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EVALUATION OF THE POTENTIAL ANTI-CARCINOGENIC EFFECT OF ETHANOLIC *CAPPARIS SPINOSA* L. LEAVES EXTRACT ON ORAL SQUAMOUS CELL CARCINOMA CELL LINE (SCC-4)

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ABSTRACT

INTRODUCTION: Malignancy is regarded as a potentially fatal disorder that can impact human health globally. More than 90% of all oral cavity malignancies that have been detected are invasive oral squamous cell carcinomas (OSCCs). Patients having OSCC still have an extremely poor survival rate, even though medical therapy has advanced significantly. Medicinal plants have been the subject of recent scientific research. Capparis spinosa L. (CS) is among the prominent plants found across the Mediterranean region. According to phytochemical analysis, CS leaves are considered to be a promising therapeutic plant since they are an essential spring of bioactive substances such as flavonoids and phenolic acids. Research has indicated that CS extract exhibits anti-carcinogenic properties against numerous forms of cancer.

OBJECTIVE: The current research was to the evaluation of the anti-carcinogenic effect of the ethanolic extract of CS leaves on SCC-4 cell lines contrasted with both the untreated cancer cell lines and Cisplatin treated.

MATERIALS AND METHODS: This work comprised an *in vitro* investigation. SCC-4 cell line was separated into 3 groups: group 1 was considered as negative control (untreated), group 2 was positive control (Cisplatin-treated), and group 3 (treated with CS leaves extract). Viability, proliferation and apoptosis assays were performed to evaluate the possible therapeutic effect of the CS leaves extract in duration of 48 hours.

RESULTS: Treating SCC-4 cell line with the extract of CS leaves has shown that the viability effect was in a dose-dependent fashion. The IC_{50} or the half-maximal inhibitory concentration was equal to 176.3µg/ml. Also, CS treatment has elevated the apoptotic cells' percentage and decreased SCC-4 cells proliferation rate in compare to the untreated cancer cell lines and Cisplatin action.

CONCLUSION: the extract of CS leaves may have a prominent anti-cancerous effect on OSCC.

KEYWORDS: Oral squamous cell carcinoma, Capparis spinosa L., Viability, Proliferation, Apoptosis.

Running Title: Anti-carcinogenic effect of *Capparis spinosa* L. on SCC-4.

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INTRODUCTION

Over 90% of occurrences of oral cancer are oral squamous cell carcinomas (OSCCs), which are cancerous tumors with invasive and destructive characteristics that originate in the oral mucosa's stratified squamous epithelium (1, 2). OSCCs may develop as a result number of factors, including the tobacco product consumption, alcohol, betel quid usage, areca nut and genetic modification (3). Currently, nourishment is regarded as a significant determinant of the OSCCs growth and spread (4). OSCCs may arise within the tongue, the mouth floor, the alveolar surface, the buccal mucosa, and the hard palate. However, the tongue is the most often occurring site of OSCCs and possesses one of the poorest prognosis (5).

Even with the advancement in therapeutic approaches to cancer treatment, such as surgery, chemotherapy and radiation (6, 7), the mortality and morbidity percentages haven't expressively been improved. As a result, oncology researchers have turned towards herbal extracts and natural compounds

in order to lessen the drugs side effects and increase the ability toward reduce proliferation, also to stimulate apoptosis in cancer cells (8).

The genetically programmed cell death or apoptosis can be prompted by various stimuli either pathologic or physiologic. Apoptosis primarily eliminates the altered cells which are harmful or useless to any living organism. Apoptotic morphological characteristics include cell shrinkage, cytoplasmic vacuolation, and condensation of pyknosis or nuclear chromatin (9, 10). The apoptosis activation occurs by either intrinsic or extrinsic pathway which is mediated by a few proteins which are regarded as cancer prognostic markers (10). Presently, the connection between apoptosis and cancers has attracted much attention; hence declined apoptosis is essential for cancer progression and is regarded as a vital barrier to achieving a fruitful therapy (11).

