

# In-Vitro evaluation of anti-cancer and genotoxic potential of medicinal herb *Saussurea lappa* extract in human cancer cell lines

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**Abstract** For centuries, plants are known to play part in the daily routine from providing food to the management of human health. During the last few decades, diverse phytochemicals have been characterized as agents that possess a potential to execute cancer cells. The oil extracted from the root of *Saussurea costus*; has been used in traditional medicine for a long time. Purpose: This study was carried out to assess the effect of *Saussurea lappa* aqueous extract on five different cell lines; Human Lung Fibroblast cells (MRC5), Human Dermal Fibroblast adult (HDFa), Madin-Darby Canine Kidney cells (MDCK), breast cancer cells (MCF7) and human colonic cancer (Caco2). Results: On normal cell lines; the extract showed cytotoxic activities with the half maximal inhibitory concentration; IC50 values, ranging from 0.85 to 2.5 mg/ml whereas MRC5 was the most sensitive cell line (IC50 values: 0.85). On malignant cell lines; MCF7 cells showed inhibition in cell proliferation with activity from 67.17% to 91.01% by the extract treatment concentration 0.50 to 2.0 mg/dl. However, Caco2 cells exhibited inhibition in proliferation ranging from 37.10% to 69.45% by the same treatment dose. Thus, breast cancer cells were more sensitive than colon cancer cells to *Saussurea costus* extract. The genotoxic potentialities of the crude extract showed increase in expression of proapoptotic genes; P53, IκBα, BAX and TNF and decrease in the expression of antiapoptotic genes; Bcl2, Survivin, and MMP-7. Conclusion: Our work pointed out that *Saussurea lappa* extract could be a potential candidate that has the capability to fight cancer.

**Keywords:** *Saussurea lappa*, Colon cancer, Breast cancer, Dermal fibroblasts, Genotoxic potentialities.

## Introduction

Cancer is a heterogeneous group of diseases that can begin in almost any tissue of the body when abnormal cells grow uncontrollably, go beyond their usual boundaries to invade adjacent parts of the body and/or spread to other organs. According to the International Agency for Research on Cancer; part of the World Health Organization of the United Nations, cancer is estimated to be the second most common cause of death worldwide, about 9.6 million deaths in 2018.<sup>1</sup>

Natural agents are believed to suppress the inflammatory process that may lead to neoplastic transformation, hyperproliferation, metastasis and angiogenesis.<sup>1</sup> Natural derived ingredients; medicinal plants have been used in cancer treatment in various parts of the world especially in ancient Egypt, India, China, and the Arab world for thousands of years with few complications arise from the uses of plant-derived products than that of chemotherapeutic drugs.<sup>2</sup>

Some of these products have been tested for their potency and their possible mechanism of actions suggested them as potential candidates as anti-cancer agents.<sup>3</sup>

Family Asteraceae, genus *Saussurea* has three hundred species in the world, sixty-one of them native to India. The oil extracted from the root of *Saussurea costus*, commonly known as *costus* or *kuth* has been used in traditional medicine and in perfumes for a long time. The natural ingredients derived from this plant are suggested as possible anticancer treatment against leukemia, liver, breast, ovarian, prostatic, colonic, and bladder cancers.<sup>4-7</sup>

The dried roots of *Saussurea lappa* (*costus* root) have been used as traditional medicine in India, China, Japan, and Pakistan<sup>8</sup>. Its main active ingredient,  $\beta$ -elemene, which belongs to the sesquiterpenes group has been confirmed to inhibit the mouse pancreatic cancer and neoplastic metastasis and to possess antitumor effect. Moreover, *costunolide* and *dehydrocostus lactone* (DL); two active ingredients of *Saussurea lappa* have a good therapeutic effect against a wide range of cancer types through inhibition of cancer cell proliferation,<sup>6</sup> inducements of cancer cell apoptosis and differentiation.<sup>9</sup>

