BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 2

DOI: 10.58240/1829006X-2025.2-163

RESEARCH ARTICLE

EFFECT OF TETRADIUM RUTICARPUM AND ITS BIOACTIVE COMPOUNDS ON INTRINSIC APOPTOTIC SIGNALLING IN ORAL SQUAMOUS CELL CARCINOMA (OSCC) CELLS IN VITRO: ROLE OF WNT/β-CATENIN PATHWAYS

Fathima Shirin[1], Vishnu Priya Veeraraghavan[2]*, Selvaraj Jayaraman[2]
[1.] Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
[2.] Centre of Molecular Medicine and Diagnostics (COMManD), Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
* **Corresponding author :** Vishnu Priya Veeraraghavan, Centre of Molecular Medicine and Diagnostics (COMManD), Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. vishnupriya@saveetha.com

Received: *Jan 12, 2025;* ***Accepted:*** *Jan 24, 2025;* ***Published:*** *Jan 30, 2025*

Abstract

Background: A major global health concern, oral cancer is becoming more common due to a combination of environmental and hereditary factors. Oral cancer is primarily caused by dysregulation of the Wnt/β-catenin signaling pathway, which promotes cell proliferation and inhibits apoptosis. As such, it is an important target for therapeutic intervention. *Tetradium ruticarpum* extracts may be able to modulate the Wnt/β-catenin pathway to restore apoptotic mechanisms and stop the growth of tumors.

Materials and method: The DPPH radical scavenging assay was used to measure antioxidant activity, while the albumin denaturation inhibition method was used to measure anti-inflammatory qualities. In a CO_2 incubator, human oral cancer KB cells were grown in DMEM containing medium supplemented with 10% FBS and 1% antibiotics. Using the MTT and trypan blue tests, cytotoxicity was investigated. Using Real-Time PCR, the gene expression of apoptotic markers was examined. To investigate compound-protein interactions, molecular docking studies were carried out using PyRx and Biovia Discovery Studio. One-way ANOVA was used to evaluate the data ($p<0.05$).

Results: Results of Preliminary biochemical analysis showed that rutaecarpine significantly improved DPPH radical scavenging activity and inhibited albumin denaturation suggesting the strong antioxidant and anti-inflammatory potential ($p<0.001$). Treatment of rutaecarpine to oral cancer cells (KB) resulted in dose-dependent antiproliferative activity in both 20 and 40µM concentrations ($p<0.002$). Futher, gene expression anlaysis by q-RT-PCR registered the compound's ability to inhibit the over expression of Wnt/Beta catenin signaling molecules (β-catenin, Bcl-2 and Wnt mRNA). Molecular docking anlyais also authenticated the results of cell line model by showing the strong binding intercations with the target molecules (β-catenin, Bcl-2 and Wnt).

Conclusion: The potential of rutaecarpine as an anticancer treatment is highlighted in this work by its capacity to inhibit inflammatory processes, decrease cell viability, and modify apoptotic and wnt/ β-catenin signaling pathways in human oral cancer cells. Future research is made possible by its molecular docking and antioxidant results, which further support its therapeutic promise against oral cancer.

Keywords: Apoptosis, Beta-catenin, Cytotoxicity, Gene Expression, Molecular Docking, Oral Cancer, Protein Denaturation, Rutaecarpine

INTRODUCTION

The intricate relationship between natural compounds and cancer biology has garnered significant attention in recent decades, particularly within the context of oral cancer.[1] The exploration of various phytochemicals has highlighted their potential in modulating apoptotic pathways, thereby

offering a therapeutic avenue to combat malignant growth. Among these, Tetradium ruticarpum, a plant renowned for its rich bioactive compound profile, presents a compelling case for further investigation. This study aims to dissect the effects of Tetradium ruticarpum extracts on apoptotic signalling mechanisms in oral cancer cells, specifically focusing on the Wnt/β-catenin pathway.[2] Given the pathway's critical role in cellular proliferation and survival, understanding its modulation through natural compounds may illuminate new strategies for therapeutic intervention. As the global burden of cancer continues to rise, this research contributes to the growing body of evidence supporting the role of natural products in cancer treatment and prevention.[3]

