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Thuja Occidentalis as a Promising Natural Therapy in Oral Squamous Cell Carcinoma: A Literature Review

Saranya Ramsridhar[1], Chandini Rajkumar[2], Vishnu Priya Veeraraghavan[3*], Arul Prakash Francis[3], Murali Balasubramaniam[2], Vidya Kaliyan[4]

[1]*Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 600 077, India*

[2]*Department of Oral Pathology and Microbiology, Sathyabama Dental College and Hospital, Chennai, India*

[3]*Centre of Molecular Medicine and Diagnostics (COMManD), Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 600 077, India*

[4]*Department of Prosthodontics, Sathyabama Dental College and Hospital, Sathyabama Institute of Science and Technology, Chennai, 600119, Tamil Nadu, India*

Abstract

Oral cancer continues to be one of the leading causes of death globally, being more prevalent in developing countries. Radiation, chemotherapy, targeted therapy, immunotherapy, hormone-based therapies, and surgery are common treatment plans for oral cancer that lead to a range of short- and long-term side effects resulting in an urgent need to develop treatment options with minimal or no adverse effects. Recently, numerous bioactive compounds derived from various plants have garnered attention as potential therapeutic options for cancer treatment.Thuja occidentalis, also known as Eastern White Cedar, has been traditionally used for its medicinal properties. The components of this plant like thujone, flavonoids, and polysaccharides, have demonstrated significant pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and anti-cancer effects. These compounds work through various mechanisms, such as promoting apoptosis, reducing oxidative stress, and enhancing the immune response. There is no literature available assessing the role of T.occidentalis in Oral Squamous Cell Carcinoma (OSCC) and the current article will be a novel overview discussing its pharmacological properties and its potential role in the treatment of OSCC. However, further research is needed to understand the precise molecular mechanisms, optimal dosage, and evaluate the synergistic effects with conventional cancer therapies.

Keywords: *Anti-cancer Properties, Immunomodulation, Oral Squamous Cell Carcinoma, Thuja occidentalis, Thujone.*

Introduction

Cancer, often referred to as the "king of all maladies," is swiftly emerging as the most prevalent non-communicable disease globally and is the leading causes of mortality and morbidity in both developed and developing countries [1,2]. Despite considerable advancements in oncology research, cancer remains a formidable challenge and is often viewed as a 'hopeless case.' Over the past decades, extensive studies have explored diverse approaches to cancer treatment. However, the search for a definitive cure continues to be elusive, highlighting the

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****Corresponding Author: vishnupriya@saveetha.com***

complexity and heterogeneity of this disease [3]. Oral squamous cell carcinoma (OSCC) is the most prevalent type of oral cancer, comprising over 90% of cases. It exhibits varying degrees of histological differentiation and is characterized by a high potential for invasion and metastasis [4]. It arises in the mucosal epithelium of the oral cavity and leads to functional impairments, which in turn affect the physical appearance, speech, ability to swallow and sense of taste, all of which impacting the quality of life. OSCC is marked by its aggressive behaviour, frequent recurrence, and tendency to spread to nearby and distant areas (metastasize), often resulting in late diagnosis and a bleak outlook. It poses a significant public health challenge due to its high mortality rate and poor prognosis, along with the ineffectiveness of standard chemotherapy treatments administered to the patients [5].

According to data from the Global Cancer Observatory, there were 3,77,713 cases of OSCC reported globally in 2020, with a significant diseased population reported in Asia. OSCC predominantly affects middle-aged to older adults, with males being more susceptible than females. The incidence of OSCC is expected to rise by approximately 40% by 2040, accompanied by an increase in mortality rates [6]. Clinically, OSCC manifests as distinctive lesions with a combination of red and white patches featuring uneven surfaces and irregular borders. In the early stages, these lesions are often asymptomatic, but they can progress to exhibit symptoms such as ulceration, nodule formation, and tissue attachment in the later stages of the disease [7].

