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# ANTICANCER EFFECTS OF METHANOL EXTRACT OF LIBYAN *SALVIA FRUTICOSA* MILL ON MCF7, T47D AND (MDA-MB-468) BREAST CELLS LINES

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## ABSTRACT

Because of the well-known health benefits of the *Salvia species* plant, this plant has been used globally for traditional medicine for long time. More importantly however many extracts of *Salvia species* have been exhibited significant *in vitro* and *in vivo* anticancer performance on a range of different cancer cell types. This *in vitro* study as the first one highlighted the anti-proliferative activity of methanol extract of bark of the Libyan *Salvia fruticosa* on three breast cancer cells lines (MCF-7, T47D and MDA-MD-468) to reveal the potential of this plant species to be used as natural anticancer agent and add new insight for further research of this species of the plant Salvia, specifically. SRB assay was used in this study and the results showed good cytotoxic activity against tested breast cell lines. **Conclusion**: Our results suggest that polar methanol extract of bark of *Salvia fruticosa* Mill can produce good therapeutic effect in treatment of breast cancer of MCF-7, T47D and MDA-468 cell lines types and could be considered as the potential source of raw material in pharmaceutical industry for the extraction and isolation of natural compounds with a good spectrum of biological cytotoxic activity in breast malignancy therapy.

**KEYWORDS:** Anticancer activity, Breast cell lines, *Salvia fruticosa* Mill, $IC_{50.}$

## INTRODUCTION

Cancer is a major problem to human health and well-being which considered as one of the main diseases leading to death. According to the report of International Agency for Research on Cancer in 2012 the rate of cancer burden rose to approximately 14 million cases per year with expectation to rise to 22 million cases within next 2 decades (Stewart, 2014). According to World Cancer Report the rates of cancer disease could further increase by 50% to 15 million new cases in the year 2020 (Tundis, 2017). More than 60% of the world's total cases of cancer and account for ~70% of the world's cancer deaths were in developing countries which suffer from social and economic burden due to cancer incidence due to the lack of early detection and access to treatment. Thus the effective prevention and treatment of cancer remain imperative issue (Heyninck *et al*., 2014).

Worldwide, breast cancer is the most common invasive cancer in women. The most common form of cancer is non-invasive non-melanoma skin cancer; non-invasive cancers are generally easily cured, cause very few deaths, and are routinely excluded from cancer statistics.) Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancers. In 2008, the World Cancer Report documented that 458,503 deaths worldwide were suffered breast cancer (13.7% of cancer deaths in women and 6.0% of all cancer deaths for men and women together). The number of cases worldwide has significantly increased. Breast cancer documented worldwide as the most common invasive cancer in women. Breast cancer comprises 16% of all female cancers and 29% of invasive cancers in women. (Yuanyuan *et al*., 2018, International Agency for Research on Cancer, 2008).

Over years, Anticancer agents have been derived either from natural or synthetic compounds. It is well known that the synthetic agents need to pass through regulatory stages to be ready for recommendation for public use and also they have a risk for side effects which limit their use. In contrast, compounds from natural dietary sources realized as nontoxic are cuter than those from synthetic sources. *Salvia* plant usually referred to as "sage" is belonging to the family Lamiaceae and constitutes the largest genus of this family and having about 1000 species throughout the world (Yuanyuan *et al*., 2018). Many of *Salvia* species are used in food as flavouring

