



# Investigation Of Anti-Tumour Activity Of *Orthosiphon Stamineus* On Human Oral Squamous Cell Carcinoma

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## Abstract

**Introduction:** Oral Cancer contributes to 1.11% of total deaths in Malaysia, with 1,865 cases reported in 2020. In this study, the standardized extract of Nuvastatic<sup>TM</sup> (C5OSEW5050ESA) showed antitumor activity in oral cell carcinoma in vitro and in vivo. The active ingredient of Nuvastatic<sup>TM</sup> is derived from a standardized extract of *Orthosiphon stamineus* based on 6% rosmarinic acid

**Objective:** The present study was conducted to evaluate the anticancer potential of C5OSEW5050ESA OS against human oral squamous cell carcinoma (CAL27) ectopically implanted into Nu/Nu nude mice.

**Methods:** The cytotoxicity of C5OSEW5050ESA OS against human oral squamous cell carcinoma CAL27) was evaluated using the MTT assay. The antitumor activity of C5OSEW5050ESA OS was performed by ectopically implanting CAL27 cells into athymic NCR Nu/Nu nude mice. The diseased animals were treated with 200 and 400mg/kg C5OSEW5050ESA OS daily for 3 weeks.

**Result:** The study showed that C5OSEW5050ESA was weakly cytotoxic against CAL27 with an IC<sub>50</sub> of 899.2 µg/ml. At a dose of 200 and 400 mg/kg, compound

C5OSEW5050ESA showed significant anti-tumor effect in xenograft cancer model. C5OSEW5050ESA showed a dose-dependent suppression of oral cancer growth with 74.1.1±1.1 and 81.7±2.1% compared to the negative control group at 200 mg/kg and 400 mg/kg, respectively. In addition, the cancer growth of the positive control (imatinib) treated animals showed that the size of cancer growth reduced significantly with 52.4±2 compared to the negative control (untreated) group.

**Conclusion:** The result of this study highlights the potential of Nuvastatic<sup>TM</sup> in the treatment of oral squamous cell carcinoma.

**Keywords:** Oral squamous cell carcinoma (OSCC), C5OSEW5050ESA, *Orthosiphon Stamineus*, xenograft tumor model, botanical drug

## 1. Introduction

The most typical form of oral Cancer is called oral squamous cell carcinoma (OSCC). It accounts for over 94 percent of all oral malignancies and is the sixth and fifteenth most prevalent malignancy in men and women, respectively (Zhong and Bowen 2006). OSCC can quickly penetrate the nerves and bone. The prognosis for this disease's metastatic spread is appallingly poor (Choi et al.,2008). Within the first two years of a patient's life, almost half of those with head and neck squamous cell carcinoma

**Significance** | The potential of Nuvastatic in the treatment of oral squamous cell carcinoma.

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have recurrence and metastasis (Parkin et al., 2005; Johnson et al., 2020). The cervical lymph nodes on the same side of the face typically experience this metastatic dissemination via lymphatics (Xia et al., 2022). Additionally, extracapsular expansion spread in some OSCC patients results in a poor prognosis, including a higher chance of distant metastasis and regional recurrence as well as a worse survival rate (Gutschner and Diederichs 2012). Chemoradiotherapy, surgery, EGFR inhibition and COX-2 inhibitors, photodynamic therapy, and other current treatment options for OSCC are still insufficiently successful. Therefore, a more potent medication is required to treat this illness Bugshan, A., & Farooq, I. (2020). The use of this herbal extracts is complementary to modern medicine and is slowly gaining popularity. As the knowledge of the benefits of *Orthosiphon stamineus* (OS) is mainly by personal communication, therefore, OS Botanical Herb remains unknown to the general population. There is a need to do more Scientific studies on the effects of this herbal extracts on cancer cells; to promote the use and application of herbs for anticancer agent. To the knowledge of the author, there are no experiments to evaluate the effects of OS on human Oral Squamous Cell Carcinoma (OSCC). Angiogenesis is development of new budding blood vessel and it plays a critical part in oral Cancer leading to the onset of the disease and its metastasis (Dhanuthai et al., 2018). Inflammation can activate angiogenesis and this can be contributed by free radicals (Al-Salahi, et al., 2013; Alshehade, et al., 2023) Nuvastatic™ is a botanical drug derived from *Orthosiphon stamineus*, a well-known herb is Southeast Asia. Nuvastatic™ is potentially antiangiogenic and this is mainly due to its active ingredient rosmarinic acid which has anti-oxidant and antiinflammatory activity (Al-Suede et al., 2014b). The drug has successfully completed a phase 2/3 clinical studies on advanced cancer patients for the treatment of cancer fatigue and currently it is undergoing phase 3 clinical studies for metastatic breast and colon (Ng et al., 2023).