One important chemotherapeutic medication that is frequently used in the treatment of OSCC is cis-diamminedichloroplatinum (II), sometimes referred to as cisplatinum or Cisplatin. It is regarded as the first-generation anti-cancer agent (12-15). The action method of Cisplatin is through a cross-linkage formation with the purine bases on DNA to produce DNA adducts that interfere with DNA repair mechanisms via P_{53} signaling induction (16). Accordingly, both cell cycle arrest and repair apoptosis occur (16, 17). Some patients do not respond to Cisplatin treatment owing to the malignancy's chemical resistance and rapid progression. Numerous other adverse effects have also been reported, such as nephropathy and severe renal injuries, digestive toxicity, auto-toxicity, neuropathy, myelo-suppression and vascular injury (18-20).

Capparis spinosa L. (CS) is a part of the perennial plants family which has about 250 different species commonly applied for ornamental, culinary and cosmetics along with both medicinal and pharmaceutical purposes (21, 22). CS bears large white to pinkish-white flowers and rounded fleshy leaves (21). Generally, CS is among the most abundant aromatic plants that can be grown anywhere on slopes, rocky and stony, and to the dry zones' basin and they are well-adapted, (21). Moreover, CS wild species are found in the Mediterranean countries extending as far as the North African great desert to the western and central Asian dry regions (23, 24).

Due to their different biological activities, diverse parts of CS such as roots, leaves, fruits, and even buds are known for their health-enhancing properties including anti-rheumatism, anti-arthritis, anti-inflammatory, anti-oxidant, anti-anemia, hepato-protective, anti-mutagenic and anti-carcinogenic (25). CS leaves are regarded as an excellent source of numerous flavonoids and phenolic substances such as quercetin, catechin, coumarin, chlorogenic acid, caffeic acid, luteolin, rutin, ferulic acid, kaempferol, syringic acid, resveratrol and vanillic acid (26). Previous literature has indicated that phytochemicals exert anti-cancer action via modifying the different activities (Kinases Tyrosine), receptors in the expression of the genes which control both apoptosis and cellular proliferation (27, 28).

The effect of the ethanolic CS extract has been evaluated by several researchers on various cell line types such as hepatocellular carcinoma cell line (HepG2) (29), cervix carcinoma cell line (Hela), osteosarcoma cell line (Saos) breast cancer cell lines (MCF7) (30), epithelial cervical cancer cell line (HeLa) (31), cancer cells derived from fibroblast cell-2 (28), human gastric cancer cell line cells (SGC-7901) (32) and colon carcinoma (HT-29) (33). Despite these studies, the exact mechanism via which CS toxicity in tumor cells occurs is not well understood. Furthermore, it is currently unknown how well CS leaf extract may work on OSCC.

This research's objective was to assess the anti-carcinogenic effect of the CS leaves ethanolic extract on the SCC-4 cell line through evaluating the cellular viability, cellular proliferation and apoptosis. The null hypothesis denotes that ethanolic CS leaves extract has no role in the treatment of OSCC.

MATERIALS AND METHODS

Ethanolic *CS* leaves Extract and Cisplatin Preparation

Matruh region (280 Km west of Alexandria) was the selected region for collecting the CS leaves. Botanical identification and authentication for the collected CS plants was performed by the Department of Botany and Microbiology, Faculty of Science, Alexandria University. Then, for 25–30 days, plants were dehydrated at a temperature between 30 and 40°C. Afterwards, plants were converted into fine powder. The dried plant powder (100 g) was then extracted using 90% v/v ethanol by a soxhlet device (40mm ID, with 500 ml round bottom flask). The crude extract was then filtered and concentrated at around 40°C with less pressure (34). Then, the sterilized extract was used by the autoclave. The Cisplatin vials (1 mg/mL) were purchased from CiplaMed (a pharmaceutical company) by Mylan.

Cell line and Analysis

The SCC-4 cell lines were obtained from ATCC (American Type Culture Collection) and it was prepared from the primary culture of tongue SCC of a male patient who was 55 year old. Then, the cells were grown in Dulbeco's Modified Eagle's Medium (DMEM) at pH =7.2. A 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/ streptomycin) in 95% air and 5% CO2 were used in a humidified environment at 37°C. all these procedures were done at Alexandria, Faculty of Medicine at the Center of

Excellence for Research in Regenerative Medicine and its Applications, CERRMA.

