

## Short Communication

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

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## 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.

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

## 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 vinca 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* L. Benth. (Lamiaceae), *Hedera helix* (Araliaceae) and *Thapsia garganica* (Apiaceae) were chosen for their possible anticancer activity. In previous studies, the aerial part of *B. pseudodictamnus* showed antimicrobial, antioxidant activity and the presence of caryophyllene oxide, phytol and gamma-murolene (Couladis *et al.*, 2002; Citoğlu *et al.*, 2004). Four phenylpropanoids were isolated from *T. garganica* and found to be active against prostate cancer cell lines (Liu *et al.*, 2006). When tested on mice and rat models, the leaf extract of *H. helix* showed analgesic and anti-inflammatory activity (Mandade *et al.*, 2010). In another study, three saponins isolated from *H. helix* showed antimutagenic activity against benzo-[a]pyrene and mutagenic urine concentrate from a smoker (Elias *et al.*, 1990). Previously, none of these plants has been investigated against human breast adenocarcinoma cell and therefore were chosen for this study.

## 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.

*Shinoda 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 de-oxy sugars in cardenolides:* The crude extract (50mg) was dissolved in 2mL chloroform. Addition of H<sub>2</sub>SO<sub>4</sub> 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-glutamide, 1.0mg/mL NaHCO<sub>3</sub>, 100units/mL penicillin, and 100units/mL streptomycin in a humidified incubator at 37°C and 5% CO<sub>2</sub> atmosphere. Each plate was seeded with 5×10<sup>3</sup> 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% CO<sub>2</sub>.

### 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 taxenes (paclitaxel and

Table I. Percentage of dead cells of tested plants extracts

Tested plant	% dead of cells
<i>B. pseudodictamnus</i>	90
<i>H. helix</i>	5
<i>T. garganica</i>	60
DMSO	5

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, flurouracil 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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