## Materials and Methods

### Plant materials

Nuvastatic™ (C5OSEW5050ESA OS) (Catalogue No. 931886-P) was obtained from NatureCeuticals Sdn. Bhd, Kedah, Malaysia. The extract was prepared according to NatureCeuticals proprietary in house method.

Chemicals, reagents and cell line

Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Matrigel growth factor was obtained from BD Bioscience USA. Heat inactivated foetal bovine serum (HIFBS), trypsin, penicillin/streptomycin (PS), phosphate buffered saline (PBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent was purchased from

Sigma, Germany. 96-well flat bottom tissue culture plates, 10 mL serological pipettes, vacuum filtration system with PES membrane, syringe filters (0, 22 µm), cryo tubes and tissue culture flasks (25 cm<sup>2</sup> and 75 cm<sup>2</sup>) with 0.2 µm polysulphone filter cap were purchased from Techno Plastic Products (Trasadingen, Switzerland). 15 and 50 mL plastic centrifuge tubes were obtained from Corning Life Sciences (Lowell, Massachusetts, USA). AMG EVOS fl inverted microscope was purchased from Electron Microscopy Sciences (Hatfield, PA, US). Micro plate reader Infinite pro200 was obtained from Tecan (Männedorf, Switzerland). CO<sub>2</sub> incubator was obtained from Binder, USA. Oral squamous carcinoma CAL-27 cells were purchased from ATCC, USA. DMEM medium was obtained from (Invitrogen, Gibco, Waltham, MA, USA). The CAL27 cell line was maintained in DMEM medium with 10% heat-inactivated fetal bovine serum in 5% CO<sub>2</sub> at 37°C. Cell culture was done in sterile environment in ESCO class II biosafety cabinet and was maintained in an incubator at 37 °C, 95% relative humidity and 5 % CO<sub>2</sub>.

### In vitro study

#### Preparation of complete medium

DMEM supplemented with 10 % heat-inactivated fetal bovine serum (FBS), and 1 % penicillin/streptomycin. Cell culture was carried out in sterile environment in ESCO class II biosafety cabinet and was maintained in an incubator at 37 °C, 95% relative humidity and 5 % CO<sub>2</sub>. When the cells were 70% confluent, they were trypsinized and harvested

#### Cell preparation

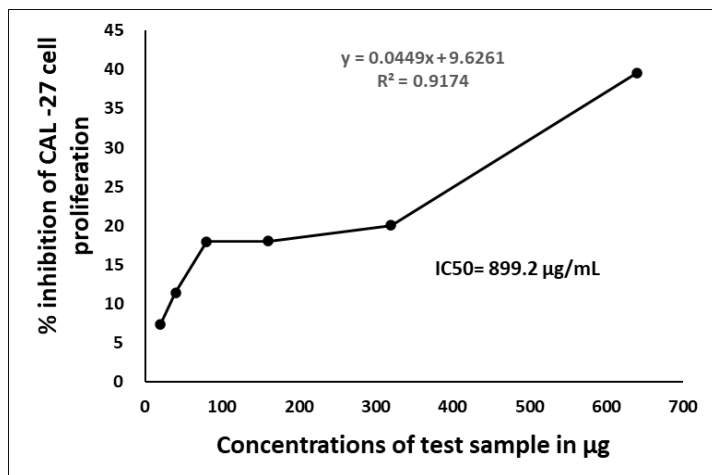
The cell was harvested from 70-80% confluent flask by 1x trypsin and resuspended in 3mL fresh media. The cells were counted and the cell count diluted to get final concentration 1-2 X 10<sup>5</sup>/mL. About 1-2 x 10<sup>4</sup> cells/well in 100 µL fresh media were inoculated into 96 wells plate. The cells were incubated at 37°C incubator under 5% CO<sub>2</sub> for 24 h in order to allow the cells for attachment.