To initiate the experiment, the SCC-4 cell line was separated into 3 groups; Group 1 negative control (no treatment), group 2 positive control (Cisplatin-treated), and group 3 (extract-treated).

In each group, the percentage of SCC-4 cells viability, proliferation and apoptosis were assessed. The researcher conducted each test 3 times and the mean value was taken from the 3 trials in order to guarantee the results' accuracy.

Cell Viability Analysis for the Determination of the half-maximal inhibitory concentration

A total of 5,000 SCC-4 cells per well were planted in 96 well plates. The following day, SCC-4 cells were subsequently exposed to the following CS leave extract concentrations: 1000, 500, 250, 125, 62.50, 31.25, 15.625, 7.813 µg g/mL and (40, 20, 10, 5, 2.5, 1.25, 0.625, 0.313 µg/ml of Cisplatin. After 48 hours, each well received an addition of the MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) reagent and they were incubated at 37°C for 4 hours. The cell supernatant was discarded and the formazan precipitates were dissolved in 100 µl dimethyl sulfoxide (DMSO). A spectrophotometer (ELISA reader, Infinite F15 TECAN, Switzerland) was used for measuring the absorption rate at 570 nm wavelength. The values of half-maximal inhibitory concentration (IC_{50}) were calculated by means of GraphPad Prism software (version 6.0).

Proliferation Analysis by Flow Cytometry

In a 6-well plate, cells were deposited and allowed to develop overnight. Following treatment with the ethanolic CS leaves extract (176.3µg/ml) and Cisplatin (12.4 µg/ml), the cells were incubated for 48 hours and then rinsed with phosphate-buffered saline (PBS). The cells were fixed in 0.5 ml of 95% frozen methanol for 1 hour. The cells were then treated with PBS and allowed to incubate for 1 hour with an anti-Ki67 antibody labeled with Alexa Fluor 488. Next to the period of incubation, the cell pellet was re-suspended in 0.5 ml of PBS after the cells were cleaned with PBS and the supernatant was discarded, and measured on a BD (Becton-Dickinson) FACS (Fluorescence-activated cell sorting) Calibur flow cytometer (BD Bioscience, Heidelberg, Germany). A minimum of 10,000 events were acquired for Ki67 determination. Data were analyzed using BD CellQuest Pro Software, version 5.1 (BD Biosciences, San Jose, CA, USA).

Apoptosis Analysis by Flow Cytometry

The Annexin V-FITC Apoptosis Detection Kit / Propidium Iodide (PI) were employed in accordance with the protocol of the manufacturer. To summarize this, both the floating cells and the harvested cells were mixed together, washed twice with cold PBS, and finally re-suspended in a binding buffer at a final density of 10^6 cells/ml. Annexin V- FITC(5 µl) and PI (5 µl) were added to 100 µL of the cell suspension that contains 10^5 cells. This cell suspension was mixed by gentle vortexing and then 15 min of incubation at 37 C^0 in a dark area. Consequently, 400 µl of binding buffer was added and cells were immediately measured by flow cytometry utilizing a FACS Calibur type (BD Bioscience, Heidelberg, Germany). Afterward, it was analyzed by the BD CellQuest Pro Software, version 5.1 (BD Biosciences, San Jose, CA, USA).

Statistical Analysis

The obtained outcomes were analyzed by means of IBM Statistical Package for Social Sciences (SPSS), version 20 (SPSS Inc., Chicago, IL, USA). For pairwise group comparisons, the post-hoc Tukey test was employed. The P-values that were significant at $P \leq 0.05$.

RESULTS

Viability Assay

For 48 hours, SCC-4 cells were subjected to different concentrations of both ethanolic extract of CS leaves and Cisplatin. Their cellular viability was evaluated through an MTT assay. The IC_{50} value for ethanolic extract of CS leaves and Cisplatin were respectively: 176.3µg/ml and 12.04µg/ml (Figure 1 a, b). The ethanolic extract inhibitory effect on cellular proliferation reeled a decrease in the cellular viability percentage in a dose-dependent pattern.