Furthermore, *Saussurea lappa* extract has been reported by Choi *et al.* to be an efficient product to induce arrest in G2/M phase of ovarian cancer (SK-OV-3) cells by p21 up-regulation and Cdk1 down-regulation.<sup>10</sup> Meanwhile, Kuo and Kretschmer *et al.* showed its eligibility of blocking S-phase progression through Cdk inhibitor up-regulation and cyclin inhibition pathways.<sup>11-13</sup>

Further studies revealed that the anti-cancer activity of *dehydrocostuslactone* on the ovarian cancer SK-OV-3 cells has been attributed to a marked increase in the expression of the apoptotic protein BAX, cell cycle arrest is via CDK1 down-regulation, activation of p53 and release of cytochrome c.<sup>10,14</sup>

In the MCF7 cell line (Breast Cancer Cell line), the growth inhibitory activity can be linked to the inhibition of the signal transduction pathway involved in cell proliferation; by suppressing the expression of cyclin D, cyclin A, cyclin-dependent kinase 2, and Cdc25A and increases the amount of p53 and p21, resulting in cell cycle arrest.<sup>15</sup> A dose-dependent antiproliferative activity relationship has been observed by Degtarev *et al.* and Bocca *et al.* in their study.<sup>16</sup>

Programmed cell death; apoptosis is essential for normal development and maintenance of tissue homeostasis in multicellular organisms. The anti-apoptotic proteins BCL-2, BCL-X, and BCL-XL and the pro-apoptotic BAX, BAK, BID, and BAD that belong to the BCL-2 family of proteins can trace the pathways of apoptotic mitochondria through mitochondrial membrane permeability regulation.<sup>17,18</sup>

The apoptotic process is mediated by the activation of specific proteases; the caspases, however, programmed cell death can occur independently of caspases activation in cases of autophagy, paraptosis, and mitotic catastrophe. Caspase-independent cell death pathways are important safeguard mechanisms to protect the organism against unwanted and potentially harmful cells when caspase-mediated routes fail but can also be triggered in response to cytotoxic agents or other death stimuli.<sup>19</sup>

Dehydrocostus lactone (DL), the major sesquiterpene lactone isolated from the roots of *Saussurea lappa*, could ameliorate nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B) activation and enhanced tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-induced apoptosis through caspase-8 and caspase-3 activities. Also, DL renders human leukemia-60 cells susceptible to TNF- $\alpha$ -induced apoptosis by enhancing caspase-8 and caspase-3 activities.<sup>20</sup> Furthermore, it inhibited survival signaling by activating caspase-3 induced apoptosis in lung cancer cells through growth inhibition.<sup>21</sup>

## **Aims**

The aim of the present study was to check the possible use of *Saussurea lappa* species as natural anticancer remedies in breast and colon cancer cell lines.

## **Materials and methods**

### *Plants*

*Saussurea lappa* species were collected from a local Herbal Store and the plant was identified and authenticated by the taxonomists of the biology department, faculty of science, King Khalid University, Abha, Kingdom of Saudi Arabia.

### *Preparation of crude extracts*

The fruits of *Saussurea lappa* were extracted by maceration technique as has been described by Harbone protocols.<sup>22</sup> About 150 grams were macerated at room temperature with continuous shaking in 1 Liter of distilled water. The supernatant was filtered and subjected to evaporation at 60°C for 16 hours and residue was weighed, dried in the oven, and stored at 4°C until used.

### *Determination of phytochemical compounds in Saussurea lappa extract*

The phytochemical compounds such as tannins, phenols, flavonoids, alkaloids, reducing sugars, volatile oils, glycosides, amino acids, proteins, saponins, and terpenoids were determined in the licorice extract according to the procedure described by Harbone *et al.*<sup>22</sup>

*Determination of total phenolic compounds and antioxidant activity in Saussurea lappa extract*

The concentration of total soluble phenolics in Saussurea lappa extract was estimated according to the method described by Malick and Singh,<sup>23</sup> using Gallic acid as standard. The ability of licorice extract to scavenge DPPH free radicals was evaluated according to the method described by Braca *et al.*, and Kumarasamy *et al.*<sup>24, 25</sup>

*Preparation of mammalian cell lines*

Human Lung Fibroblast cells (MRC5), Human Primary Dermal Fibroblasts adult (HDFa), Madin-Darby Canine Kidney (MDCK) Cells, Michigan Cancer Foundation-7 (MCF7) Breast Cancer Cells and Human Colonic Carcinoma (Caco2) cells were cultured on Dulbecco's Modified Eagle media. Media were supplemented with 200 mM L-glutamine and 10% fetal bovine serum (Gibco-BRL, Germany). This work has complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects and/or animals.

