Short Communication

CYTOTOXIC ACTIVITY OF SELECTED LIBYAN MEDICINAL PLANTS ON HUMAN BREAST ADENOCARCINOMA CELL LINE (MCF-7)

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INTRODUCTION

Breast cancer is one of the most common diseases affecting women worldwide especially in western countries (Coleman and Tsongalis, 2002). For thousands of years, natural plant products have an important role throughout the world in prevention and treatment of many human diseases (Chin et al., 2006). More than 60% of anticancer drugs used now are derived from natural sources (Reddy et al., 2003). The first search for anti-cancer drugs from natural plants started in the 1950s with the discovery of the vincristine alkaloids (Reddy et al., 2003). It has been estimated that less than so far 5-10% of between 200,000 and 600,000 species of higher plants occurring in the world have been investigated for their chemical constituents and/or for their biological activity (Cowan, 1999). Investigating plants with the aim of discovering therapeutic agents including anticancer agent is still of interest to the scientists (Bauer, 2000). The present work was aimed at investigating the selected extracts from Libyan flora against human breast adenocarcinoma (MCF7) using MTT assay.

Libyan traditional medicine enjoys the use of various plants for the treatment of diseases. Many medicinal plants growing in Libya have been used in folk medicine for the treatment of many diseases as inflammation. In the present study, three plants, namely Ballota pseudodictamnus, Hedera helix, Thapsia garganica were tested for anticancer activity. The plants, collected from Aljabal-Al-Akhdar area of Libya in April 2010 were shed dried and subjected to Soxhlet extraction by methanol. Phytochemical screening indicated the presence of 2-deoxy sugars, flavonoids, saponins and tannins but absence of alkaloids in all three plants under investigation. Antiproliferative activity was done on human breast adenocarcinoma cell line (MCF7). At the concentration of 100µg/mL, B. pseudodictamnus, T. garganica and H. helix showed 90, 60 and 5% cell death, respectively as compared to the control.

Key words: Antiproliferative; Breast adenocarcinoma; Cell line MCF-7

ABSTRACT

Three Libyan plants namely Ballota pseudodictamnus, Hedera helix, Thapsia garganica were tested for anticancer activity. The plants, collected from Aljabal-Al-Akhdar area of Libya in April 2010 were shed dried and subjected to Soxhlet extraction by methanol. Phytochemical screening indicated the presence of 2-deoxy sugars, flavonoids, saponins and tannins but absence of alkaloids in all three plants under investigation. Antiproliferative activity was done on human breast adenocarcinoma cell line (MCF7). At the concentration of 100µg/mL, B. pseudodictamnus, T. garganica and H. helix showed 90, 60 and 5% cell death, respectively as compared to the control.

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METHODOLOGY

Plant materials

The plants were collected from Aljabal-Al-Akhdar area, Libya in April 2010 and identified by the experts at Department of
Botany, Faculty of Science, University of Tripoli. The plants used in this study were *Ballota pseudodictamnus* (Lamiaceae), *Hedera helix* (Araliaceae) and *Thapsia garganica* (Apiaceae). Voucher specimens (J.122/2010, J.125/2010 and J.124/2010) respectively, were deposited in the herbarium of the Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.

**Extraction**

Air-dried and finely powdered aerial parts (100g) of the selected plants were extracted with 95% methanol (1.5L) using Soxhlet apparatus. The extracts were concentrated using rotary vacuum evaporator at 50°C under reduced pressure to get the extract.

**Phytochemical screening**

An alcoholic solution of the extract was prepared by mixing 1g of the crude extract with 10mL of ethanol. Tests were performed with this solution (Harborne, 1984; Ikhiri et al., 1992).

- **Frothing test for saponins:** Alcoholic solution of the extract (1mL) was mixed with 20mL of distilled water and shaken vigorously in a graduated cylinder for 15min. Formation of 1cm of foam indicated the presence of saponins.
- **Mayer’s test for alkaloids:** Alcoholic solution of the extract (1mL) was mixed with 0.2mL of dilute HCl followed by the addition of 1mL of Mayer’s reagent. Formation of yellow precipitate indicated the presence of alkaloids.
- **Dragendorff’s test for alkaloids:** Alcoholic solution of the extract (1mL) was mixed with 0.2mL of dilute HCl followed by the addition of 1mL of Dragendorff’s reagent. Formation of orange red precipitate indicated the presence of alkaloids.
- **Shimada test for flavonoids:** Few drops of concentrated HCl was added to 1mL of the alcoholic solution of the extract followed by the addition of 0.5g of Magnesium turning. Formation of pink color indicated the presence of flavonoids.
- **Ferric chloride test for tannins:** Alcoholic solution of the extract (1mL) was mixed with 1mL of 5% Ferric chloride solution. Greenish black precipitate indicated the presence of tannins.