#### Treatment with different doses of test sample

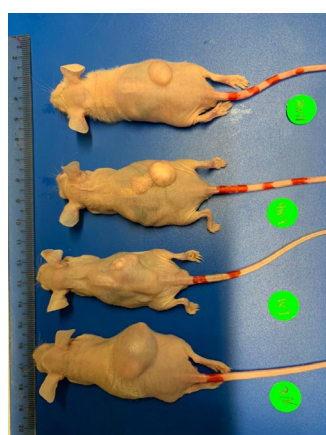
The stock solution of the test sample was prepared at 10 mg/mL in distilled water (DW). 100 µL of stock was added to each well that contain cells in 100 µL medium at different concentrations after diluted in cell culture medium. The total volume was 200 µL in each well. Medium was used as negative control and free culture media was used as a blank. A test sample was performed in six serial concentrations as follows; 20, 40, 80, 160, 320 and 640µg/mL. Each treatment concentration was done in triplicate. The plates was incubated at 37 °C incubator with an internal atmosphere of 5% CO<sub>2</sub> and 95% O<sub>2</sub> for 48 h.

#### MTT assay for assessment of cell viability

Cytotoxicity of test sample was evaluated against human oral squamous carcinoma (CAL 27) cell lines. CAL 27 cell line was used to assess the anti-proliferation property of OS using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

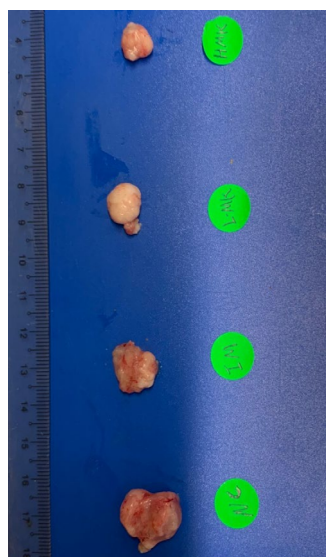


**Figure 1:** Dose-dependent inhibitory of C5OSEW5050ESA OS on CAL-27 cells  
Values are expressed as mean ± SD, n = 3



**Figure 2:** Anti-tumor CAL 27 effect of C5OSEW5050ESA OS after three weeks post-inoculation day.

- NC) Untreated Animals bearing the oral tumour showing enlarged tumor in the mice.
- PC) Animals treated with positive drug (20mg/kg Imatinib)
- LD) Animals treated with 200 mg/kg C5OSEW5050ESA OS, bearing the tumor in the mice. Treatment has shown reduction of tumor size than compared to that of negative control group.
- HD) Animals treated with 400 mg/kg C5OSEW5050ESA OS demonstrated remarkable anti-tumor effect against the human oral cancer. Treatment has caused a drastic suppression of tumor growth. The tumor size was significantly reduced than compared to that of negative control group.



**Figure 3:** Anti-tumor CAL 27 effect of C5OSEW5050ESA OS after three weeks post-inoculation day.

- A. Tumors harvested from the animals of negative control group. Anatomical studies revealed significantly enlarged tumors with enormous vasculature.
- B. Tumor harvested from the animals treated with positive control. Anatomical analysis of the tumors showed that the size of the tumors reduced significantly when compared to the negative control group.
- C. Tumor harvested from the animals treated with 200 mg/kg C5OSEW5050ESA OS. Anatomical analysis of the tumors showed that the size of the tumors reduced significantly when compared to the negative control group.
- D. Tumor harvested from the animals treated with 400 mg/kg C5OSEW5050ESA OS. Anatomical analysis of the tumors showed that the size of the tumors reduced significantly when compared to the negative control group.