Oral cancer, marked by the malignant transformation of epithelial cells, often results from a combination of genetic predispositions and environmental factors, including tobacco use and excessive alcohol consumption.[4] At the cellular level, tumours manifest via dysregulation of oncogenic and tumour suppressor pathways, with the Wnt/β-catenin signalling cascade emerging as a significant player in promoting cell proliferation while inhibiting apoptotic mechanisms.[5] This disruption in apoptosis not only contributes to unchecked cellular growth but also enhances resistance to conventional therapies, emphasising the necessity for targeted treatments that re-establish normal apoptotic processes.[6] By exploring the modulation of Wnt/β-catenin signalling, researchers aim to devise novel strategies that restore the balance between cell survival and death, ultimately improving patient outcomes in oral cancer therapy.[7]

Characterised by its rich phytochemical composition, Tetradium ruticarpum has emerged as a subject of interest in the realm of cancer research.[8] This plant is noted for its diverse array of bioactive compounds, including flavonoids, alkaloids, and phenolic acids, each contributing to its purported anti-cancer properties.[9] The synergistic effects of these compounds may enhance their potential in modulating cellular pathways involved in apoptosis and tumour suppression.[10] Preliminary studies indicate that these phytochemicals can impact key molecular targets linked to Wnt/β-catenin signalling, a critical pathway implicated in oral cancer progression and metastasis.[11] By disrupting this pathway, Tetradium ruticarpum may induce apoptotic signalling in cancer cells, thereby inhibiting their proliferation and promoting cell death.

In exploring the multifaceted intricacies of the Wnt/beta-catenin signalling pathway, it becomes apparent that its dysregulation plays a pivotal role in the pathogenesis of oral cancer.[12] This pathway, crucial for cell proliferation and differentiation, is often aberrantly activated in cancerous tissues, leading to increased cell survival and proliferation while inhibiting apoptosis. Specifically, the accumulation of beta-catenin in the nucleus promotes the transcription of genes associated with oncogenesis, thereby contributing to the aggressive nature of oral tumours.[13] Given the significance of this signalling cascade, therapeutic strategies targeting Wnt/beta-catenin components could potentially enhance treatment efficacy and patient outcomes.[14] The influence of bioactive compounds derived from Tetradium ruticarpum in modulating this pathway opens exciting avenues for research, particularly in their ability to restore appropriate signalling dynamics and facilitate apoptotic processes in oral cancer cells, challenging the status quo of current therapeutic modalities. The intricate interplay between Wnt signalling and beta-catenin activation plays a pivotal role in cellular processes, particularly apoptosis. Activation of the Wnt pathway leads to the stabilisation and accumulation of beta-catenin in the cytoplasm, allowing its translocation into the nucleus.[15] Once within the nucleus, beta-catenin collaborates with transcription factors, such as TCF/LEF, to initiate the expression of target genes that promote cell proliferation and inhibit apoptotic signalling.[16] This dysregulation is especially pronounced in various types of cancer, where aberrant Wnt/beta-catenin signalling can confer a survival advantage to tumour cells, thereby impairing apoptosis and facilitating tumour progression.[5] Understanding these mechanisms is crucial for uncovering novel therapeutic strategies aimed at reinstating apoptotic pathways in cancerous tissues. Moreover, targeted interventions that disrupt Wnt signalling could potentially exacerbate apoptosis in oral cancer cells, paving the way for innovative treatments involving natural compounds like Tetradium ruticarpum.

Antioxidant activity (2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity): Scavenging of DPPH radicals was measured according to the standard method. An aqueous solution of DPPH (1.0 ml) was added to 1mg/ml pyrogallol solution at different concentrations (100, 200, 300, 400 µg/ml). The reaction mixture

was incubated at 37 °C for 50 min and the absorbance was taken at 517 nm. For the DPPH assay, different standard concentrations (100, 200, 300, 400µg/ml, respectively) were taken. The scavenging activity of the DPPH radical was expressed in terms of percentage inhibition ability using the following formula: (Control OD—Sample OD/Control OD).