The examination of phase contrast inverted microscope as well as the MTT findings have demonstrated that SCC-4 cells in the control group with malignant properties, almost cells with prickle and spindle profiles with enhanced ratio of nuclear cytoplasm and prominent daughter cells (Figure 2a). On the other hand, the positive control of SCC-4 cells (Cisplatin-treated) displayed significant morphological alterations in comparison to the untreated SCC-4 cells. The cells shrank in size and were rounded out, lacking their capability to adhere to each other (Figure 2b). However, for 48 hours, CS leaves-treated cells also exhibited modifications such as cell detachment and nuclear size decline, the cells became more rounded. Moreover, a decreased number of cells were noticed (Figure 2c).

Flow Cytometry Proliferation Assay

Cells were treated with Alexa Fluor 488-conjugated anti-Ki67 antibody in order to track the proliferation prompted by both CS leaves extract and Cisplatin by flow cytometry for 48 hours (Figure 3 a, b, c). The cellular proliferation percentage was expressively alleviated in the group treated with CS leaves (50.13 ± 0.82%) compared to the untreated one (97.34 ± 0.43%) and the Cisplatin group (78.62 ± 1.16%) (Table 1).

Flow Cytometry Apoptosis Assay

Annexin V-FITC / PI assay was performed to assess the percentage of both apoptosis and necrosis. For 48 hours, cell lines were treated with 176.3 µg/ml of ethanolic CS leaves extract and with Cisplatin concentration of 12.04µg/mL (Figure 4 a, b, c).

In the studied groups, the OSCC-4 cells treated with the ethanolic extract of CS leaves group showed a significant elevation in the total apoptosis percentages (26.04 ±1.17%) and necrosis percentages (19.59 ±0.68%) in comparison with the Cisplatin group; total apoptosis (17.06 ±o.45%) and necrosis (10.58 ± 0.14%) and untreated total apoptosis (2.08 ±0.63%) and necrosis (0.87 ±0.14%) (Table 2).

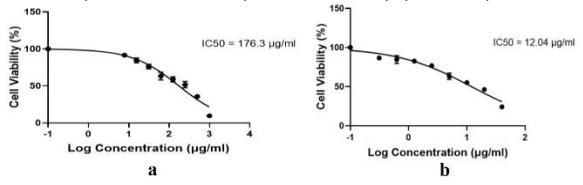

Figure 1: Line graph showing the viable cell percentage of ethanolic CS leave extract (a) and Cisplatin (b) with different doses. The IC_{50} values (representing the lowest toxic doses) detected at 48 were 176.3µg/ml and 12.04µg/ml respectively.

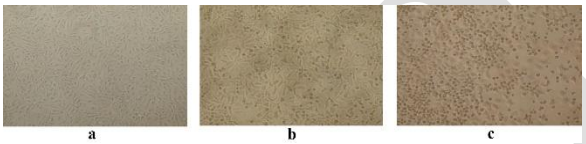

Figure 2: Inverted light microscope photomicrographs showing the morphology of the cells of the untreated group (a), Cisplatin-treated group (b) extract treated group(c).

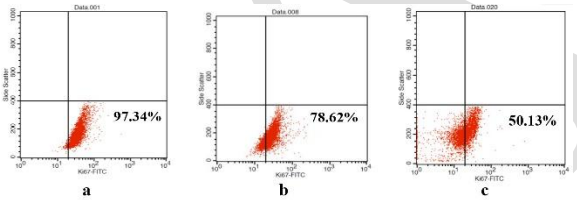

Figure 3: Flow cytometry scatter for Alexa Fluor 488-conjugated anti-Ki67 antibody staining to evaluate the proliferative status of SCC-4 cells in the different studied groups. a) untreated cells b) cells treated with Cisplatin 12.04µg/ml. c) cells treated with ethanolic CS leaves 176.3 µg/ml for 48 hours, the lower right quadrant in each profile represents the cells positive for Ki-67 (the proliferating cells).

