

Comparison Between the Effects of *Syzygium Jambolanum* Leaf and Fruit Extracts on Estrogen Positive and Estrogen Negative Breast Cancer Cell Lines

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Abstract: Medicinal plants have widely been used to treat different kinds of ailments including cancer. *Syzygium jambolanum*, Linn (Myrtaceae) is a tropical plant used traditionally in the treatment of diabetes. However the effect of this plant on cancer has not been reported. Breast cancer is the most prevalent type of cancer among women and the incidence of this cancer has increased significantly around the world. In the present study, aqueous extract of *S. jambolanum* leaves and methanolic extract of its fruits were tested on breast cancer (MCF-7 and MDA-MB-231) cell lines for anti-proliferative effect. The MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used to quantitate the viable cells. The aqueous extract of *S. jambolanum* leaves and the methanolic extract of the fruits exerted dose-dependent inhibition (dose range of 100-500 $\mu\text{g mL}^{-1}$) on both cell lines. The leaf extract exerted more potent inhibitory effect on the MDA-MB-231 (estrogen negative) cell line as well as the MCF-7 (estrogen positive) compared with the methanolic extract of the fruit. The concentration of leaf extract that caused 50% inhibition (IC_{50}) of MDA-MB-231 was 270 $\mu\text{g mL}^{-1}$. The IC_{50} for the rest of the incubations could not be determined.

Key words: *Syzygium jambolanum*, breast cancer

INTRODUCTION

Breast cancer is one of the most common causes of cancer related deaths among women around the world. All women are at a risk of getting breast cancer as they get older. Breast cancer with Estrogen Receptor positive (ER+) and estrogen Receptor Negative (ER-) have different clinical, pathologic and molecular features^[1]. The prognosis for ER- is poorer in comparison to the ER+ and usually is associated with shorter duration of survival of patients^[1].

One of the current strategies for the treatment of human cancer is to activate the cellular apoptotic death programme^[2,3]. Currently applied radiation therapy and standard chemotherapeutic drugs kill some tumour cells through induction of apoptosis. Plant-derived compounds have great potential to be developed into anticancer drugs because of their multiple mechanisms and low side effects^[4-8]. The ER- breast tumors are known to be highly invasive and often produce distant metastases. Therefore, there is an urgent need to find an alternative or adjuvant therapy for the treatment of ER- breast cancer.

Syzygium Jambolanum Linn, commonly known as Jambol, originates from the family Myrtaceae. It is widely

found in India, Indonesia and Malaysia. The seeds are traditionally used to treat patients with diabetes mellitus and glycosuria^[9]. The fruit is stated to be astringent, stomachic, carminative, antiscorbutic and diuretic^[9]. The constituents in the seed, leaf and bark of Jambol have been identified. The seed contains fatty oils and tannins, whereas the bark's constituents include tannins, steroids, triterpenes and flavonoids. The seed has been reported to possess anti-inflammatory activity^[10]. The leaves are rich in tannins and contain the enzymes esterase and galloyl carboxylase^[9]. In this study, the fresh leaves and fruits were used to assess the anti-proliferative effect on both the estrogen positive and estrogen negative breast cancer cell lines.

MATERIAL AND METHODS

MCF-7 and MDA-MB-231 adenocarcinoma cell lines were obtained from American Type Culture Collection, USA. RPMI culture medium, trypsin, fetal bovine serum were from FLOWLAB, Australia. MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was from MERCK chemicals, Germany. All other chemicals used were of pure analytical grade.

The leaves and fruits were collected from Petaling Jaya, Selangor in March, 2004. The samples were authenticated by a Pharmacognosist in the Department of Pharmacy, Faculty of Medicine, University of Malaya.

The leaves were dried, homogenized in water, filtered and then lyophilized using a freeze dryer. The fruits were extracted using methanol, lyophilized with a rotary evaporator to obtain methanolic crude extract. The extracts were stored at -20°C.