assay as described earlier (Mosmann, 1983, Al-Suede et al., 2014a). The assay based on the ability of the live cells to convert the yellow tetrazolium salt to water insoluble formazan salt with violet color by cellular mitochondrial dehydrogenases. The stock solution of MTT reagent was prepared at 5mg/mL in sterile PBS. The reagent was diluted by the culture media (1:10). Following discarding the old culture media from plate, 200 µL of medium was mixed with 20 µL of MTT reagent and added into each treated well. The 96well plate was incubated at 37 °C, 5% CO<sub>2</sub> in a humid atmosphere for 2-3 h. After the incubation period, the supernatant was removed carefully and the water insoluble formazan salt was solubilized in 200 µL DMSO per well and further incubation for 15 min. Absorbance was read at wavelength of 570 nm as measurement and wavelength of 620 nm as reference. Untreated cells concern as negative control. The experiment was repeated three times with four replicates for each concentration. The cell viability was calculated using the following formula:

$$\text{Percentage cell viability} = \frac{[(\text{Abs Sample} - \text{Abs Blank}) / (\text{Abs Control} - \text{Abs Blank})] \times 100}{\dots}$$

Where, As: Abs Sample is the MTT absorption reading for cells which was treated with samples at a specific concentration.

Abs Blank is the blank absorption reading only media without cells

Abs Control is the absorption of untreated cell.

**In vivo antitumor study**

**Animals**

Twenty four athymic NCR nu/nu nude mice aged 5-6 weeks with a weight of 23 ± 2 g were obtained from EMAN Biodiscoveries Sdn Bhd. Specific pathogen free cages provided with high efficiency particulate air filter in animal transport unit (Allentown, USA) was used to keep the mice. Sterile food, water and bedding were provided and mice were housed under a standardized 12/12 h light/dark cycle at room temperature of 24 ± 2°C and a humidity of 60%. Sterile bedding was changed twice a week. All *in-vivo* studies were conducted according to ethical guidelines and were approved by the Eman research ethical committee.

**Preparation of Human oral squamous carcinoma CAL-27**

Human oral squamous carcinoma CAL -27 cells were selected as a model for oral cancer. Cells were cultured in DMEM medium containing 10% FBS and 1% PS solution. 90% confluent human oral squamous carcinoma cells cultures in T75 flasks were trypsinized and resuspended in 10 ml of fresh medium, the cells were collected by centrifugation at 1500 rpm for 5 minutes and washed with sterile PBS to remove the trypsin. The cell pellet was resuspended in 200 µL complete DMEM culture medium and

stored on ice. Nude mice aged 5-6 weeks were ectopically implanted with 6 × 10<sup>6</sup> cells in 200 µL culture medium into the right flank using a 1ml insulin syringe (25G needle). The injection site was inspected with a sterile cotton swab for 30 seconds to prevent cell leakage before the animals were returned to the cages.

**Treatment and tumor size measurement**

Two weeks after tumor implantation, animals were randomly divided into four groups of 6 animals each. Group 1 received 0.2 ml vehicle as a negative control, group 2 received 20 mg/kg imatinib as a positive control, and groups 3 and 4 received oral treatment with 200 and 400 mg/kg body weight C5OSEW5050ESA OS, respectively. Tumor size and body weight were recorded before the start of treatment and every three days. Animals were treated orally by gavage (wt/wt) daily for a period of 3 weeks. Tumor size was measured with a digital caliper at three angles: Length, width, and depth (Sayles et al., 2018). Tumor size was calculated using the following formula: Tumor volume (mm<sup>3</sup>) = (L x W x D) / 2, where L is length, W is width, and D is depth. At the end of the experiment, animals were euthanized by CO<sub>2</sub> followed by neck dislocation. The tumors were harvested and cut into two parts. One half was preserved in 4% paraformaldehyde for histopathological studies and examined for vascularization, tissue morphology, and necrosis/apoptosis. The other half was immediately stored at -80°C until use.

**Statistical methods**

Excel software (Microsoft, USA) were used for statistical analysis. Data were expressed as mean ± S.D for three data sets. The data analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey’s honest significant difference (HSD) post hoc test to assess the significant differences among all groups. P < 0.05 was considered

**Result**

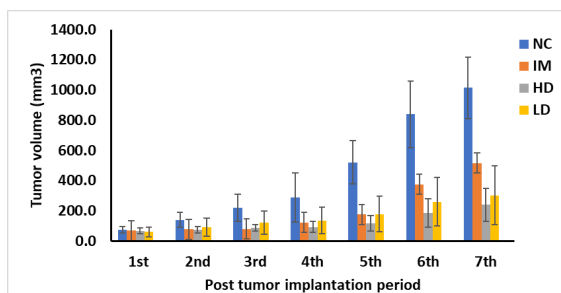
**Antiproliferative activity of C5OSEW5050ESA OS**