Despite all the advancements in research and high-end technologies, the 5-year survival rate is low, with a mortality rate of around 40%, majorly due to tumour metastasis and subsequent recurrence, and the treatment of OSCC remains a significant challenge. Developing effective therapies is difficult, as current treatments have limited efficacy and substantial side effects [8]. The lack of reliable biomarkers and the dire need for early prognosis also makes it challenging to identify patients for targeted interventions for personalized medicine [9]. The high incidence and mortality of OSCC underscore the pressing need for novel, more effective therapeutic strategies to combat this devastating disease.

The use of complementary therapies is gaining attraction as an increasingly common approach to treatment. Among these, the utilization of plants for the management of various medical conditions, including cancer, has emerged as a significant modality. Folk medicine, relying on the application of plants (flora) and their extracts, has been widely practised all over the world for ages despite not being officially recognized in many countries. Over 80% of the population in Africa and Asia rely on plants and plant-derived products as their primary means of treating a wide range of diseases [10]. One such plant is *Thuja occidentalis*, also known as 'Eastern White Cedar'. It is a coniferous tree native to Eastern North America. Thuja genus under the family of Cupressaceae belongs to the order Pineales that are 3 to 60 m tall and includes five species: *Thujakoraiensis*, *T. occidentalis*, *Thujaplicata*, *Thujastandishii*, and *Thujasutchuenensis*. Fig. 1 shows *T. occidentalis.*

Figure 1.*T. occidentalis* in USA [pic courtesy: Joshua Mayer from Madison, WI, USA]

All of these species have been known for their well-established medicinal uses. *T. koraiensis* has antimicrobial and antioxidant properties. *T. occidentalis* has a wide range of applications, from antimicrobial, antioxidant, anti-inflammatory, anti-atherosclerotic, anti-diabetic, antipyretic and anti-cancer properties. *T. pilata* is a well-known agent of immunomodulation and tissue remodelling. Similarly, *T. standishii* and *T. sutchuenensis* have also been found to have antimicrobial and anti-tumor properties. *T. occidentalis* has been a blessed herb when it comes to healing a wide range of ailments and its chemical composition has been explored to a great depth. A fresh plant of *T. occidentalis* is composed of 0.6% essential oils that are majorly monoterpenes like Thujone (65%), isothujone (8%), fenchone (8%), sabines (5%), and α-pinene (2%). The essential oil Thujone is composed of α-thujone (85%) and β-thujone (15%). Additionally, around 2.07% reducing sugar, 2.11% minerals, 1.67% free acids, 4% proteins and polysaccharides and around 1.31% tannic agents form the rest of the composition. However, apart from a plethora of essential oils, the dried herbal form yields other bioactive components like a wide range of flavonoids, coumarins, proanthocyanidins, and tannins [11].

T. occidentalis has been used in traditional medicine for centuries, with evidence of its use dating back to the early 17th century. *T. occidentalis* leaves are rich in vitamin C and were utilized by American indigenous people and early European explorers to treat scurvy back in history. The leaves and bark of the tree were used to treat liver diseases, bronchitis, and rheumatism, to name a few.The plant also showed effective results in treating skin ailments such as eczema and psoriasis [11]. Recent research has demonstrated that extracts derived from the *T. occidentalis* plant exhibit a wide range of beneficial properties, including immunostimulatory, anti-cancer, antibacterial, antifungal, and antiviral effects, making it a potentially valuable resource for the development of new medicinal treatments [12].

Additionally, the leaves and the seeds of the plant have been found to possess antimicrobial activity against various bacterial strains, and the ethanolic extract of the leaves has been shown to have anticonvulsant potential, suggesting its possible use in the management of epilepsy. Few studies have collected evidence on the pharmacological effects of *T. occidentalis* on cancer. However, no literature has been documented that evaluates the impact of T. occidentalis on oral cancer [13]. Therefore, the present review will discuss the link of pharmacological properties of*T. Occidentalis* with the pathophysiology of OSCC. Moreover, the review will also shed light on the mechanism of action involved in the modulation of cancer and the counter effects of *T. occidentalis,* along with the preclinical and clinical studies related to *T. occidentalis* and OSCC.