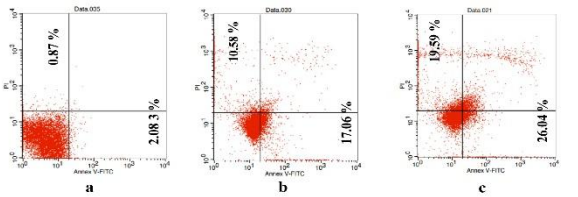

Figure 4: Flow cytometry scatter plots for Annexin V-FITC / PI staining to evaluate apoptosis in SCC-4 cells in the different groups. a) untreated cells. b) cells treated with Cisplatin 12.04µg/ml. c) cells treated with176.3 µg/ml ethanolic CS leaves extract.

Table (1): Alexa Fluor 488-conjugated anti-Ki67 antibody Assay Results Showing the Percentages of Proliferation% (Mean ± SD)

	Untreated cell	Cisplatin	Ethanolic extract
	Mean ± SD	Mean ± SD	Mean ± SD
Proliferation %	97.34 ± 0.43	78.62 ± 1.16	50.13 ± 0.82
p-value	*P_1 < 0.001 ,****P_2 < 0.001,***P_3 < 0.001		

SD: Standard Deviation
The Post Hoc Test was used to compare each of the two groups pairwise (Tukey)
P_1: p-value for the variation between the Cisplatin group and the untreated group
P_2: p-value for the variation between untreated and ethanolic extract group
P_3: p-value for the variation between Cisplatin and ethanolic extract group
Statistically significant at $p \leq 0.05$ (*P < 0.05, **P < 0.01, ***P < 0.001, ****p < 0.0001)

Table (2): Annexin/PI Assay Results Showing the Percentages of Apoptosis and Necrosis % (Mean ± SD)

	Untreated cell	**Cisplatin**	**Ethanolic extract**
	Mean ± SD (%)	Mean ± SD (%)	Mean ± SD (%)
Necrosis%	0.87 ±0.14	10.58 ±0.14	19.59 ±0.68
Early apoptosis%	1.65 ±0.32	13.62 ±0.24	12.17 ± 0.72
Late apoptosis %	0.43 ±0.31	3.44 ± 0.21	13.87 ±0.44
Total apoptosis%	2.08 ±0.63	17.06 ±0.45	26.04 ±1.17
p-value of the total apoptosis and necrosis.	****P_1 < 0.001 , ****P_2 < 0.001,****P_3 < 0.001		

SD: Standard deviation
Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
P_1: p-value for the variation between untreated and Cisplatin group

P_2: p-value for the variation between untreated and ethanolic extract group
P_3: p-value for the variation between Cisplatin and ethanolic extract group
Statistically significant at $p \leq 0.05$ (*P < 0.05, **P < 0.01, ***P < 0.001, ****p < 0.0001)

DISCUSSION

In many populations, A highly frequent cancer of the head and neck is called OSCC. Despite the noteworthy progression in malignancy treatment, the mortality rates in such individuals are still great (35). As a chemical agent, Cisplatin is one of many choices for cancer management including OSCC (16). Taking in account that these chemical agents have adverse effects, Herbal medication has been recommended as a probable alternative due to its exceptional characteristics, for instance availability, safety and low cost. Recently, several reports have verified various pharmaceutic properties of herbal medicines which are vital aspects in the palliation of cancer therapies (36-38). Therefore, the present research is regarded as a pioneer in investigating the anti-carcinogenic potential of CS leaves extract on the SCC-4 cell line.

Previously, various phytochemical agents have been isolated from the whole CS plant and they have been reported to be anti-carcinogenic agents (30). Based on these researches, this study was conducted to explore the anti-carcinogenic properties of CS leaves extract against the viability of the SCC-4 cell line by means of MTT assay as well as cellular proliferation and apoptosis by means of flow cytometry.

In the present research, the findings revealed that the viability of SCC-4 was pronounced in a dose-dependent pattern in response to the influence of ethanolic extract of CS leaves in the following doses (1000, 500, 250, 125, 62.50, 31.25, 15.625, 7.813 µg/ml) at a period of 48 hours. In other words, the higher the dose the greater the efficacy of the extract against the SCC-4 cell line. Also, the IC_{50} value of the CS extract was found to be 176.3µg/ml. In line with the work of Sheikh et al. where they used the ethanolic extract of all parts of the plant on HepG2 cell line. Their findings confirmed the significant inhibition effect of CS in a dose-dependent manner (from 310 to 5000 µg/ml) in 48 hours duration (29).