The antiproliferative assay is used to evaluate the cytotoxic effect of the test sample on cancer cells. Hence, CAL 27 cell lines were used to test the antiproliferative effect of C5OSEW5050ESA OS. Imatinib was used as a positive control and the medium was used as a negative control. The IC<sub>50</sub> of C5OSEW5050ESA OS was compared with the reference drug (imatinib). The result demonstrated that the C5OSEW5050ESA OS exhibited poor cytotoxicity with IC<sub>50</sub> 899.2 µg/mL (Figure 1).

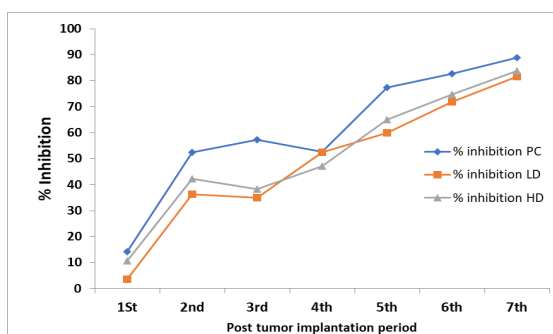
**In vitro study**

**Antitumor activity of C5OSEW5050ESA OS**

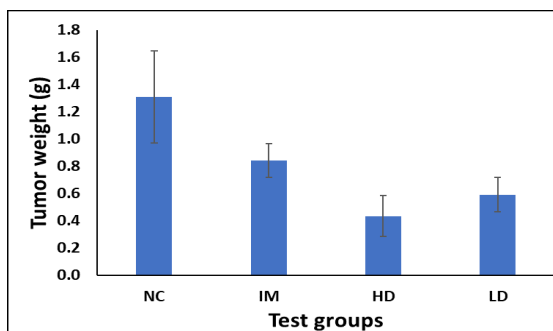
Tumor-bearing Nu/Nu nude mice were orally administered C5OSEW5050ESA OS once daily for 3 weeks (Figure 2). The results showed that mice treated with C5OSEW5050ESA OS exhibited a dose-dependent decrease in cancer size (Figure 3). Figure 4 shows the cancer development in each group studied.



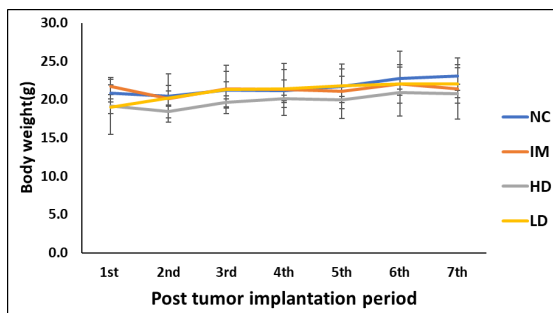
**Figure 4:** Graphical representation of the tumor profile in different groups of animals (n = 6). The values are presented as mean tumor size (mm<sup>3</sup>) ± SD.



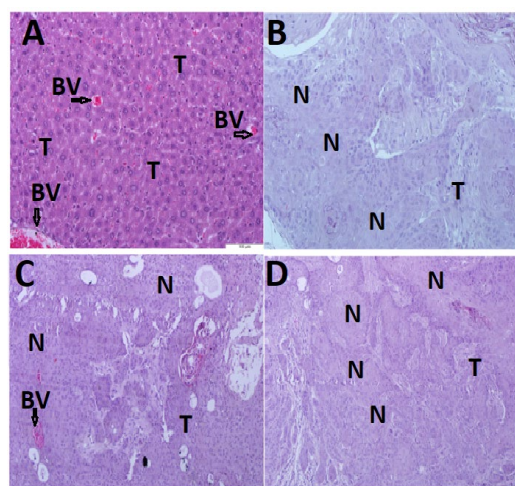
**Figure 5:** Graphical representation of the % inhibition in different groups of animals (n = 6). The values are presented as mean tumor inhibition ± SD.



**Figure 6:** Comparative analysis of tumor weight in different groups of animals (n = 6). The values are presented as mean tumor size (mm<sup>3</sup>) ± SD.