The breast cancer cell lines (MCF-7 and MDA-MB-231) were routinely grown in RPMI 1640 medium supplemented with 2 mM L-glutamine and 5% Fetal Bovine Serum (FBS) maintained at 37°C in humidified air containing 5% CO₂ for 48 hours. The cell suspension was diluted with RPMI medium to yield a concentration of 3000 cells per 100 µL in each well of the 96-well culture plate. The leaf extract was solubilized in water whereas the fruit extract was solubilized in dimethyl sulfoxide (DMSO) and filter-sterilized using 0.22 µL pore-size syringe filters (Millipore). The diluted extracts (20 µL) were introduced into the cell culture and incubated for 96 hours. The control incubation contained either water or DMSO. The cell growth was quantitated by using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay based on the method described by Mosmann^[11]. The MTT solution (10 µL of 5 mg mL⁻¹ MTT in phosphate-saline buffer) was added to each well and was incubated for 4 hrs. Then, the medium was removed and the formazon formed was solubilized with isopropanol. The absorbance was measured at 560 nm. This absorbance was proportional to the number of viable cells.

RESULTS AND DISCUSSION

The leaf extract of *Syzygium jambolanum* showed more potent, dose-dependent inhibitory effect on both the MCF-7 (estrogen receptor positive) and MDA-MB-231 (estrogen receptor negative) cell lines after a 4 day incubation period compared with the fruit extract (Figs. 1 and 2). The extracts (both leaf and fruit) did not exert significant cytotoxic effects in 2 or 3 day incubation period (results not shown). Untreated cells (control) were healthy and were almost 70% confluent on the 4th day of incubation. The leaf extract of *S. jambolanum* exerted potent inhibition on MDA-MB-231 cell line with an IC₅₀ (Concentration that caused 50% inhibition) of 270 µg mL⁻¹ and 70% inhibition at the maximum dose of 500 µg mL⁻¹ (Fig. 2), whereas the extract exerted a maximum of 45 % inhibition throughout the concentration range of 200-500 µg mL⁻¹ on MCF-7 cell line (Fig. 1). The present

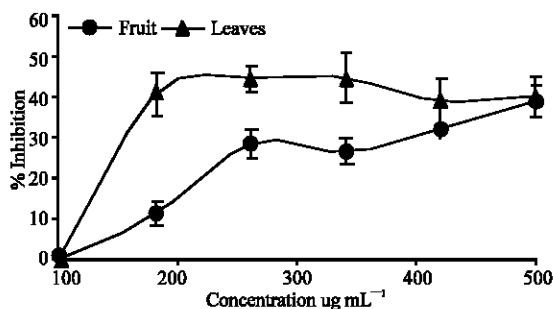


Fig. 1: Effect of *Syzygium jambolanum* leaf and fruit extracts on MCF-7

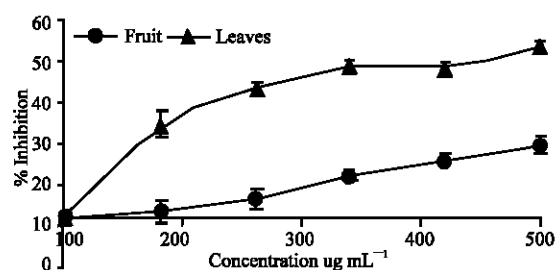


Fig. 2: Effect of *Syzygium jambolanum* leaf and fruit extracts on MDA-MB-231

study shows the potential of *S. jambolanum* in inhibiting the ER-cell line. We speculate that upon prolonged incubation, the crude extracts were able to undergo modifications possibly via oxidation and/or polymerization that render them to be more cytotoxic. Tannins are polymers and they are known to polymerize to form larger complexes in solution. The *S.jambolanum* leaves are rich in tannins^[9]. Further investigation is necessary to evaluate the role and possible cytotoxic effect of polymerized tannins from *S.jambolanum* on breast cancer. There have been evidences linking diabetes to cancer^[12]. *Syzygium jambolanum* is best known for its anti-diabetic use in traditional medicine and our present study shows the potency of this plant extracts on breast cancer cells. Further investigations would be necessary to elucidate the mode action and to identify the active components of these crude extracts.

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