Pathophysiology of OSCC

OSCC is a complex and multifaceted disease that originates from the epithelial cells of the oral cavity. The development and progression of OSCC involve the intricate interplay of various genetic and epigenetic mechanisms. These molecular pathways and their alterations contribute to the malignant transformation and aggressive behavior exhibited by OSCC. The onset and progression of this disease are influenced by a multitude of factors, including genetic mutations, epigenetic modifications, immunological components, and environmental exposures. It commonly originates from prolonged exposure to carcinogenic agents, primarily tobacco and alcohol, which can cause genetic mutations in the oral epithelium. The inherent genomic instability of these mutated cells results in the further accumulation of genetic aberrations, facilitating the stepwise transition from normal healthy epithelium to dysplasia and ultimately to the establishment of carcinoma [14,15].

Genetic mutations, particularly in the H-Ras gene, majorly contribute to the development OSCC. A systematic review of H-Ras expression and mutation in OSCC found that H-Ras mutations are prevalent in OSCC and are associated with poor prognosis. Furthermore, elevated H-Ras expression is often observed in OSCC, which can contribute to the progression of the disease [16]. This increased expression of H-Ras can drive disease progression by promoting uncontrolled cell growth, evading programmed cell death, and activating PI3K/AKT and MAPK signaling pathways, which are crucial for the development and progression of OSCC [17].

Similarly, the presence of the YAP1 gene in the Hippo Signalling pathway has been found to be linked with OSCC differentiation, suggesting its role in tumor progression and development [18]. The Hippo signaling pathway is crucial for regulating cell proliferation and determining cell fate, thereby controlling organ growth and regeneration. Many components of this pathway are found at cell-cell junctions and help regulate Hippo signaling through cell polarity, cell contacts, and the cytoskeleton. When Hippo signaling is downregulated, it can lead to uncontrolled cell proliferation and impaired differentiation, which is linked to cancer [19]. Epigenetic alterations, including aberrant DNA methylation, histone modifications, and micro RNA dysregulation, play a crucial role in the development and progression of OSCC by influencing gene expression and cellular behavior [20,21]. Another important component which contributes to the progression of OSCC is the extracellular matrix (ECM). It not only provides structural support but also regulates various cellular functions via biochemical signalling [22]. Changes in the composition of the ECM can impact the growth and dissemination of tumours. The dynamic interactions between cancer cells and the ECM components enable malignant cells to invade surrounding tissues and metastasize to distant sites, which, in turn, enhance tumour growth [23].

Pharmacological Properties of *T. Occidentalis*

Modern-day research has thoroughly investigated the pharmacological properties of *T. occidentalis,* highlighting its active compounds, including essential oils, flavonoids, tannins, and polysaccharides. These bioactive constituents have shown a variety of significant biological activities such as antimicrobial, anti-inflammatory, antioxidant, and anti-cancer effects. The essential oils, particularly rich in α-thujone and β-thujone like isothujone, fenchone, sabinene and others, exhibit strong antimicrobial properties. Coumarins like p-coumaric acid and umbelliferone, along with flavonoids like quercetin, kaempferol, and myricetin, are potent antioxidants and anti-inflammatory agents. Tannins such as catechins, proanthocyanidins and tannic acid contribute to the antiviral and astringent properties of the plant. At the same time, polysaccharides and proteins are recognized for their immunomodulatory and anti-tumor activities [11,24]. This comprehensive profile emphasizes the medicinal potential of *T. occidentalis* in modern pharmacology.

A study conducted by Kim et al. (2007) showed that polysaccharides derived from *T. occidentalis* can stimulate macrophages and enhance the generation of nitric oxide, an essential molecule in the body's defence against pathogens. Furthermore, these polysaccharides have been observed to demonstrate anti-tumour effects by stimulating the growth of immune cells and triggering programmed cell death in cancer cells [12,24,25].

Following this direction, Saha et al. (2014) found that *T. occidentalis* extract triggered programmed cell death by the activation of the caspase enzymes and inducing the apoptotic pathways in human breast cancer cells. Apart from the pro-apoptotic roles, the antioxidant properties of flavonoids and other plant compounds found in *T. occidentalis* play a significant role in its anti-cancer effects by reducing oxidative stress, which is a known factor in cancer development [26].

Phytodynamic Effects of *T. occidentalis* in OSCC

Several research studies have shown that *T. occidentalis* has potential anti-cancer properties, particularly in its thujone-rich fraction, which has demonstrated enhanced cytotoxic, anti-proliferative, and pro-apoptotic effects compared to the whole plant extract. These effects have been observed in various cancer cell lines, such as melanoma, lung, and prostate cancer. Contemporary research has well established the activation of the Gamma delta (γδ) T cells in the success of cancer immuno therapeutics. The significance of the γδ T cells lies in their direct recognition and targeting of the transformed cells without depending upon the HLA-antigen status of the cells [26].

In OSCC, the role of γδ T cells has been already explored in depth previously [27]. In this context, the ability of Thujone to proliferate γδ T cells and subsequently up-regulate the expression of perforin [28] suggests its potential as an adjunctive therapeutic agent in relieving OSCC prognosis. The upregulation of the perforin allows cytolytic T-cells to effectively target and damage cancer cells by perforating their cell membranes, leading either to apoptosis or cell lysis. Therefore, from understanding the importance of the increased proliferation of these γδ T cells, the application of Thujone and its standardization have the potential to be utilized as an immunomodulator that may complement other cancer therapeutic modalities like chemo- or radiotherapy. Thujone might be effectively increasing the population of γδ T cells in the tumour microenvironment [28,29] thereby enabling these cells to recognize and damage tumours even in the metastasis stage. However, given the clinical utility of Thujone in tumour treatment, more research and trials, especially in OSCC settings, are warranted to support its safety, efficacy, mechanism, and therapeutic potential.

Additionally, Thujone, especially α-Thujone, has been reported to increase the expression of cytotoxicity markers CD107a and other cell damage markers p-ERK1/2 and p-Akt in colon cancer cell lines. Meanwhile, α-Thujone was able to achieve this feat even without damaging the normal proliferation of the cell lines HCT116 and SW620 cells under study [29,30]. Such a mechanical and molecular approach is expected to be involved in the activity of α-Thujone in OSCC as well. The effects of *T. Occidentalis* in the pathophysiological mechanism of OSCC have been extracted from some of the major studies (Table 1) [26-30].

Table 1. Findings of the Major Studies

PathophysiologicalMechanism in OSCC	**Effect of *T.Occidentalis***
Genetic Mutations	Thuja components, like thujone, reduce genetic mutations bypromoting apoptosis and inhibiting cell proliferation.
Epigenetic Alterations	Compounds in Thuja can modulate epigenetic alterations,influencing gene expression favorably.
TumorMicroenvironment	Thuja enhances immune cell activity, improving the tumor microenvironment.
Inflammation	Anti-inflammatory properties of Thuja reduce inflammation, slowing tumor progression.
Oxidative Stress	Antioxidant compounds in Thuja reduce oxidative stress, which is a factor in cancer development.
Immune Response	Thuja stimulates the immune system, enhancing the activity of macrophages, T-cells, and NK cells.
Apoptosis	Thuja induces programmed cell death (apoptosis) through the activation of caspase enzymes.

There have been numerous studies that have explored the molecular-level anti-tumour changes that are caused by the administration of the bioactive components derived from *T. occidentalis*. For instance, a study done to evaluate the anti-cancer properties of the polysaccharides derived from *T. occidentalis* was found to improve the cell-mediated immune response in metastatic tumour-bearing animals. The polysaccharide compounds were reported to cause an increase in the proliferation of T cells, NK cells, and especially γδ T cells, thereby enhancing the production of cytokines like IFN-γ, TNF-α, and IL-2 [30]. In another study conducted on the A375 melanoma cells, it was found that the thujone-rich fraction of *T. occidentalis* exhibited significant anti-proliferative, pro-apoptotic, and anti-metastatic effects. The bioactive component, Thujone, was able to perform these functions by targeting the apoptotic pathway through the activation of caspase-3 and PARP cleavage, meanwhile inhibiting the migration and invasion of A375 cells [31].

Another mechanism of action was identified in context to the anti-tumor functioning of *T.occidentalis*is autophagy. Such a mechanism was deciphered in a study done on cervical cancer cells using the mother tincture of *T.occidentalis.* The researchers reported that in addition to the induction of apoptosis via activation of caspases, thujone tincture was able to induce autophagy through a Beclin-1-dependent pathway, leading to the inhibition of cervical cancer cell growth [31]. However, one of the significant anti-cancer findings of *T.occidentalis* is its ability to increase the levels of p53 and induce apoptosis, as was reported in the case of mammary epithelial carcinoma cells. *T.occidentalis* was able to cause this anti-tumour effect via the activation of a highly specialized ROS-p53 feedback loop [32].

Apart from polysaccharides, the monoterpene α-thujone, the main compound of *T.occidentalis* essential oil, has demonstrated a significant therapeutic potential against various cancer cell lines, including glioblastoma [33]. Similarly, another study explored the ethyl acetate-soluble extract of *T.occidentalis* leaves and twigs that contained (+)-7-oxo-13-epi-pimara-8,15-dien-18-oic acid and was found to exhibit potential anti-tumor-promoting activity [34]. Through these mechanisms, it is believed that *T.occidentalis* and its derivatives have the capabilities to be utilized in the clinical management of OSCC [35].

Challenges and Future Directions

Despite the advantages and applications, there are challenges in using *T.occidentalis* as an anti-cancer treatment, and that majorly includes the unavailability of reports on its efficacy, safety, and proper dosage guidelines as it has been reported that the anti-cancer effects of *T.occidentalis* and its bioactive compounds like Thujone are dependent on the p53 status of the cancer cells. However, the compound had improved efficacy in cells with functional p53 expression compared to p53-deficient cells [32]. This further suggests the need to carefully select cancer types and patient populations that are most likely to respond to Thuja-based treatments. Although Thuja or Thuja-based compounds are natural products, yet ensuring their consistent quality, potency, and bioactive composition of extracts or preparations can be a challenge. Rigorous quality control measures would be required for clinical development.

Additionally, when it comes to the potential synergistic effects of *T.occidentalis* with standard cancer treatments like chemotherapy or radiation therapy we have almost no literature available to understand this significance. Therefore, evaluating such combinatorial approaches would be an important future direction. Another limitation has been the unavailability of details on the absorption, distribution, metabolism, and excretion of the key bioactive compounds from *T.occidentalis*. Understanding the pharmacokinetic profile would be crucial for optimizing dosing and administration routes [36].

Challenges and hurdles like these warrant numerous future directions that should majorly focus on elucidating molecular mechanisms [35,36]. This involves understanding the precise molecular pathways and signalling cascades by which *T.occidentalis* and its bioactive compounds exert their anti-cancer effects, especially in relation to p53 status and other genetic factors. Secondly, the evaluation of the synergistic combinations of *T.occidentalis* with conventional cancer therapies could expand its clinical utility and improve treatment outcomes. Most importantly, conducting well-designed, randomized, and controlled robust clinical trials is necessary to conclusively demonstrate the safety and efficacy of *T.occidentalis* or its extracts as anti-cancer agents in human patients [35].

Studies aimed at improving and developing optimized formulations and delivery systems for Thuja-based anti-cancer agents could enhance their bioavailability, stability, and targeted delivery to tumour sites. As the anti-cancer implication of the Thuja-based treatment modalities has been scarce in numerous types of cancers, expanding the evaluation of the anti-cancer potential of *T.occidentalis* across a broader range of cancer types would be an important future direction [37]. Mineralisation of artificial substitutes likecalcium carbonate, prf, nano hydroxyapatite have shown to have clinical benefits across various fields [38-40]. To summarize, while *T.occidentalis* shows promise as an anti-cancer agent, addressing the challenges related to specificity, standardization, combination therapies, and pharmacokinetics will be crucial for realizing its full clinical potential.

Conclusion

In conclusion, *T. occidentalis* shows promise as an adjunctive therapeutic agent for OSCC. Addressing current research gaps and conducting comprehensive clinical trials are critical steps towards integrating *T. occidentalis* into cancer treatment regimens, potentially enhancing the efficacy of existing therapies and improving patient outcomes.

Conflict of Interest Statement

None.

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None.

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