*Assay of cytotoxicity in Saussurea lappa extract*

The non-toxic doses of Saussurea lappa crude extract were tested on three different normal cell lines as MRC5, HDFa, MDCK according to the method as described by Borenfreund and Puerner.<sup>26</sup>

*Assay of cell proliferation in Saussurea lappa extract*

The cell proliferation assay in Saussurea lappa extract was performed according to the manufacturer's protocol of cell proliferation ELISA BrdU (colorimetric) kit (Roche Diagnostics, USA), cell proliferation in response to treatments was assayed using the measurements of 5-bromo-2-deoxyuridine incorporated into cellular DNA.

*Determination of gene expression by using RT-qPCR*

The anticancer activity of Saussurea lappa extract was examined using quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) for several genes in the treated breast cancer and human colonic cancer cells compared with the non-treated cells. The cells were treated with the resultant non-toxic concentration of Saussurea lappa extract for 48 hours as previously described by Yang *et al.*<sup>27</sup> The RNA extraction from the treated and non-treated cells using RNA extraction kit (Qiagen, Germany). The first cDNA strand was synthesized using oligo(dT) primer (Thermo scientific) and Master Mix (Qiagen, Germany). GAPDH gene was used as an internal control reference and the RT-qPCR was performed using Syber Green master mix (Qiagen, Germany). The primers used in this study are listed in Table 2.

**Results**

**Table 1: Phytochemical analysis of Saussurea lappa extract**

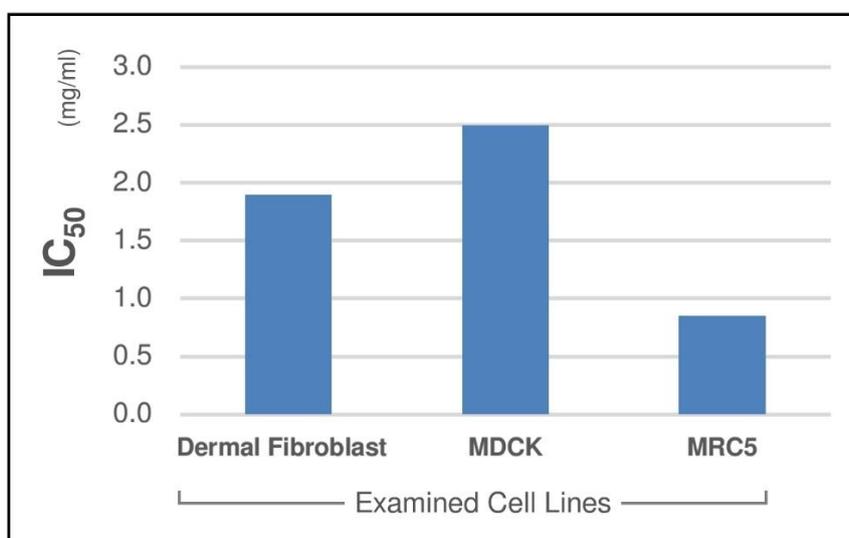
Tests	<i>Saussurea lappa</i> aqueous extract
Tannins	-
Reducing sugars	+

Glycosides	+
Alkaloids	++
Flavonoids	+++
Volatile oils	+
Terpenoids	+++
Protein + amino acids	+
Saponins	+

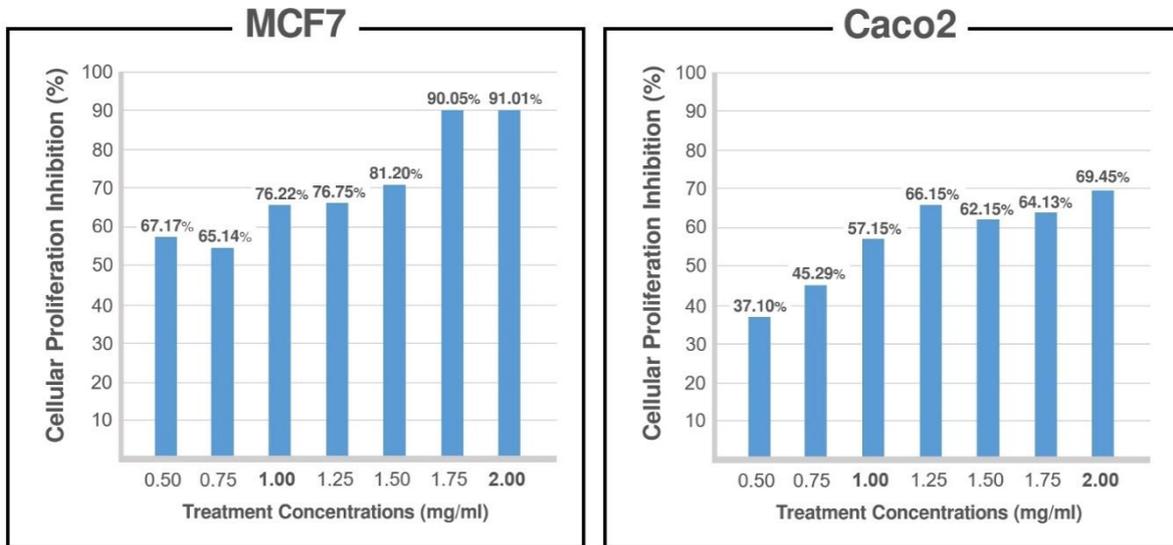
**Table 2: List of primers used in gene expression analysis**

Primers		The nucleotide sequence 5' to 3'	Annealing Temp.
GPDH	F	ATTGACCACTACCTGGGCAA	60°C
	R	GAGATACACTTCAACACTTTGACCT	
Bcl2	F	TATAAGCTGTTCGAGAGGGGCTA	60°C
	R	GTAATCAGTCATCCACAGGGCGAT	
P53	F	AACGGTACTCCGCCACC	60°C
	R	CGTGTCACCGTCGTGGA	
Survivin	F	TGCCCCGACGTTGCC	56°C
	R	CAGTTCTTGAATGTAGAGATGCGGT	
IκBα	F	CATGAAGAGAAGACACTGACCATGGAA	56°C
	R	TGGATAGAGGCTAAGTGTAGACACG	
BAX	F	CCTGTGCACCAAGGTGCCGGA	55°C
	R	CCACCCTGGTCTTGGATCCAGCCC	
MMP-7	F	GATGGTAGCAGTCTAGGGATTA	53°C
	R	GGAATGTCCCATACCCAAAGAA	
TNFα	F	TCTCTAATCAGCCCTCTGGCC	53°C
	R	TGGGCTACAGGCTTGTC	

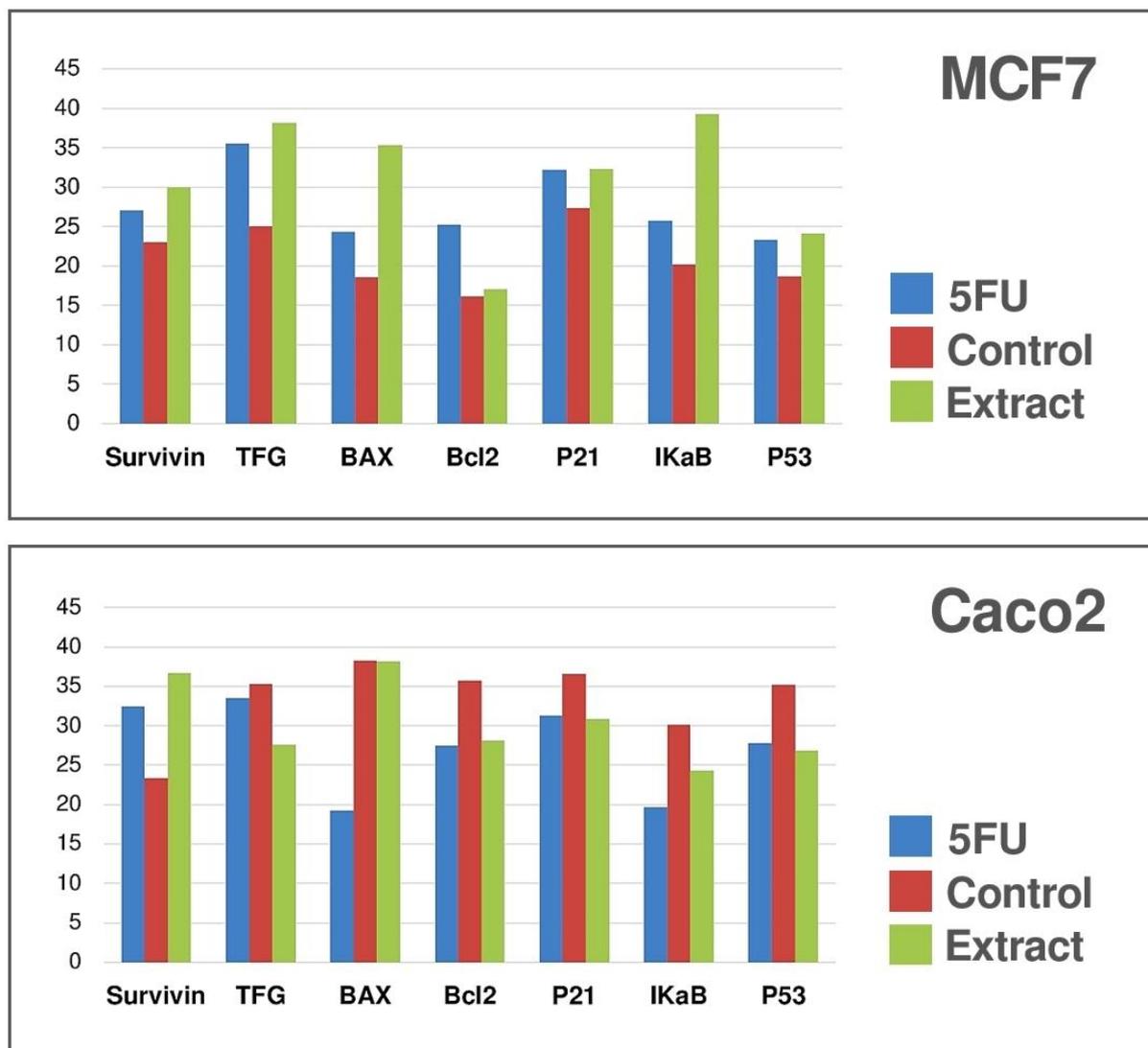
F, forward; R, reverse



**Figure 1: Cytotoxicity of Saussurea lappa aqueous extract on normal cells. The data presented revealed the effect of treatment half maximal inhibitory concentration (IC50) on normal cells.**



**Figure 2: Anti-proliferation activities of Saussurea lappa aqueous extract on MCF7 cells (on the left), and Caco2 cells (on the right).**



**Figure 3: Gene expression pattern of Saussurea lappa extract on breast cancer and Caco2 cell lines.**

### Discussion

Saussurea lappa is traditionally known as one of the potent plant derived products that is considered for its medicinal uses in treatment of cancer, inflammatory and digestive tract disorders.<sup>4-7</sup> To date, many active ingredients have been isolated from Saussurea lappa appa.<sup>6-9</sup>

Our analysis indicated that the Saussurea lappa aqueous extract contained flavonoids, terpenoids, alkaloids, glycosides and saponins as bioactive ingredients which could be a potential source for new molecules used in plant derived medicine. Both phenolic and flavonoid components were extracted in concentration of 6.5 mg/g of catechol-equivalent phenolics and 13.0 mg/g of gallic acid-equivalent flavonoid respectively. (Table 1)