Also, Mansour et al. has explored the inhibition effect of hydro-ethanolic extract of CS (the plant aerial parts) on the HeLa. For 72 hours, they treated cells with numerous extract concentrations varied from (0-to-3.75 mg/mL). Their findings indicated that the CS extract has displayed inhibitory actions on the development of human cells in a dose-dependent pattern (31). This study's results are similar to the previous report of Moghadamnia et al. where their obtained findings showed that the hydro-alcoholic extract of the above-ground organs of CS had a toxic action on the three cell lines; Hela, Saos and MCF7 and the highly toxic dose was at 1000, 500 and 250 µg/ml respectively (30).

Another research was previously conducted by Al-Janabi et al. on cancer cells obtained from fibroblast cell-2 (cell murine fibroblast). They employed an alcoholic extract from CS leaves to assess the anti-tumor efficacy at 250, 500 and 1000 mg L^{-1} concentrations. In May, the alcoholic extract sample from the gathered complex displayed the greatest anti-tumor activities at 500 mg L^{-1} in compare to samples collected at 250 mg L^{-1} concentration in September (28).

Apoptosis is regarded as the process in which cells undergo death in order to manage the occurring cellular proliferation or in response to DNA injury (39). In the current work, it has been shown that the ethanolic CS leaves extract may lead to the anti-proliferative effect and may facilitate apoptosis. These results indicated the enhanced therapeutic efficacy of CS extract in human OSCC-4 cells. The proliferation percentage of Alexa Fluor 488-conjugated anti-Ki67 antibody and the apoptotic cells percentage with Annexin V-FITC / PI staining in ethanolic CS leaves extract after 48 hours was (50.13 ± 0.82 %) and (26.04 ±1.17 %) respectively as well as being significantly greater than the cells of the extract treated group with Cisplatin and the untreated group.

This work's results are in accordance with *Ji and Yu* study that they have conducted their research which showed that the n-butanol extract from CS on SGC-7901 decreased cellular growth and promoted apoptosis by activating the mitochondrial apoptosis mechanism through the activity of caspase-9/caspase-3 over the course of 48 hours in a dose-depending manner (32). Also, a previous report conducted by Sheikh et al. has indicated the inhibition of HepG2 proliferation and apoptosis via the up-regulation of caspase-9/caspase-8 for 48 hours and the down-regulation of bcl-2 with gradual elevation of concentrations (29).

Additionally, Mohammed et al. findings have revealed that the treatment with CS leaves, rutin, and hesperidin-induced apoptosis of Ehrlich ascites carcinoma in adult female Swiss albino mice led to Caspase-3 activity increase along with bcl-2 level decrease compared with the positive control (40).
In agreement with the current obtained results, Kulisic-Bilusic et al. revealed the inhibitory effect of the essential oil extract of caper flower buds and leaves (diluted with ethanol 0.4%) on HT-29 cell proliferation effect from within 15 -72 hours. On the other hand, their results didn't display any action on HT-29 cell apoptosis (33). Moreover, a study done by Karamallah et al. has estimated the apoptotic outcome of the

ethanolic extract of all the fragments of CS plant on the MCF-7. The rate of expression of both the mRNA of the Bax gene and apoptotic protein were significantly elevated besides their expression rate wasn't changed (34).

It is worth mentioning that the present results were conducted on a specific cell line SCC-4 and the Cisplatin adjuvant therapy as an anti-tumor therapeutic agent wasn't employed. Further techniques such as western blot analysis and gene expression analysis were also required for detecting the molecular mechanism behind the cellular proliferation and apoptosis. Also, further *in vivo* research would be recommended in order to support the present research results. Considering the limitations of this work, the null hypothesis was rejected.

CONCLUSION

The present research demonstrated the potential inhibitory effect of CS leaves extract on the SCC-4 cell line in a dose-dependent pattern in addition to its efficacy which was more than the Cisplatin drug efficacy. This research shows that CS leaves extract could be a promising anti-cancer agent which may be employed to decrease the adverse effects of the chemotherapeutic drugs.