**Figure 7:** Graphical representation of the body weights in different groups of animals (n = 6). The values are presented as mean Body weight ± SD.



**Figure 8:** Histological Analysis: Tumor sections from the representative groups analysed for the antitumor effect.

A) Tumor section from negative control group showing compact arrangement of live tumor cells (T) with large number of blood vessels (BV).  
 B) Tumor section from animals treated with positive drug (Imatinib) exhibits strong antitumor efficacy. The histology sections revealed a large necrotic section (N) with prominent islands of live tumor cells (T) without blood vessels.  
 C) Tumor section from animals treated with 200 mg/kg C5OSEW5050ESA OS, bearing the tumor in the animals, demonstrating a large necrotic section (N) with islands of live tumor cells (T). The tumor section shows significantly few numbers of blood vessels (v) surrounded by few tumor cells that form a shape of islands of tumor cells without blood vessels.  
 D) Tumor section from animals treated with 400 mg/kg C5OSEW5050ESA OS exhibits strong antitumor efficacy. The histology sections revealed a large necrotic section (N) with prominent islands of live tumor cells (T) without blood vessels.

Compared with the negative control group, C5OSEW5050ESA OS at 200 and 400 mg/kg showed a dose-dependent inhibition of oral cancer growth of 74.1 and 81.72%, respectively (Figure 5). Tumor harvested from the animals treated with positive control at 20 mg/kg showed that the size of the tumors reduced significantly when compared to the negative control group with  $52.4 \pm 2\%$ . Figure 6 shows the comparative evaluation of tumor weight in different groups of animals. The current study provides evidence that C5OSEW5050ESA OS has potent anti-cancer activity against oral cancer. Figure 7 shows the graphical representation of body weights in different animal groups. Figure 8 shows the histological Analysis of cancer growth sections from the representative groups analyses for the antitumor effect. The cancer growth section from the negative control group displays a dense cluster of living tumour cells and a significant number of blood vessels (Figure 8 A). Cancer growth sections from animals treated with the positive drug (imatinib) showed potent anticancer activity. Histological sections showed a significant necrotic area with distinct islands of live cancer cells that lacked blood vessels (Figure 8 B). A tissue sample from mice administered 200 mg/kg C5OSEW5050ESA OS shows an extensive necrotic area with islands of viable cancer cells. The tumour section has strikingly few blood vessels surrounded by few cancer cells and takes the form of islands of cancer cells without blood vessels (Figure 8C). A strong anticancer effect can be seen in the cancer growth sections of mice treated with 400 mg/kg C5OSEW5050ESA OS. The histological sections showed a significant necrotic area with distinct islands of live cancer cells that lacked blood vessels (Figure 8D).

#### Discussion

Multiple dysfunctional molecular signaling pathways are involved in complex cancer. Activation of oncogenes and/or deactivation of tumor suppressor genes are features of disease development (Zhong et al., 2006). This can be caused by viruses or by chemical or physical agents that lead to mutations in DNA. The usual approach is to remove the tumor if it has not spread and is in an operable region. This is combined with the use of radiation therapy and cytotoxic chemotherapeutic agents (Ahmad et al., 2021). Even though much work has been done in cancer research, the prognosis of survival has only improved marginally. The development of drug resistance is a common phenomenon that often leads to treatment failure. Even more often, patients tend to succumb to drug induced toxicity. Recently, a new class of agents has been introduced using monoclonal antibodies that target key receptors specific to cancer cells. These include Avastin, a monoclonal antibody against VEGF, and sunitinib, which targets the EGFR receptor (Babiker et al., 2020). Although these drugs show promise, they are too expensive, making them accessible only to a privileged few. However promising they may be, the