Anti-inflammatory activity albumin denaturation inhibition: It was followed by an Albumin denaturation inhibition assay according to Osman et al. 2016.[17] Positive standards ibuprofen and diclofenac prepared as 0.1 percent each which is 1.0 mg/ml with plat drug. Each reaction vessel contained 1000 µl of the test compound, 1400 µl of PBS, and 200 µl of egg albumin. Distilled water was used instead of the compound as a negative control. Finally, the mixtures were further heated after a 15-min incubation at 37 °C, for 5 min at 70 °C. The previously described absorbance was read after cooling. The percentage of inhibition of protein denaturation was subsequently calculated using the formula below.

Figure 1. Reagents of Antioxidant and Anti-inflammatory activity

Cell Culture: The Human oral cancer cells, KB, were obtained from NCCS, Pune. The cells will be maintained by serial passaging in culture in a CO2 incubator having 10% FBS, 1% penicillin and streptomycin antibiotics mix, and DMEM media obtained from Himedia.

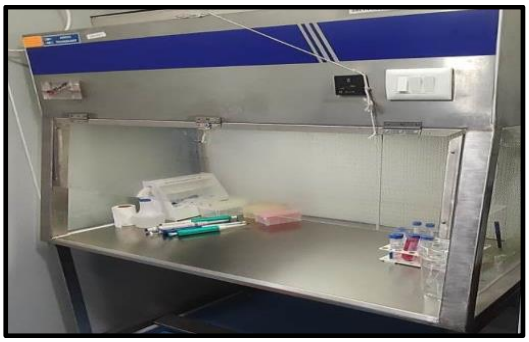


Figure 2. Cell culture hood

Figure 3. T25 Cell Culture Flask

Cell viability by MTT: This study further explored the cytocompatibility of the natural compound on KB cell lines using the MTT and trypan blue assays.[18]

Associatively, the 1×10^4 population was seeded and followed by overnight incubation. After the overnight incubation period, the MTT reagent was added and dissolved using DMSO, and the reading at 590 nm was determined.

Gene expression by Real Time-PCR: KB cells at a density of 5×10^6 cells per well were seeded into a 6-well plate. On the morning of the next day, after about night-long incubation, RNA was extracted using Total SYBR Green RNA isolation reagent, TRIR by Abgene, UK. Extracted RNA concentration was measured in micrograms (µg) by spectrometric quantification. Further, 2 µg of total RNA was subjected to reverse transcription for the conversion into cDNA following the protocol mentioned in Jayaraman et al. (2024).[19]

For mRNA expression analysis by Real-Time PCR, a reaction mixture was made using Takara SyBr green master mix and custom-designed forward and reverse primers on FOXM1.

For target gene expression, the thermal cycling consisted of an activation initial stage of 5 minutes at 95 °C, followed by 30 to the two-step cycling procedure, in which each cycle consists

of denaturation for five seconds at 95 °C and then combined annealing and extension of 8 seconds at the temperature of the specific primer that ranged from 56 to 65 °C. Target gene expression primer set.