agent. *Salvia* plants show different pharmacological effects due to their high diversity in their secondary metabolites. They included in many Pharmacopeias because they are widely used in folk medicine (Tepe *et la*., 2004, Sivropoulou, 1997). Many studies were studied the biological properties of the extracts of different species of *Salvia* and revealed that this plant has anti-inflammatory, antitumor, antidiabetic and antioxidant activities (Duletić-Laušević *et al*., 2018). *Salvia fruticosa* is one of ten species of the genus Salvia were represented in the flora of Libya (Jafri and El-Gadi, 1985) and little data were presented about where they are very scarcely investigated. Libyan *Salvia fruticosa* used wide for medicinal and cooking purposes due to its essential oils (Al Sheef *et al*., 2013) and also for the chemical composition, antimicrobial and anti-oxidant activities of the essential oil (Giweli *et al*., 2013). The toxicity of Libyan *Salvia officinalis* L. was more screened on different cancer cell lines and reported extensively. An antioxidant activity and cytotoxic effect have been reported from water extract of the Libyan *Salvia fruticosa* (Alimpić *et al.*, 2015), but no study have been done to investigate the anti-proliferative effect of methanol extract of bark of *Salvia fruticosa* plant on cell lines used in this study. In this sense this study was carried out in aim to provide data on the cytotoxic activities of Libyan *Salvia fruticosa* (stems) on a panel of three human breast cancer cell lines; MCF-7 and T47D luminal breast cell lines and MDA-MB-468 aggressive breast cell line.

## 2. MATERIALS AND METHODS

### Plant collection and preparation

The plants was collected with collaboration with Herbalists in August 2016 from around Al-Bayda city; located in the Green Mountain area in the Northeast of Libya. The plant was cleaned from dust with tap water, dried at room temperature and then powdered using mixer and kept in closed clean container.

### Extraction of plant material

In order to get crude extract one hundred grams of coarsely powdered *Salvia fruticosa* bark materials was extracted by Soxhlet apparatus with enough quantities (250-300 ml) of methanol for (6 – 10 hours). The extract was filtered and organic solvent was evaporated under reduced pressure using Rota-evaporator. After that the yield extracts were air dried and kept in closed well labelled clean containers to be ready for use.

### Cell lines and culture condition

Three tumour cell lines were used in this study; breast luminal (MCF-7), breast luminal (T47D) and aggressive breast basal (MDA-MB-468) cell lines. They were all obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained at the National Cancer Institute, Cairo, Egypt. They were cultured in RPMI 1640 medium supplemented with 10 % Fetal bovine serum, 2 mM L-glutamine, 100 µg/ml penicillin/streptomycin and 1.5 g/L sodium bicarbonate and incubated at 37 C in 5 % carbon dioxide.

### Cytotoxicity testing

Cells were seeded at a density of $2.5*10^3$ per well in 96-well microtiter plates. Cells were left for 24 h before incubation with various concentrations of Salvia fruticosa extract (12.5, 25, 50 and 100 µM) for 48 h. Cytotoxicity was determined using SRB assay according to a previously explained method (Sekehan *et al*., 1990). Fixation of cells was performed by the addition of 10 % cold trichloroacetic acid. After 1 h incubation at 4 _C, cells were washed five times with deionized water. The cells were then stained with 0.4 % SRB dissolved in 1 % acetic acid for at least 30 min and subsequently washed four times with 1 % acetic acid to remove unbound stain. The plates were left to dry at room temperature and bound protein stain was solubilized with 100 µl/well Tris base (10 mM, pH 10.5) and the optical density (OD) of each well was measured spectro-photometrically at 570 nm with an ELISA microplate reader (Techan SunriseTM reader, Germany). The percentage of cell survival was calculated as survival fraction = OD (treated cells)/OD (control cells).

### Determination of IC50 values

Data from cell viability analysis were analyzed using Prism Software program (Graphpad Software incorporated, version 3) to determine IC50 values of *Salvia fruticosa* in the four different cancer cell lines.

## RESULTS AND DISCUSSION

The effects of *Salvia fruticosa* methanol-soluble fraction on tumour cells viability have been evaluated against three human breast cancer cell lines, namely TD47, estrogen receptor positive (ER+) MCF-7 and triple negative MDAMB-468 at different concentrations (12.5, 25, 50 and 100µg/mg) using SRB assay. Many species of Salvia have been extensively studied for their cytotoxic activity, but no previous data has found explain the cytotoxic effect of methanol extract of *Salvia fruticosa* on tested cell lines and this study is the first and it provides that methanol extract of bark of *Salvia fruticosa* showed an interesting cytotoxic activity on the above mentioned cancer cell lines where the extract able to inhibit the *in vitro* proliferation of human breast cancer cell line MCF-7. Also in a concentration-dependent manner, good anti-proliferative activity was shown from Salvia extract against T47D and MDA-MB-468 as shown in tables and figures 1 and 2.