impact of drug resistance still cannot be avoided. Avastin, for example, is generally ineffective beyond a 6-month period. Use of Avastin can extend life expectancy by only 6 months. Other agents designed to treat key receptors such as VEGFR and EGFR are selective for only a small group of patients. Common names for *Orthosiphon stamineus* include "cat's whiskers" and "kumis kucing". It is mainly cultivated in the tropics, Europe and Southeast Asia. The leaves of this plant are used to prepare herbal tea and to treat a variety of human diseases and conditions, including cystitis, diuretic, rheumatism, abdominal disorders, some kidney diseases, oedema, gout, and some kidney diseases (Tabana et al., 2016). *Orthosiphon stamineus* possesses several properties such as gastroprotective, hypoglycemic, antihypertensive, anti-obesity, hepatoprotective, antimicrobial, antioxidant, anti-inflammatory and analgesic, cytotoxic, antiangiogenic and antiproliferative activities (Alshawsh et al., 2011; Al-Suede et al., 2021; Nazari et al., 2022). The antioxidant ability of phenolic compounds in C5OSEW5050ESA is essential for the destruction of free radicals present in the human body. *Orthosiphon stamineus* was reported to exhibit free radical scavenging activity, which is probably due to the higher concentration of caffeic acid derivatives, especially rosmarinic acid.

Therefore, in this study, an oral tumour xenograft model was performed in humans to examine the anti-tumour efficacy of the herbal drug Nuvastatic™ (C5OSEW5050ESA), which is currently in clinical trial, against human colon and breast cancer (Ng et al., 2023). Oral squamous cell carcinoma (CAL27) cells were ectopically implanted into athymic NCR Nu/Nu nude mice. The aim of the study is to evaluate the anticancer activity of this herbal drug both in vitro and in vivo. According to the cytotoxicity tests on the CAL27 cancer cell line, Nuvastatic™ showed little anti-cancer efficacy. The IC<sub>50</sub> result was higher than 899.2 mg/ml, indicating that the cytotoxic potential of the drug is limited. However, the anti-tumor effect in vivo was remarkable, as the tumor volume decreased compared to the control. Histological examination of tumor tissue treated with the extract and positive control showed significant necrosis. The data showed no significant differences between the treatments with the high dose (400 mg/kg) and the low dose (200 mg/kg). In addition, the result showed no significant differences have been found between low and a high dose of C5OSEW5050ESA in the inhibition of tumor growth and weight. Data from other trials with the Nuvastatic™ tumor model, including pancreatic, breast and colon cancer, are consistent with this data (Al-Suede et al. 2021). In our previous study, Nuvastatic™ showed poor cytotoxic activity against many of the cancer cell lines tested (Al-Suede et al. 2021; Yehya et al. 2022). Nuvastatic™ tumoricidal activity is largely due to its antiangiogenic activity. The cancer cells present in the tumor are

deprived of nourishment due to the absence of new blood vessels supplying the tumor. This leads to the starvation of the cancer cells and eventually to their death. This may be due to the high concentration of phenolic compounds in C5OSEW5050ESA, which induce senescence of oral cancer cells. These effects of C5OSEW5050ESA may be attributed to its antioxidant property and the presence of phytonutrients such as polymethoxylated flavonoids, polyphenolic constituents, caffeic acid derivatives, and terpenes, particularly eupatorin, sinensetin, rosmarinic acid, 3'-hydroxy-5,6, 7,4'-tetramethoxyflavone, and betulinic acid (Tabana et al 2016). These series of compounds possess their own unique anti-angiogenic properties, which may afford a powerful combination in preventing or treating tumor development. The result of this study is consistent with the findings of a previous study, which reported that the antiangiogenic and antitumor efficacy of the 50% ethanol extract of OS may be mediated via inhibition of the VEGF pathway (Ahamed et al., 2012). In addition, C5OSEW5050ESA exerted antiangiogenic activity by inhibiting vascular endothelial Growth factor (VEGF), epidermal growth factor (EGF), Fibroblast growth factor (FGF), interleukin 2 (IL -2) & Interleukin 7 (IL -7), nerve growth factor  $\beta$  (NGF- $\beta$ ), Transforming growth factor- $\alpha$  (TGF- $\alpha$ ) and tumor necrosis Factor-  $\beta$  (TNF- $\beta$ ) (Al-Suede et al., 2021). C5OSEW5050ESA has also been shown to cause significant upregulation of interferon  $\alpha$  (IFN- $\alpha$ ), interferon  $\beta$  (IFN- $\beta$ ) and granulocyte macrophage colony-stimulating factor (GM-CSF). The OS extract was found to have a dual effect: It inhibits the production of essential proangiogenic molecules in cancer cells and suppresses the phosphorylation of VEGFR-2 in HUVECs (Ahamed et al., 2012; Al-Suede et al., 2014a). Polyphenolic compounds such as caffeic acid are one of the active compounds in C5OSEW5050ESA extract. They have been shown to suppress the growth and colony formation of human lung, skin, bladder, breast, and prostate cancer cells by promoting cellular senescence. In patients with oral cavity cancer, most patients underwent surgical treatment and irradiation with therapeutic neck dissection to remove the tumor (Kramer, Simon et al. 1987). At the site of the main origin, the cancer may occasionally return due to remnants of the microscopic daughter cells (Arrangoiz, Rodrigo et al. 2018). This drug can significantly slow the spread of cancer by preventing cancer cells from reappearing by targeting the angiogenesis mechanism (Al-Salahi, et al., 2013). Under these circumstances, this drug may also help slow the rate of cancer cell progression and counteract the progression of metastasis Bugshan, A., & Farooq, I. (2020). This may help to improve the survival of cancer patients and prolong their lifespan. The use of natural products to treat cancer has been known since ancient times. Natural products usually contain a large number of active compounds that enable them to target the multiple carcinogenic pathways (Almoustafa et