**Keller-Kiliani test for deoxy sugars in cardenolides:** The crude extract (50mg) was dissolved in 2mL chloroform. Addition of H2SO4 led to the formation of brown ring at the interphase in presence of deoxy sugars in cardenolides.

**Assessment of cytotoxicity**

Cytotoxic activity was evaluated *in vitro* by MTT assay (Robinson et al., 1980). Cell line from human breast cancer (MCF7) was cultured in EMEM (EBSS) medium supplemented with heat-inactivated 10% FBS, 0.2mg/L L-glutamine, 1.0mg/mL NaHCO3, 100units/mL penicillin, and 100units/mL streptomycin in a humidified incubator at 37°C and 5% CO2 atmosphere. Each plate was seeded with 5x10^4 cells. After 48h, the plant extracts, sterilized by passing through 0.22µm millipore filter were added to plates at the concentration of 100µg/mL. Untreated plate was used as negative control. Each plate was incubated for another 72h at 37°C in a humidified incubator with 5% CO2.

**RESULTS AND DISCUSSION**

**Result of phytochemical screening:** All three plant extracts indicated the presence of saponins, tannins, flavonoids and 2-deoxy sugars but absence of alkaloids.

**Result of cytotoxicity test:** Among the three plant extracts B. pseudodictamnus showed 90% death of the breast adenocarcinoma (MCF7) cells while T. garganica and H. helix showed 60 and 5% death of the cells, respectively (Table I).

Cancer is the leading cause of death in developed countries and second leading cause of death in developing countries. About 12.7 million cases and 7.6 million cancer deaths has been estimated to occur in 2008. Breast cancer accounted for 23% of this total cancer cases and 14% death of the total cancer deaths. In females, breast cancer is most frequently diagnosed cancer and leading cause of death. Increase in the incidence of breast cancer is associated with the use of oral contraceptives, post menopausal hormone therapy, late age first birth, alcohol consumption (Jemal et al., 2011).

For the treatment of metastatic breast cancer, the anthracyclines (doxorubicin and epirubicin) and taxanes (paclitaxel and...
docetaxel) are considered to be first and second line therapy. Treatment options are very limited for breast cancer. Although some other drugs including mitomycin, vinorelbine, fluorouracil are used for benefit from further chemotherapy, these agents require hospitalization and data from controlled trials are limited. Therefore, there is a clear need of newer therapeutic agents for the treatment of breast cancer with the opportunity of oral administration, fewer side effects and treatment from home rather than hospital (Crown et al., 2002).

Cytotoxicity study provide the preliminary results to help selecting the most active plant extracts with potential anticancer activity for future work to isolate the agent(s) responsible for anticancer activity (Cardellina et al., 1999). From the present study, it was found that B. Pseudodictamnus and T. garganica may have potential beneficial effect against breast adenocarcinoma cell line (MCF-7). In a previous study, phenylpropanoids isolated from T. garganica were active against prostate cancer cell lines. It is not clear whether the same compounds are responsible for the observed activity or some other compounds are responsible for it. Present investigation revealed the presence of tannins, saponins and flavonoids in all the three plant extracts. Previous studies have found that such chemical class of compounds or plant extract or fraction containing these types of compounds can show anticancer activity (Madhuri and Pandey, 2009). Therefore, it is possible that the observed activity could belong to these chemical groups.

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REFERENCES

Table I. Percentage of dead cells of tested plants extracts

<table>
<thead>
<tr>
<th>Tested plant</th>
<th>% dead of cells</th>
</tr>
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<tbody>
<tr>
<td>B. pseudodictamnus</td>
<td>90</td>
</tr>
<tr>
<td>H. helix</td>
<td>5</td>
</tr>
<tr>
<td>T. garganica</td>
<td>60</td>
</tr>
<tr>
<td>DMSO</td>
<td>5</td>
</tr>
</tbody>
</table>

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