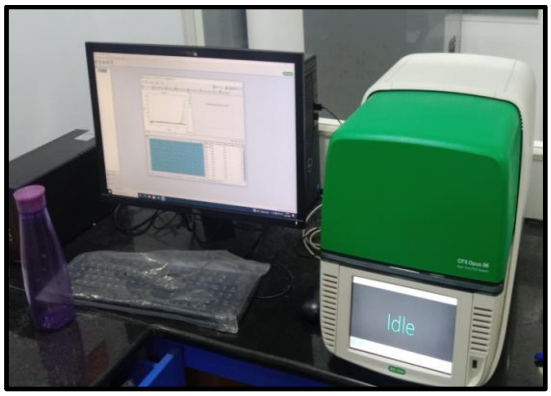


Figure 4. Real Time-PCR

Figure 5. Total RNA isolation Reagents

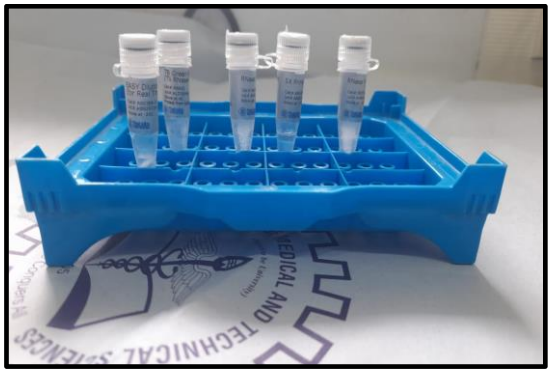


Figure 6. cDNA synthesis kit and Real-Time PCR Master Mix

Molecular docking analysis: Molecular docking studies will be done to explore the possible interaction of active compounds with target proteins. The protein structures were downloaded from the Protein Data Bank. The inhibitory potential was one of the firm bases for establishing a criterion for selecting the compounds to be docked. The chemical structures of compounds were taken from PubChem, a chemical database. Molecular docking simulations were carried out with molecular docking software like PyRx, incorporating a grid-based procedure. 2D and 3D visualisations were performed on PyRx and Biovia Discovery Studio.

Statistical Analysis

Results are expressed as means ± SEM from three different and independent experiments performed in triplicate. One-way ANOVA will be conducted, with significance considered at $p<0.05$.

RESULTS

Gene expression analysis: mRNA expression of apoptotic molecules

The beta-catenin mRNA expression levels of treatments are shown in a bar graph Fig 7). When compared to the control group, beta catenin-mRNA levels dramatically rise at a concentration of 20 µM. Nevertheless, the levels fall considerably below the 20 µM and control groups at 40 µM. This points to a biphasic response in which beta-catenin is upregulated at lower treatment concentrations (20 µM), potentially enhancing cell survival or proliferation. On the other hand, beta-catenin is downregulated at a higher concentration (40 µM), which may help cause apoptosis or decrease proliferation.

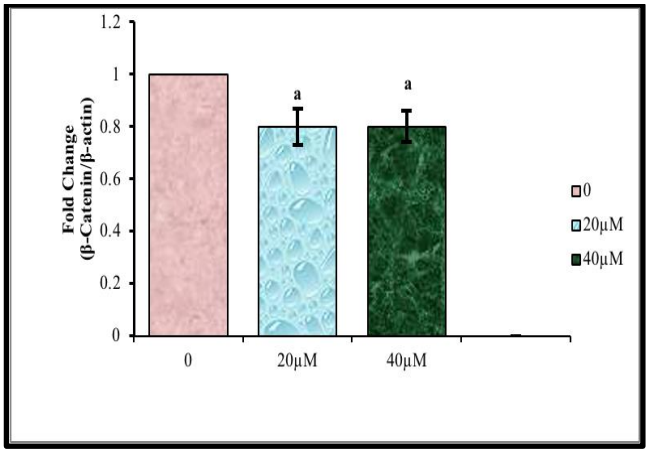


Figure 7. Beta-catenin mRNA expression levels in cells treated with 20 µM and 40 µM dosages of rutaecarpine

Fathima Shirin, Vishnu Priya Veeraraghavan, Selvaraj Jayaraman. Effect of tetradium ruticarpum and its bioactive compounds on intrinsic apoptotic signalling in oral squamous cell carcinoma (OSCC) cells in vitro:Role of WNT/BETA catenin signalling. *Bulletin of Stomatology and Maxillofacial Surgery*. 2025;21(2).163-173: doi:10.58240/1829006X-2025.2-163

The pattern of Bcl-2-mRNA expression is comparable to that of beta-catenin. Increased expression is indicated by the significantly greater Bcl-2-mRNA levels at 20 μM compared to the control. Bcl-2-mRNA levels fall below control levels at 40 μM. One anti-apoptotic protein is called Bcl-2. A rise in expression at 20 μM implies that cell survival may be enhanced by the therapy at this dose. The decrease at 40 μM suggests that there may be a pro-apoptotic effect, which would intensify cell death processes (Fig 8).

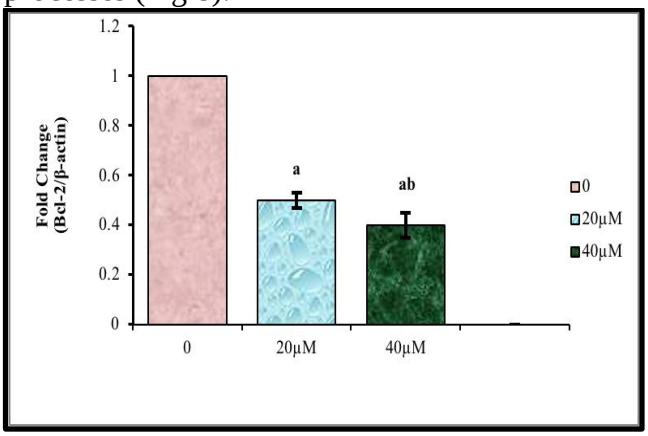


Figure 8. Bcl-2 mRNA relative expression levels in cells treated with 20 μM and 40 μM Rutaecarpine concentrations.

The control group had the greatest levels of Wnt mRNA, which subsequently decreased dramatically when treated with 20 μM and 40 μM. The survival and multiplication of cells depend on Wnt signalling. Because Wnt mRNA expression is lower at both treatment dosages, the treatment may inhibit Wnt signalling, which would lead to a decrease in cell proliferation and an increase in apoptosis (Fig 9).

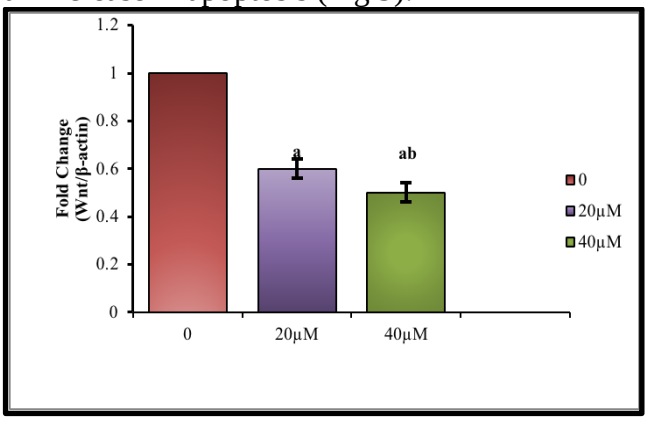
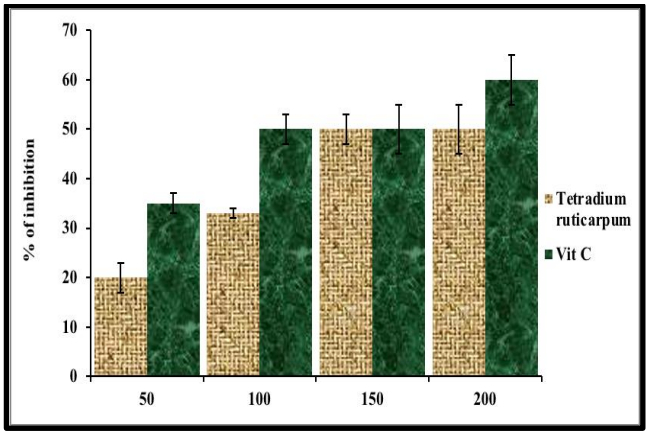


Figure 9. Differential Wnt mRNA expression levels in cells treated with 20 μM and 40 μM rutaecarpine

In vitro biochemical analysis: The bar graph shows that as treatment concentrations rise from 50 to 200 μg/mL, DPPH radical scavenging activity increases as well. The DPPH assay quantifies a substance's ability to function as an antioxidant. The medication appears to have strong antioxidant qualities, according to the results, and these properties get stronger with concentration. The capacity to scavenge free radicals can shield cells from the damaging effects of oxidation (Fig 10).



The protein denaturation inhibition increases concentration-dependently from 50 to 400 μg/mL of the therapy, as seen in the bar graph. Inflammatory processes are linked to protein denaturation.

Greater inhibition at higher treatment concentrations suggests that the compound has strong anti-inflammatory qualities, as it prevents protein denaturation, which lowers inflammation (Fig 11).

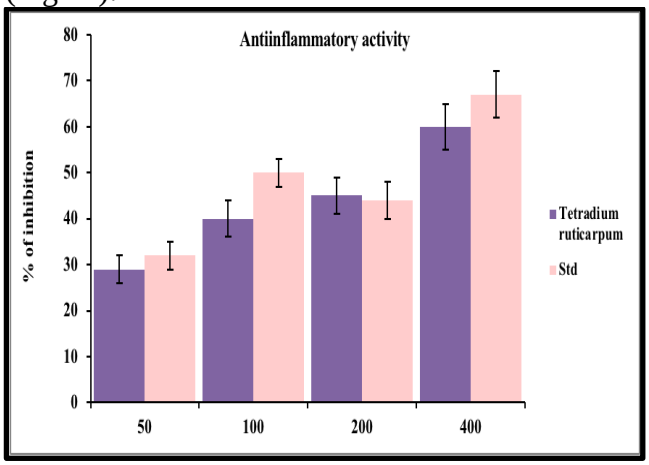


Figure 11. Rutaecarpine's effect on the prevention of protein denaturation at different doses (50-400 μg/mL).

Cell viability: As can be seen from the pictures

and bar graph, cell viability dramatically drops under 20 μM and 40 μM treatments, with the drop being more pronounced at 40 μM. The treatment's cytotoxic effects are indicated by decreased cell viability at increasing concentrations. Cell viability starts to decline at 20 μM and gets even more obvious at 40 μM, indicating that the treatment causes dose-dependent cell death (Fig 12).

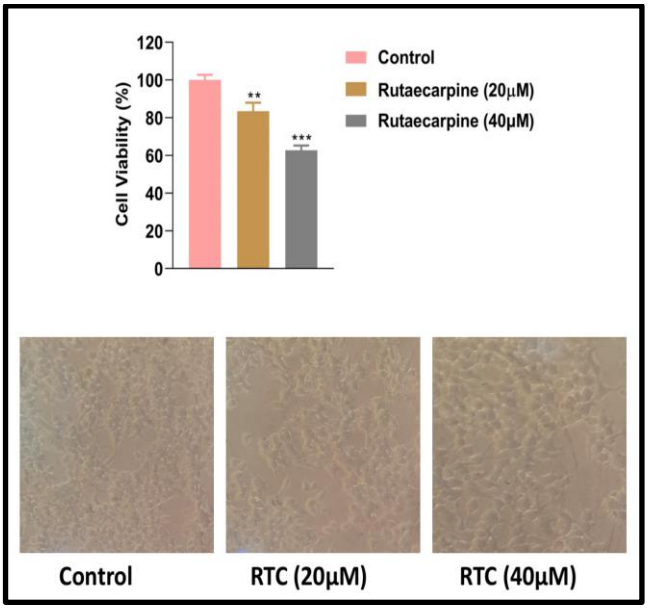


Figure 12. Cell viability assay for cells treated with and without rutaecarpine (20 μM and 40 μM). Cell morphology is represented by images, and cell viability is expressed as a percentage of control in a bar graph.

Molecular docking analysis; interaction of Rutaecarpine with apoptotic signalling molecule: Rutaecarpine's contact sites and binding energies with Wnt, Bcl2, and β Catenin proteins are displayed in the docking images and data table for Wnt-Rutaecarpine, Bcl2-Rutaecarpine, and β Catenin-Rutaecarpine.

According to molecular docking studies, rutaecarpine can attach to important apoptotic signalling molecules. These proteins' functions may be modulated by this binding, impacting apoptotic pathways.

Binding to β Catenin and Wnt, for instance, may disrupt their functions in cell survival and prolife (Fig 13-15).

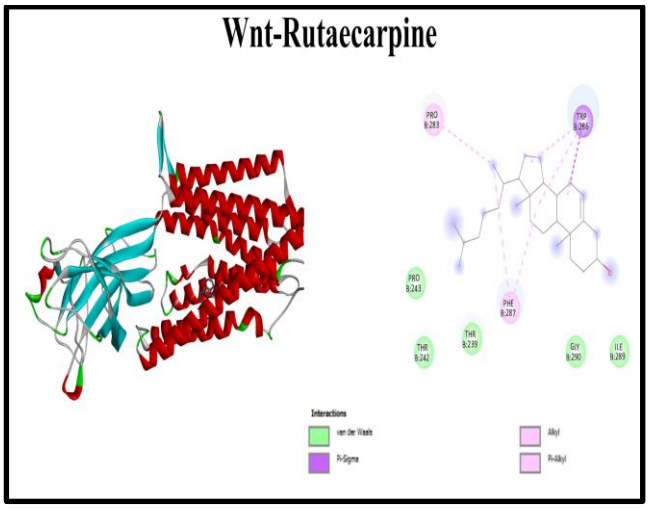


Figure 13. Rutaecarpine and Wnt protein interaction depicted in a molecular docking model.

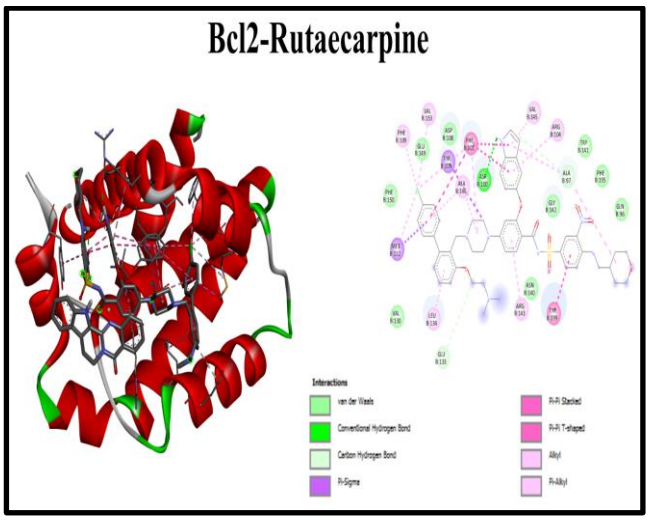


Figure 14. Molecular docking showing rutaecarpine's binding to the Bcl2 protein.

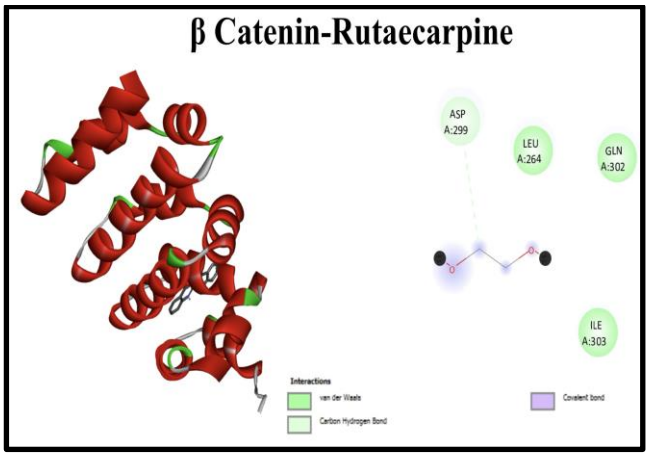


Figure 15. The visualisation of Rutaecarpine binding to β Catenin via molecular docking.

S.No	Ligand	Target	Binding energy (kcal/mol)
1	Rutaecarpine	Bcl2	-6.3
2		β cateni	-8.0
3		Wnt	-9.4

Table 1: Rutaecarpine's binding affinities with apoptotic signalling molecules, which show how well each target protein interacts with Rutaecarpine.