al., 2023). Therefore, numerous claims have been made about the efficacy of natural products in the treatment of cancer (Demain, A.L. and Vaishnav, P., 2011). The efficacy of these natural products has also been demonstrated by several in vitro and in vivo studies. Unfortunately, the number of clinical studies conducted with natural products is very limited. An important factor that is often cited is the inadequate intellectual property protection of natural products, which prevents private investors from funding the expensive clinical trials. The first herbal drug based on a natural product was filed in 2000, namely saw palmetto for its use in prostate enlargement. The number of patents filed for natural product-based medicines is still limited but steadily increasing (Suzuki et al., 2009). However, there is still a misconception that natural products are not patentable. In addition, natural products fall under the category of "food" in certain countries such as the United States. As a result, they cannot be classified as registered drugs, leaving patients without funds to cover their expenses for herbal medicines. For this reason, many researchers have resorted to isolating the active ingredient from natural products, and many have synthesized improved analogs that have better activity (Yehya et al., 2019). Through this approach, the products have better i.p. protection and fall well within the FDA classification as a "drug." However, the results of the clinical trials have not been a great success, with a large number of patients responding poorly to the compounds tested. Therefore, to improve the response rate, a new approach is being introduced using patients with a specific genotypic background instead due to the limited activity of the drug. While this approach improves therapeutic success, long-term efficacy and/or a complete cure is still not achieved.

The most common malignant tumour of the oral cavity is oral squamous cell carcinoma (OSCC) (Choi et al., 2008). In general, OSCC is considered as an adult disease, and ulcerated lesions with malignant nuclei are more commonly found in older patients (Close et al., 1989; EG al., 2001). Smoking, alcohol consumption, UV radiation associated with lip cancer, human papillomavirus (HPV), Candida infection, betel nut, and poor diet are risk factors for OSCC (Diendéré, Jeoffray et al. 2020). In addition, the lower lip and floor of the mouth account for 38% and 40% of oral squamous cell carcinoma cases, respectively. Currently, surgical intervention, radiation, and chemotherapy are commonly used as treatment options for oral cavity cancer (Guha et al., 2007). Although advanced therapeutic strategies are used in the treatment of oral cavity cancer, patients who have metastases often have poorer outcomes and lower survival rates. Therefore, it is necessary to find innovative treatments for oral cavity cancer.

Conclusion

The present study was conducted with the intention of testing the anticancer potential of C5OSEW5050ESA OS against human oral carcinoma using CAL27 cells. The results of the study showed that C5OSEW5050ESA OS has a potent property to inhibit the cancer and further suppress its spread in the body. Therefore, the compound C5OSEW5050ESA OS at a dose of 200 and 400 mg/kg showed significant tumor suppressive effect in animals. Thus, C5OSEW5050ESA OS can be considered as a potential source for further development of an effective chemotherapeutic agent against metastatic oral cancer.

Author Contributions

SKBJD, FSRA, FA, JTCK and ASAM have designed the experiments. FSRA and SKBJD, have conducted the experiments. FSRA, SKBJD, and AMSA,FA have drafted the manuscript. All the authors read and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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