CONFLICT OF INTEREST

Conflicts of interest have not been disclosed by the authors.

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REFERENCES

1. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol Lett. 2014;8:7-11.
2. Tampa M, Mitran MI, Mitran CI, Sarbu MI, Matei C, Nicolae I, et al. Mediators of inflammation - A potential source of biomarkers in oral squamous cell carcinoma. J Immunol Res. 2018;2018:1061780.
3. Aghiorghiesei O, Zanoaga O, Nutu A, Braicu C, Campian RS, Lucaciu O, et al. The World of oral cancer and its risk factors viewed from the aspect of microrna expression patterns. Genes (Basel). 2022;13:594.
4. Rodríguez-Molinero J, Migueláñez-Medrán BDC, Puente-Gutiérrez C, Delgado-Somolinos E, Martín Carreras-Presas C, Fernández-Farhall J, et al. Association between oral cancer and diet: An update. Nutrients. 2021;13:1299.
5. Listl S, Jansen L, Stenzinger A, Freier K, Emrich K, Holleczek B, et al. Survival of patients with oral cavity cancer in Germany. PLoS One. 2013;8:e53415.
6. Rusthoven K, Ballonoff A, Raben D, Chen C. Poor prognosis in patients with stage I and II oral tongue squamous cell carcinoma. Cancer. 2008;112:345-51.
7. Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, et al. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021;9:20503121211034366.
8. Safarzadeh E, Sandoghchian Shotorbani S, Baradaran B. Herbal medicine as inducers of apoptosis in cancer treatment. Adv Pharm Bull 2014;4:421-7.
9. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35:495-516.
10. Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY). 2016;8:603-19.
11. Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Front Oncol. 2022;12:985363.
12. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov. 2005;4:307-20.
13. Ueta E, Kamatani T, Yamamoto T, Osaki T. Tyrosine-nitration of caspase 3 and cytochrome c does not suppress apoptosis induction in squamous cell carcinoma cells. Int J Cancer. 2003;103:717-22.
14. Michaelis M, Bliss J, Arnold SC, Hinsch N, Rothweiler F, Deubzer HE, et al. Cisplatin-resistant neuroblastoma cells express enhanced levels of epidermal growth factor receptor (EGFR) and are sensitive to treatment with EGFR-specific toxins. Clin Cancer Res. 2008;14:6531-7.
15. Bourhis J. New approaches to enhance chemotherapy in SCCHN. Ann Oncol. 2005;16 Suppl 6:vi20-4.
16. Cheng Y, Li S, Gao L, Zhi K, Ren W. The molecular basis and therapeutic aspects of cisplatin resistance in oral squamous cell carcinoma. Front Oncol. 2021;11:761379.
17. Sancho-Martínez SM, Prieto-García L, Prieto M, López-Novoa JM, López-Hernández FJ. Subcellular targets of cisplatin cytotoxicity: an integrated view. Pharmacol Ther. 2012;136:35-55.
18. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008;8:726-36.
19. Astolfi L, Ghiselli S, Guaran V, Chicca M, Simoni E, Olivetto E, et al. Correlation of adverse effects of cisplatin administration in patients affected by solid tumours:

A retrospective evaluation. Oncol Rep. 2013;29:1285-92.
20. Dasari S, Njiki S, Mbemi A, Yedjou CG, Tchounwou PB. Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. Int J Mol Sci. 2022;23:1532.
21. Yu L, Yang J, Wang X, Jiang B, Sun Y, Ji Y. Antioxidant and antitumor activities of *Capparis spinosa* L. and the related mechanisms. Oncol Rep. 2017;37:357-67.
22. Fici S. Intraspecific variation and evolutionary trends in *Capparis spinosa* L.(Capparaceae). Plant Syst Evol. 2001;228:123-41.
23. Zarei M, Seyedi N, Maghsoudi S, Nejad MS, Sheibani H. Green synthesis of Ag nanoparticles on the modified graphene oxide using *Capparis spinosa* fruit extract for catalytic reduction of organic dyes. Inorg Chem Commun. 2021;123:108327.
24. Inocencio C, Rivera D, Obón MC, Alcaraz F, Barreña JA. A systematic revision of capparis section Capparis (Capparaceae) 1, 2. Ann Mo Bot Gard. 2006;93:122-49.
25. Tlili N, Feriani A, Saadoui E, Nasri N, Khaldi A. *Capparis spinosa* leaves extract: Source of bioantioxidants with nephroprotective and hepatoprotective effects. Biomed Pharmacother. 2017;87:171-9.
26. Vahid H, Rakhshandeh H, Ghorbani A. Antidiabetic properties of *Capparis spinosa* L. and its components. Biomed Pharmacother. 2017;92:293-302.
27. Zhao CY, Liu X, Shen C, Wang Y, Qiao L. Treatment of hepatocellular carcinoma with traditional chinese medicine: possible molecular mechanisms. In: Molecular Aspects of Hepatocellular Carcinoma. Sharjah, U.A.E: Bentham Science Publishers; 2012. 167-73.
28. Al-Janabi NM, Al-Halbosiy MM, Al-Badri SR. The inhibitory action of *capparis spinosa* leaves against the tumor and leishmania. Al-Nahrain J Sci. 2019;10:53-8.
29. Sheikh M, Eshraghi HR, Khoshnia M, Mazandarani M, Moradi A. Cytotoxicity effect of *Capparis spinosa* L. on the HepG2 human hepatocellular carcinoma cell line. Indian J Tradit Knowl. 2017;16:73-7.
30. Moghadamnia Y, Mousavi Kani SN, Ghasemi-Kasman M, Kazemi Kani MT, Kazemi S. The anti-cancer effects of *Capparis spinosa* hydroalcoholic extract. Avicenna J Med Biotechnol. 2019;11:43-7.
31. Mansour RB, Jilani IBH, Bouaziz M, Gargouri B, Elloumi N, Attia H, et al. Phenolic contents and antioxidant activity of ethanolic extract of *Capparis spinosa*. Cytotechnology. 2016;68:135-42.
32. Ji YB, Yu L. N-butanol extract of *Capparis spinosa* L. induces apoptosis primarily through a mitochondrial pathway involving mPTP open, cytochrome C release and caspase activation. Asian Pac J Cancer Prev. 2014;15:9153-7.
33. Kulisic-Bilusic T, Schmöller I, Schnäbele K, Siracusa L, Ruberto G. The anticarcinogenic potential of essential oil and aqueous infusion from caper (*Capparis spinosa* L.). Food Chem. 2012;132:261-7.
34. Karamallah MH, Kalantar M, Babaei J, Goudarzi M, Kalirad A, Sabaghan M, et al. Apoptotic effect of *Capparis spinosa* extract on breast cancer cell line (MCF-7). Der Pharma Chemica. 2016;8:328-33.
35. Badwelan M, Muaddi H, Ahmed A, Lee KT, Tran SD. Oral squamous cell carcinoma and concomitant primary tumors, what do we know? A review of the literature. Curr Oncol. 2023;30:3721-34.
36. Safarzadeh S, Shirban F, Bagherniya M, Sathyapalan T, Sahebkar A. The effects of herbal medicines on cancer therapy-induced oral mucositis: A literature review. Phytother Res. 2022;36:243-65.
37. Eubank PLC, Abreu LG, Violante IP, Volpato LER. Medicinal plants used for the treatment of mucositis induced by oncotherapy: a systematic review. Support Care Cancer. 2021;29:6981-93.
38. Regassa H, Sourirajan A, Kumar V, Pandey S, Kumar D, Dev K. A review of medicinal plants of the himalayas with anti-proliferative activity for the treatment of various cancers. Cancers (Basel). 2022;14:3898.
39. Neophytou CM, Trougakos IP, Erin N, Papageorgis P. Apoptosis Deregulation and the Development of Cancer Multi-Drug Resistance. Cancers (Basel). 2021;13:4363.
40. Z Mohammed F, A Gurigis A, Gamal A. Assessment of antiproliferative activity of *Capparis spinosa* L extract against Ehrlich ascites carcinoma in Swiss albino mice. Biochemistry Letters. 2018;14:54-78.