**Table (1): Cytotoxic activity of Methanol extract of bark Libyan *Salvia fruticosa* against T47D breast cancer cell line.**

| Concentration µg/ml | T47D Breast tumor cell line |
|---|---|
| 0.000 | 1.000 |
| 12.500. | 0.589 |
| 25.000 | 0.470 |
| 50.000 | 0.364 |
| 100.00 | 0.413 |

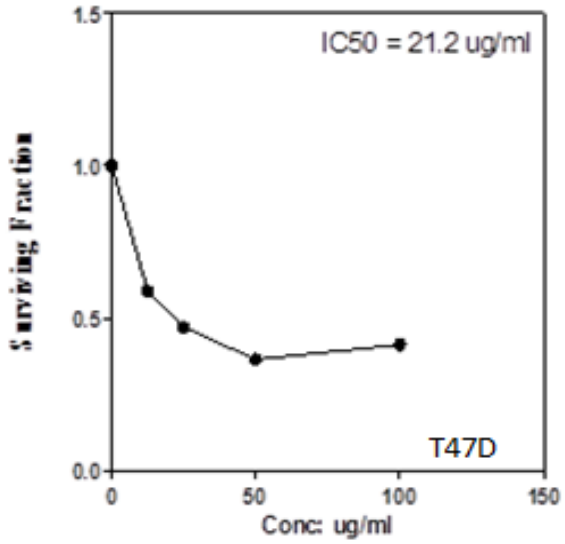


**Figure (1): Cytotoxic activity of Methanol extract of bark Libyan Salvia fruticosa against T47D breast cancer cell line.**

**Table (2): Cytotoxic activity of Methanol extract of bark Libyan *Salvia fruticosa* against MDA-MD-468 breast cancer cell line.**

| Concentration µg/ml | MDA-MD-468 Breast tumor cell line |
|---|---|
| 0.000 | 1.000 |
| 12.500. | 0.840 |
| 25.000 | 0.640 |
| 50.000 | 0.340 |
| 100.00 | 0.320 |

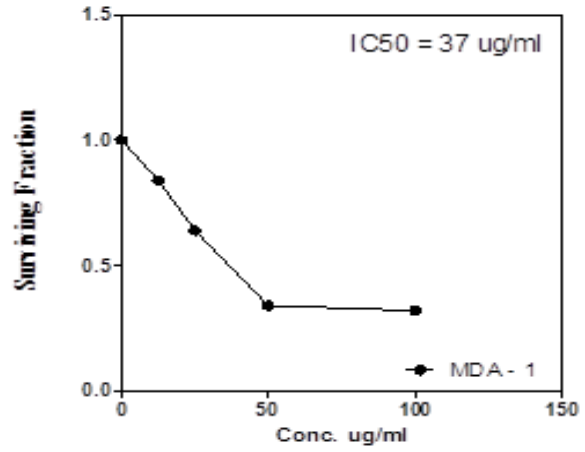


**Figure (2): Cytotoxic activity of Methanol extract of bark Libyan *Salvia fruticosa* against MDA-MD-468 breast cancer cell line.**

In form of surviving and inhibition percentages on the tested three cell lines after 48 hr., as reported in table (3), the screening of the cytotoxic effect of *Salvia fruticosa* displayed antitumor activity against MCF-7 with 35% surviving percentage and 65% inhibition percentage. Also 28% surviving percentage and 72% inhibition percentage against TD47and 24% surviving percentage and 76% inhibition percentage against MDAMB-468 were revealed in this study as showed in table (3).

