripen seeds (Figure 4D) showed anticancer potency that was comparable to that of 5-FU (Figure 4E). However, an IC\textsubscript{50} was not achieved at any concentration tested in the range of (1.5-100 µg/mL) for protein fractions isolated from seeds of *Lens culinaris* (Figures 4F), *Lupinus termis* (Figures 4J), *Trigonella foenumgraecum* (Figure 4H), *Datura stramonium* (Figure 4I), *Linum usitatissimum* (Figure 4J), *Raphanus sativus* (Figure 4K), and *Lactuca sativa* (Figures 4L).

In summary, we have isolated protein fractions from different medicinal plants on the basis of their biological significance and evaluated their anticancer activities. Out of a set of 11 protein fractions, *Momordica charantia* showed the highest anticancer potency of all tested protein fractions that are superior than that of 5-FU. Furthermore, its anticancer activity was characterised by the selectiv growth inhibitory effect on epithelial derived cell lines, HeLa, CaCo-2 and HEP-2, but not in HepG2. On the other hand, *Lactuca sativa* protein fraction exhibited a potent and a selective anticancer activity against HepG2 that could be used as a lead for further investigations in the area of liver cancer therapy.

In conclusion, although these findings are very promising and may open an avenue towards an economic plant-based selective anticancer drug, the exact chemical nature of active fractions remains to be fully explored and elucidated.
Table 1: Ethnobotanical information of selected medicinal plants used

<table>
<thead>
<tr>
<th>Genus, species</th>
<th>Family</th>
<th>Trivial name</th>
<th>Part used</th>
<th>Traditional use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Datura stramonium</em></td>
<td>Solanaceae</td>
<td>Datura</td>
<td>Seed</td>
<td>Human ailments: including ulcers, wounds, inflammation, rheumatism, gout, sciatica, bruises and swellings, fever, asthma, bronchitis and toothache (18).</td>
</tr>
<tr>
<td><em>Lactuca sativa</em></td>
<td>Asteraceae</td>
<td>Lactuca</td>
<td>Seed</td>
<td>Inflammation, gastrodynia and osteodynia (19, 20)</td>
</tr>
<tr>
<td><em>Lens culinaris</em></td>
<td>Fabaceae</td>
<td>Yellow lentils</td>
<td>Seed</td>
<td>Constipation, other intestinal affictions, ulcers that follow smallpox and other slow-healing sores (21).</td>
</tr>
<tr>
<td><em>Linum usitatissimum</em></td>
<td>Linaceae</td>
<td>Linseed</td>
<td>Seed</td>
<td>Respiratory tract disorder, eyes, infections, cold, flu, fever, rheumatism and gout (22).</td>
</tr>
<tr>
<td><em>Lupinus termis</em></td>
<td>Fabaceae</td>
<td>Lupines</td>
<td>Seed</td>
<td>The seeds are reputed to be effective for diabetes. Topically, the powdered seeds are used to treat acne (23).</td>
</tr>
<tr>
<td><em>Momordica charantia</em></td>
<td>Cucurbitaceae</td>
<td>Bitter melon</td>
<td>Seed</td>
<td>Antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, peptic ulcers kidney (stone), laxative, leprosy, leucorrhrea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies (24).</td>
</tr>
<tr>
<td><em>Raphanus sativus</em></td>
<td>Brassicaceae</td>
<td>Radish seed</td>
<td>Seed</td>
<td>Gastrointestinal, biliary, hepatic, urinary, respiratory disorders (25, 26) and in cardiovascular diseases such as hypertension (27).</td>
</tr>
<tr>
<td><em>Trigonella foenumgraecum</em></td>
<td>Fabaceae</td>
<td>Fenugreek</td>
<td>Seed</td>
<td>Antidiabetes, high cholesterol, inflammation and gastrointestinal ailments (28).</td>
</tr>
</tbody>
</table>
Figure 1: The descending order of IC₅₀ values (µg/ml) for different protein extracts against Caco-2 in comparison with 5-fluorouracil: A) Momordica charantia fully ripen fruits; B) Momordica charantia moderately ripen fruits; C) Trigonella foenumgraecum seeds; D) Momordica charantia moderately ripen seeds; E) Momordica charantia fully ripen seeds.
F) Datura stramonium seeds; G) 5-FU; H) Lupinus termis seeds; I) Lens culinaris seeds; J) Linum usitatissimum seeds; K) Raphanus sativus seeds; and L) Lactuca sativa seeds.

Figure 2: The descending order of IC₅₀ values (µg/ml) for different protein extracts against HepG-2 in comparison with 5 - fluorouracil: A) Trigonella foenumgraecum seeds; B) Lactuca sativa seeds; C) Lens culinaris seeds; D) 5-FU; E) Raphanus sativus seeds; F) Linum usitatissimum seeds; G) Momordica charantia fully ripen fruits; H) Momordica charantia moderately ripen fruits; I) Momordica charantia fully ripen seeds; J) Momordica charantia moderately ripen seeds; K) Lupinus termis seeds; and L) Datura stramonium seeds.
Figure 3: The descending order of IC₅₀ values (µg/ml) for different protein extracts against HeLa in comparison with 5-fluorouracil: A) Momordica charantia moderately ripen fruits; B) Momordica charantia fully ripen fruits; C) Momordica charantia fully ripen seeds; D) Momordica charantia moderately ripen seeds; E) Lens culinaris seeds; F) 5-FU; G) Lupinus termis seeds; H) Trigonella foenumgraecum seeds; I) Datura stramonium seeds; J) Linum usitatissimum seeds; K) Raphanus sativus seeds; and L) Lactuca sativa seeds.
Figure 4: The descending order of IC$_{50}$ values (µg/ml) for different protein extracts against HEP-2 in comparison with 5-fluorouracil: A) Momordica charantia moderately ripen fruits; B) Momordica charantia fully ripen fruits; C) Momordica charantia fully ripen seeds; D) Momordica charantia moderately ripen seeds; E) 5-FU; F) Lens culinaris seeds; G) Lupinus termis seeds; H) Trigonella foenumgraecum seeds; I) Datura stramonium seeds; J) Linum usitatissimum seeds; K) Raphanus sativus seeds; and L) Lactuca sativa seeds.
REFERENCES: