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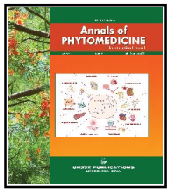

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Augmentation of cytotoxicity and apoptotic activity in human breast cancer MCF-7 cells by *Saussurea costus* (Falc.) Lipsch roots

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Abstract

Worldwide, breast cancer is one of the leading causes of mortality for women. Anti-neoplastic medicines or treatments that cure tumours can lengthen life expectancy, but because of their wide range of adverse effects, it has been shown that they also degrade the quality of life. One possible medicinal plant found in the Himalayas with notable ethnopharmacological qualities is *Saussurea costus* (Falc.) Lipsch. The phytochemicals in *S. costus* give the plant its anticarcinogenic potential and guarantee that it has no or very few negative effects. This leads to the use of the plant as a therapeutic or preventive medication candidate for cancer. By using mammary cancer MCF-7 (Michigan Cancer Foundation-7) cells at 20 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml concentrations for 24, 48, and 72 h, percentage cytotoxicity and apoptotic (caspase-3/7 and annexin V/PI) activities were investigated. According to the results of *in vitro* tests, *S. costus* root extract stimulated the apoptotic activity of caspase-3/7 and annexin V/PI on the MCF-7 cells and positively affected antiproliferative activity.

1. Introduction

Cancer is a multifaceted, heterogeneous illness with an infinite capacity for replication, resistance to growth inhibitory signals that would inhibit cell growth, an autonomous reaction to signals that would stimulate cell growth, significant angiogenesis, and evasion of apoptotic mechanisms (Adami *et al.,* 2018; Jaikaria *et al.,* 2024). Breast cancer or mammary tumours in human females is a health risk with heavy mortality linked to serious malignancies (Mandadi and Srinivas, 2023). Due to inadequate diagnosis and drug resistance to current chemotherapeutic drugs, therapy alternatives for breast cancer are crucial (Shamna and Poyil, 2023). Mammary tumour in canines is thought to be three times more common than human breast cancer and is the cause of high mortality in dogs (Hussain *et al.,* 2018). Because menopause in human women and neutering in female dogs are similar, it is possible to compare the end of hormonal impact in both species (Kim *et al.,* 2017). A variety of medicines, including the antiestrogen medication tamoxifen and chemotherapy medication including the antimicrotubule agent paclitaxel and the cytotoxic antibiotic doxorubicin, are often used in the therapeutic management of breast cancer. Despite being proven to be successful in the fight against breast cancer, chemotherapy medications come with a high risk of side effects. According to Wu (2012), doxorubicin, paclitaxel, and tamoxifen have been shown to cause major adverse effects such as cardiotoxicity, myelosuppression, and endometrial cancer. Therefore, developing novel chemotherapeutic agents or alternative compounds that have negligible side effects on normal tissues, lower treatment costs, and have a good safety profile is imperative.

Herbal medications are natural therapies made from plants; they are safe, inexpensive, and sometimes need a long course of treatment (Parveen *et al.,* 2020). Because the herbal products contain some effective phytoconstituents which can be utilized against several ailments including cancers (Srivani and Mohan, 2022). Since decades, a variety of plant extracts have been extracted as herbal medicines and used to treat ailments including general debility, indigestion, and anorexia. Several of these extracts are used by rural communities but lack scientific validation (Barman *et al.,* 2021).

In terms of the availability of flora with chemopreventive and multiple therapeutic potential, the Himalayan range is a prolific and rich source of biodiversity (Samant *et al.,* 2007). Of the about 18,440 identified plant species, about 1748 have been shown to have therapeutic potential for treating a variety of illnesses (Samant *et al.,* 1998; Joshi *et al.,* 2016). Among these native plant species, *S. costus* is found in the North Western Himalayan area at an elevation of 2000-3500 meters above sea level. Its base has leaves that range in size from 0.5 to 1.25 meters, with a rough bottom surface and smaller, sub-sessile leaves at the top. Located near the leaf axile, the plant bears purple, round blooms in clusters of two to five. On the head of the fruiting flower, the corolla is tubular and about 2cm long, accompanied by fluffy hairs. According to Pandey *et al.* (2007), the roots of this plant are around 40 cm long and dark brown.

From the genus *Saussurea*, a wide range of over 420 bioactive components have been extracted (Zhao *et al.,* 2017). The chemical

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components found in *S.costus* root extract are used to treat a variety of conditions, such as atopic dermatitis (I, 3-butylene glycol extract) (Lim *et al.,* 2014), asthma (Lee *et al.,* 2017), liver diseases (Yaeesh *et al.,* 2010), diarrhoea and dysentery (Negi *et al.,* 2013), ulcers (constunolide, dehydrocostus lactone, saussureamine A), gastritis (costunolide), and inflammation (cynaropicrin) (Venkataranganna *et al.,* 1998). A few studies have reported the anti-carcinogenic properties of cynaropicrin, dehydrocostus lactone, and costunolide extracted from *S. costus* (Jeong *et al.,* 2002).

The root extract of *S.costus* is found to exhibit anticarcinogenic potential in female rats with chemically induced mammary tumours. The antineoplastic activity of *S. costus* root extract was demonstrated by the minimal damage to the kidneys and liver enzymes, declining oxidative stress parameters, lowering the tumour growth, and barely changing the histological changes in the mammary gland tumour tissues (Kumar *et al.,* 2024). Another study conducted has shown the antiproliferative potential of *S.costus* on LC-540 cells along with inhibitory potential towards metastases of neoplastic cells to the lungs as compared with DMBA-treated rats (Kumar *et al.,* 2024).

The anticarcinogenic potential of *S. costus* is supported by only a handful of research, despite it being a remarkable medicinal herb. Therefore, the current research implies examining the potential of *S.costus* as a chemopreventive medicinal herb by *in vitro* study.

2. Materials and Methods

2.1 Procurement, and authentication of plant material

The *Saussurea costus* (Falc.) Lipsch roots were procured from Lahaul and Spiti, Himachal Pradesh, India at 4551 m altitude (Figure 1A). The plant was identified as *S.costus* at the CSIR-Institute of Himalayan Bioresource and Technology (CSIR-IHBT), Palampur, Himachal Pradesh, India (Voucher Specimen Number #PLP-15389). A semisolid material extracted from 70% ethanol was dried using a lyophiliser after it was prepared in a rotatory evaporator at 40°C temperature and vacuum pressure. The percentage yield of *S.costus* extract was 10.33%.

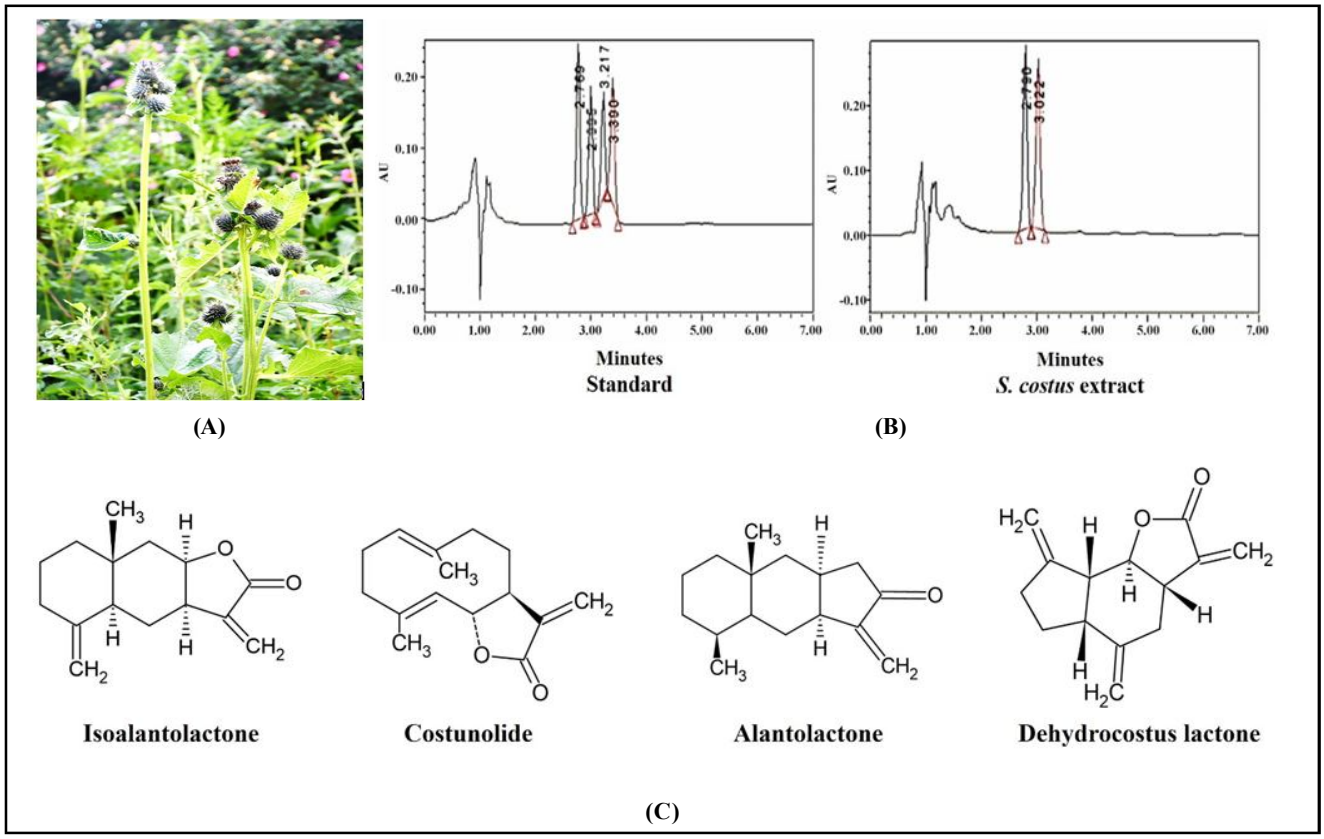


Figure 1: Figure (A) represents the *S. costus* plant with leaves and flowers. Figure (B) represents the UPLC chromatogram of standards of isoalantolactone, costunolide, alantolactone and dehydrocostus lactonepresent in *S. costus* root extract. Figure (C) illustrates the chemical structures of costunolide, dehydrocostus lactone, alantolactone and isoalantolactone.

2.2 Quantitative analysis of *S. costus* root extract

UPLC-PDA was used at CSIR-IHBT, Palampur to characterize and quantify the bioactive constituents present in the *S.costus* root extract. All standards were separated and eluted at a flow rate of 0.220 ml/min throughout a 7 min run period.The bioactive constituents present in the sample were measured by calculating the calibration curve and measuring the area of the peak.

2.3 Sulforhodamine B (SRB) colorimetric assay

The National Centre for Cell Science (NCCS) in Pune provided the human MCF-7 cells, which were then kept at CSIR-IHBT in Palampur. The human MCF-7 cells were maintained in EMEM that was boosted with 10% fetal bovine serum (HiMedia, India), penicillin (10,000 units/100 ml), and streptomycin (10 mg/100 ml) from Sigma (USA). The cell culture was maintained at 37°C in a pH 7.4 humidified

environment with 5% CO_2. Culture-grade DMSO was used to dissolve the root extract. Vinblastin (10 µM) was employed as a positive control and DMSO as a negative control. To evaluate the cytotoxicity of *S. costus* root extract (20, 50, 100, and 200 µg/ml) for 24, 48, and 72 h. Following every time interval, the cells were subjected to a 1 h dark treatment at room temperature using 50 µl of SRB dye. Following the application of 50 µl tris base to the cells, the plate was shaken for 5 min, and the optical density was determined using a microplate reader (BioTeK Synergy H1 Hybrid Reader) at 540 nm. The *in vitro* experiment was carried out in triplicates. The percentage cytotoxicity was determined by subtracting the optical density of the test material from the optical density of the control and dividing by the optical density of the control multiplied by 100.

2.4 Annexin V/PI apoptosis detection assay

Using flow cytometry, the effect of the extract on apoptosis and necrosis was assessed on MCF-7 cells, the detection kit used was annexin V-FITC (SIGMA-ALDRICH).

2.5 Assay for detecting caspase-3/7 activity

MCF-7 cells were seeded into a 96-well plate using 50 µl of complete media at a density of 1.20×10^6 cells per well. The plates were placed in a CO_2 incubator and incubated for 8 to 12 h at 37°C. Following incubation, 100 µl of caspase-3/7 reagent was added, and the mixture was then incubated for 2 h at room temperature. With the use of a Promega Apo-ONE homogeneous caspase-3/7 test kit, total caspase activity was determined. By measuring the net relative fluorescence units (RFU) at an emission and excitation wavelength of 530 nm using a microplate reader, the caspase-3/7 activity was determined:

Net relative fluorescence unit (RFU) = Assay RFU – Blank RFU

2.6 Statistical analysis

Standard error mean (SEM) ± mean (mean) has been employed to indicate all research results at $p<0.05$ and one-way variance analysis with SPSS software for the statistical analysis.

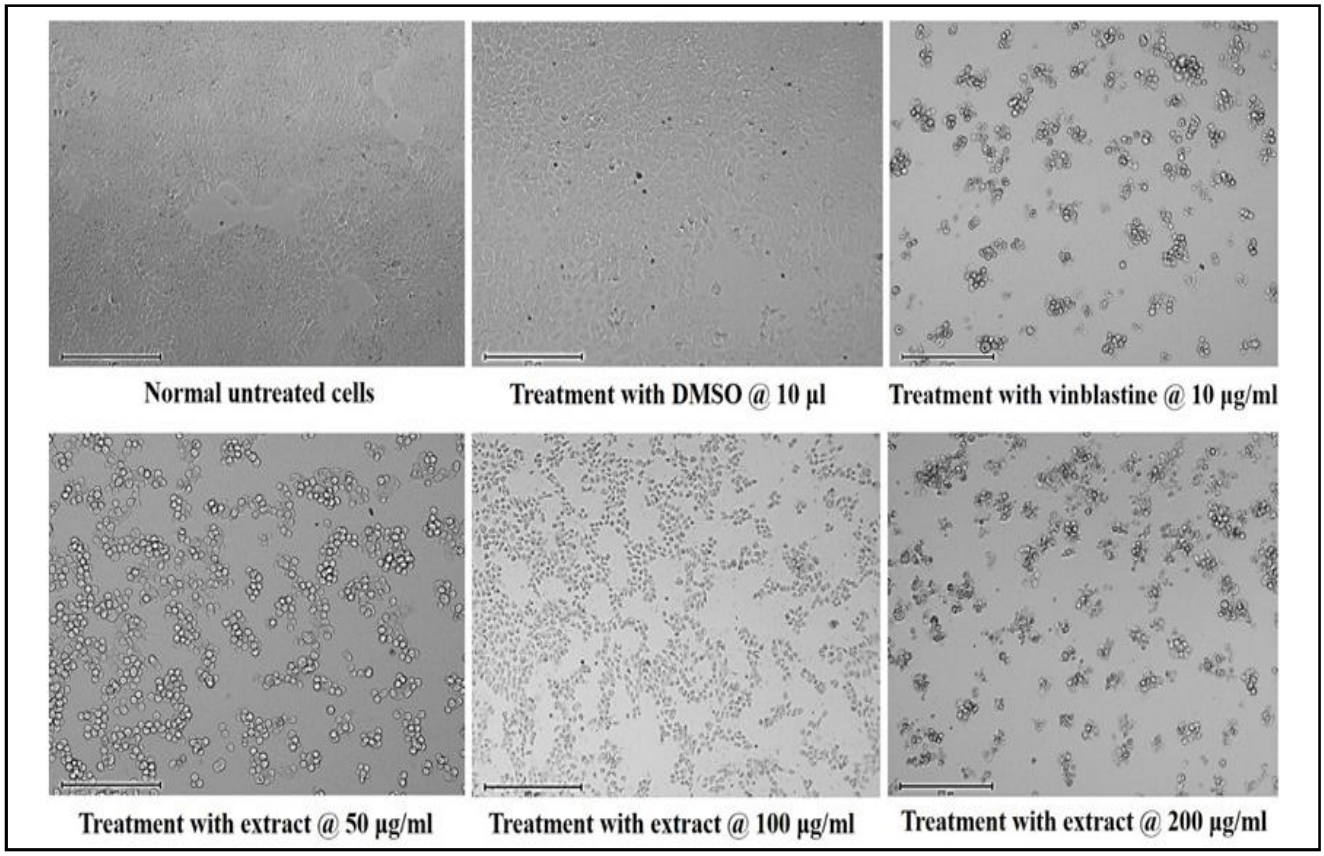
3. Results

3.1 UPLC-PDA analysis to identify phytoconstituents in *S. costus* root extract

Phytochemical screening and quantification of dried *S.costus* roots showed the presence ofcostunolide and dehydrocostus lactone at a concentration of 360.56 µg/mg and 409.71 µg/mg, respectively (Figures 1B and 1C).

3.2 Anticancer activity of *S. costus* roots

When 70% aqua-ethanolic *S. costus* root extract was introduced to MCF-7 cells in a dose-dependent manner as versus untreated cell lines, notable morphological alterationsindicative of apoptosis were noticed (Figure 2A). A time and concentration-dependent antiproliferative effect was demonstrated by the SRB assay on human breast cancer MCF-7 cell lines when tested with 70% *S. costus* root extract. Maximum percentage cytotoxicity was detected with the uppermost concentration of extract (200 µg/ml) over a 72 h incubation period (Figure 2B).



(A)

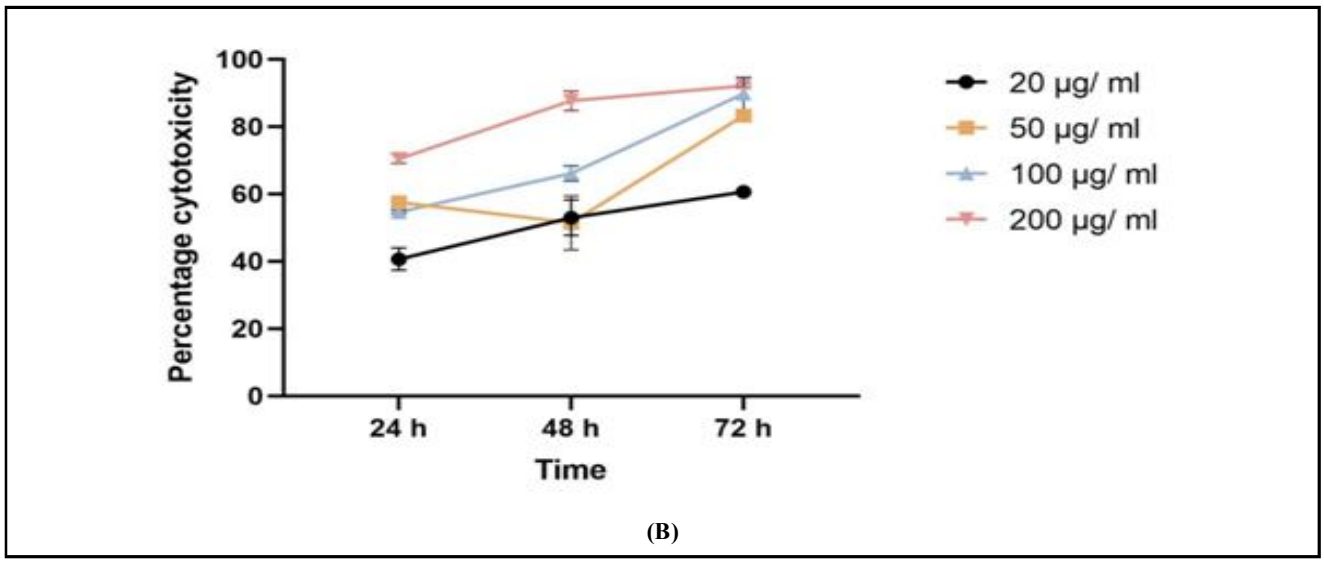


Figure 2: Figure (A) represents phase contrast micrograph fields of *S. costus* root extract treated and untreated human breast cancer MCF-7 cells. Figure (B) represents the percentage cytotoxicity of *S. costus* root extract on MCF-7 cell lines after 24, 48 and 72 h of treatment.

3.3 Impact of the *S. costus* root extract on the activity of annexin V/PI

When MCF-7 cells were subjected to the 70% aqua ethanolic *S. costus* root extract, the annexin V/PI detection test demonstrated that, in contrast to the control, there was a notable increase in apoptotic cells (Q2 + Q4) at 200 µg/ml (Figure 3A).

3.4 Impact of *S. costus* root extract on caspase-3/7 activity

After 48 and 72 h of treatment, the *S. costus* root extract (200 µg/ml) likewise, in a dose-dependent way, increased the caspase-3/7 activity on MCF-7 cells in contrast to the control group (Figure 3B).

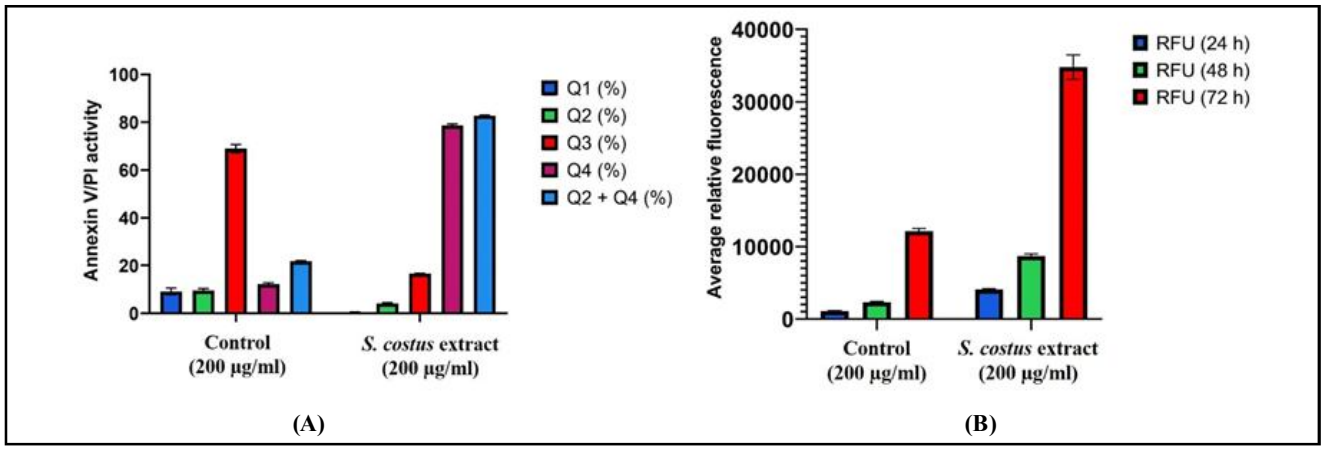


Figure 3: Figure (A) represents the average percentage of necrotic (Q1), late apoptotic (Q2), viable (Q3), early apoptotic (Q4) and average apoptotic (Q2 + Q4) cells on MCF-7 cells. Figure (B) represents the caspase-3/7 activity of *S. costus* root extract on MCF-7 breast cancer cells.

4. Discussion

A broad range of ailments can be treated with the medicinal herb *S. costus*. The haematological, cytological, immunohistochemical, histopathological and ultrasonographic evaluation of the rats administered with DMBA were altered profoundly contrary to the group that received the highest possible dose of 500 mg/kg body weightof the *S.costus* root extract (Kumar *et al.,* 2022; Kumar *et al.,* 2024).

The plant extract used at the concentration of 200 µg/ml exhibited maximal percentage cytotoxic activity on human breast cancer cells after 72 h of duration demonstrating the antiproliferative potential of *S. costus* roots, which was supposed to be attributed to the bioactive constituents namely costunolides and dehydrocostus lactones. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide test has been carried out to determine the viability of the KB cells after they had been treated with methanolic *S. costus* root extract at 30 mg/ml of concentration (Moon *et al.,* 2013).

This study established that *S. costus* reveals enhanced activity for caspase-3 and annexin-V/PI at the dosage of 200 µg/ml following 72 h. The elevated caspase-3 and annexin V/PI activities are an indication

of the killing of the neoplastic cells demonstrating the destructive action of *S. costus* roots on cancer cells. The caspase (cysteine-aspartic acid protease) family include the crucial member caspase-3 which has been linked to the control signalling pathways and cellular apoptotic processes. According to some researchers, the expression of caspase-3 is linked to the advancement of various tumours, such as glioma (Jia *et al.,* 2015; Jin *et al.*, 2015; Zheng *et al.,* 2015). As a result, caspase-3 is considered a significant biomarker for carcinogenesis and is connected with the prognosis of patients suffering from breast cancer (Nakopoulou *et al.,* 2001; Engels *et al.,* 2014).

Numerous prior investigations have demonstrated a connection between the onset of breast cancer and the attenuation of caspase-3 expression (Grigoriev *et al.,* 2002; Vegran *et al.,* 2006; Zhou *et al.,* 2013). A possible anticarcinogenic action of *S. costus* roots has been identified as evidenced by slight changes in cytopathological, haematological, and ultrasonological parameters and a lowered risk of spread of mammary tumours to the pulmonary system in rats (Kumar *et al.,* 2022; Kumar *et al.,* 2024).

5. Conclusion

The study's findings indicated that the root extract of *S. costus* at 200 µg/ml is an effective antiproliferative or antineoplastic agent. The findings were supported by reduced survival and proliferation of human breast cancer MCF-7 cells and increased cytotoxicity and caspase-3 and annexin V/ PI expression. The study also necessitates the use of active phytoconstituents of the plant against mammary tumours in animal models to further validate the antineoplastic potential.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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