**Table (3): Surviving and inhibition percentage of cells treated with Methanol extract of bark of *Salvia fruticosa.***

| Tested cell line | Surviving % | Inhibition % |
|---|---|---|
| **MCF-7** | 35 | 65 |
| **T47D** | 28 | 72 |
| **MDA-MB-468** | 24 | 76 |

The IC50 values have been calculated for all used cancer cell lines and are reported in Table (4), where the values were 30, 21.2 and 37µg/ml for MCF-7, TD47 and MDA-MB-231 respectively. The highest activity was shown against TD47 with $IC_{50}$ of 21.2µg/ml and the lowest activity was against MDA-MB-231 with $IC_{50}$ of 37 µg/ml.

This study revealed that this species exhibited cytotoxic effect against MCF-7 breast cell line with $IC_{50}$ of 30 µg/ml which is near to that found by Monica *et la*., (2014) where they studied the anti-proliferative activity of 9 different species of *Salvia* plants rather than *Salvia fruticosa* against MCF-7 cell line and their results were showed varied activity ranged from high cytotoxic, moderate to non-cytotoxic activity where the lowest $IC_{50}$ they reported was 29.1 µg/ml from *Salvia glutinosa* followed with 33.7 and 37.3 revealed from *Salvia macrisiphon* and *Salvia hydrangea* respectively. Also in another studies $IC_{50s}$ of 25.55 mg/mL and 29.89µg/ml from ethanol extracts of *Salvia fruticosa* and *Salvia triloba* respectively have shown against MCF-7 cell line (Abu-Dahab *et al*., 2014). In addition this study proved that *Salvia fruticosa* methanol extract exhibited high cytotoxic activity against T47D breast cell line with $IC_{50}$ of 21.2µg/ml which is less than $IC_{50}$ of 38.91µg/ml revealed in another study from *Salvia triloba* (Abu-Dahab *et al*., 2014).

Aysegul *et al*, 2013 in their study found that *Salvia kronenburgii* showed antiproliferative effect in a dose-dependent manner on MDA-MB-231 cell lines by inducing apoptosis-like cell death, in time that this study proved that *Salvia fruticosa* crude methanol extract exhibited cytotoxic effect in a dose-dependent manner on MDA-MB-468 with $IC_{50}$ of 37µg/ml. This little variation seen concerning the values of the $IC_{50s}$ between different *Salvia species* ssuggested to be due to different area of collection of Salvia plant which comprise different ecological factors and different cell lines subtypes. Furthermore, presence of Polyphenols, flavonoids and tannins as bio constituents of Salvia plant and their significant antioxidant performance are previously documented (Banjarnahor and Artanti, 2015), the matter which may contributed to the cytotoxic activity of this plant species; *Salvia fruticosa*..

**Table (4): $IC_{50}$ and degree of cytotoxicity of Methanol extract of bark of *Salvia fruticosa* on tested breast cell lines.**

| Used cell line | $IC_{50}$ (µg/ml) | Degree of toxicity |
|---|---|---|
| **MCF-7** | 30 µg/ml | High toxic |
| **T47D** | 21.2 µg/ml | High toxic |
| **MDA-MB-231** | 37 µg/ml | Moderate toxic |

$IC_{50}$ < 30µg/ml= High toxic, $IC_{50}$ > 100 µg/ml= No toxic, $IC_{50}$ 30-100 µg/ml= Moderate toxic

## CONCLUSION

In conclusion our results proved that the polar methanol extract of bark of Libyan *Salvia fruticosa* Mill have good anti-proliferative activity against MCF-7, T47D and MDA-468 breast cancer cell lines and this study suggests that this plant is promising as a potential source of raw material in pharmaceutical industry for the extraction and isolation of natural compounds with a good spectrum of biological cytotoxic activity in breast malignancy therapy.

## RECOMMENDATION

Purification and isolation of pure compounds presented in this crude extract and responsible for this cytotoxic activity and also study the mechanistic pathway they path to reveal this antiproliferative effect.

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