Ingredients of Saussurea lappa such as costunolide, dehydrocostus lactone, and cynaropicrin were proven to have exceptional pharmacologic properties. Some active ingredients had potency to be developed into new drugs to treat diseases.<sup>28</sup> Cynaropicrin, lappadilactone, iso-

dihydrocostunolide, costunolide, and dehydrocostus lactone could be used to inhibit angiogenesis and treat cancers.<sup>29</sup>

In the attempt to explore the anticancer activity of *Saussurea lappa* extract, we had first observed cell proliferation in three different normal cell lines; HDFa, MRC5, and MDCK by exposing these cell lines to non-toxic doses (0.85 to 2.5 mg/ml) of *Saussurea lappa* crude extract for 48 hrs. The extract showed a significant dose-dependent antiproliferative activity. The antiproliferative effect of the extract peaked for MDCK was 2.5 mg/ml, 1.9 mg/ml for human dermal fibroblast adult, and 0.85 mg/ml in MRC5 cells. These findings showed that both the human cells were more sensitive to the extract than canine kidney cells and human lung fibroblast cell line. (Figure 1)

The anti-proliferative activities of *Saussurea lappa* extract against cancer cells (breast cancer and colon cancer) were quantitatively estimated. The results detected that the highest anti-proliferation activity was 91.01% and 69.45% respectively. MCF7 cells showed inhibition in proliferation with activity of 67.17% to 91.01% by treatment concentration 0.50 to 2.0 mg/dl. However, Caco2 cells exhibited inhibition in proliferation ranging from 37.10% to 69.45% by the same *Saussurea lappa* extract concentrations. The latter finding specified that breast cancer cells showed more antiproliferative activity than those of colon cancer cells on exposure to the same *Saussurea lappa* extract concentration. The breast cancer cells were more sensitive to *Saussurea lappa* extract than colon cancer cells (Figure 2). Our results are in agreement with other studies.<sup>32,33</sup>

Finally, we have found that *Saussurea lappa* extracts effectively inhibited breast and colon cancer growth concomitant with induction of apoptosis in vitro. Extract treatment resulted in a significant increase in expression of proapoptotic genes; P53, I $\kappa$ B- $\alpha$ , BAX and TNF $\alpha$  and decreased in the expression of antiapoptotic genes; Bcl2, Survivin and MMP-7. These changes in the ratio of proapoptotic and antiapoptotic Bcl-2 family proteins might have contributed to the apoptosis-promotion activity of *Saussurea lappa* extracts (Figure 3). Our results are agreed other studies.<sup>34-44</sup>

These results are promising for alternative treatments of breast and colon cancers.<sup>30, 31</sup> However, breast cancer is a chemo sensitive malignancy, still surgery is important in the initial therapy of patients, and most of the patients still require postoperative chemotherapy to get rid of any residual microscopic or macroscopic peritoneal implants.<sup>32-34</sup>

Costunolide and dehydrocostuslactone which are major sesquiterpene lactone isolated from the roots of *Saussurea lappa* exerted anti-proliferative effects in several breast cancer cell lines by altering the cell cycle.<sup>43</sup> Their anti-cancer mechanisms, including causing cell cycle arrest, inducing apoptosis and differentiation has been studied.<sup>44</sup> *Saussurea lappa* extracts could be a promising nominate for chemotherapy in treatment of breast and colon cancer.

## Conclusion

Collectively, the results of this study suggest that *Saussurea lappa* extract possessed a significant antiproliferative activity via cell cycle arrest and apoptosis, particularly in breast and colon cancers. Because of these features, *Saussurea lappa* extract may work as a beneficial anticancer therapy through induction of cancer cell apoptosis and weaken the cell survival.

## **Compliance with ethical standards**

### **Conflict of interest**

The authors declare that they have no competing interests.

### **Informed consent**

Informed consent was obtained from all individual participants included in the study.

### **Ethical approval**

The study was conducted according to the institutional ethical standards.

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