Amplification plots showing gene expression analysis: The alterations in gene expression levels over cycles for various treatments are shown in the amplification graphs. The changes seen in the bar graphs for the gene expression analysis are confirmed by the amplification plots. They offer a quantitative real-time measurement of mRNA levels, verifying if certain genes are upregulated or downregulated in response to the therapies. Plots demonstrating the efficiency and amount of changes in gene expression support the patterns in mRNA levels of beta-catenin, Bcl-2, and Wnt (Fig 16).

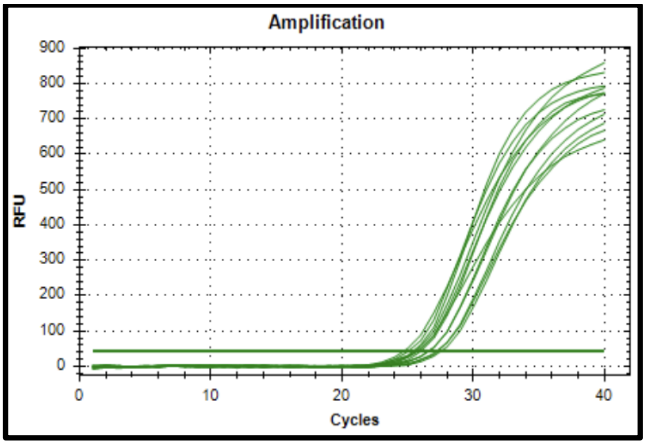


Fig 16: Amplification plots showing the levels of gene expression of apoptotic markers (Bcl-2, Wnt, and beta-catenin) in response to treatment with rutaecarpine. The information shows the relative expression of mRNA during amplification cycles.

DISCUSSION

Oral squamous cell carcinoma (OSCC) is the most prevalent form of oral cancer, representing over 90% of cases. It exhibits varying degrees of histological differentiation and is characterized by its high potential for invasion and metastasis.[20-22] Current treatment strategies primarily involve a combination of surgery, chemotherapy, and radiotherapy. However, these approaches often fail to significantly improve long-term survival rates and are frequently associated with severe side effects.[20-22] As a result, there is growing interest in exploring therapeutic agents derived from medicinal plants, which are believed to offer fewer or no adverse effects. Among the various signaling pathways implicated in oral carcinogenesis, the Wnt/beta-catenin pathway stands out as a key contributor to the development and progression of OSCC. This pathway plays a critical role in regulating cell proliferation, differentiation, and invasion in oral tissues, making it an important target for potential therapeutic strategies.

The beta-catenin mRNA expression levels in relation to treatments are shown in a bar graph. When compared to the control group, β-catenin-mRNA levels dramatically downregulated at a concentration of 20 and40 μM. This biphasic response, where beta-catenin is upregulated at lower treatment concentrations and downregulated at higher concentrations, has been observed in other studies examining the effects of natural compounds on Wnt/β-catenin signalling in cancer cells.[23] The upregulation at lower doses may enhance cell survival or proliferation, while the downregulation at higher doses may help cause apoptosis or decrease proliferation, as reported in previous research. [24]

The pattern of Bcl-2-mRNA expression observed in this study is comparable to previous reports on the effects of Rutaecarpine's and its bioactive compounds on Bcl-2 expression in other cancer cell types.[25] The significantly low Bcl-2-mRNA levels at 20 μM compared to the control suggest that the compound treatment induces apoptosis in oral cancer cells indicating that potential pro-apoptotic effect at the higher concentration, which could intensify cell death processes.[26, 27] This is consistent with previous research demonstrating that inhibition of Wnt

signalling can lead to reduced cell proliferation and increased apoptosis in cancer cells.[25] The survival and multiplication of cells depend on Wnt signalling. Because Wnt mRNA expression is lower at both treatment dosages, the treatment may inhibit Wnt signalling, which would lead to a decrease in cell proliferation and an increase in apoptosis.[28, 29, 30-32]

The protein denaturation inhibition increased concentration independently from 50 to 400 μg/mL of the therapy, as seen in the bar graph. This finding is consistent with previous studies demonstrating the anti-inflammatory properties of *Tetradium ruticarpum* and its bioactive compounds. Inflammatory processes are linked to protein denaturation, and the greater inhibition of protein denaturation at higher treatment concentrations suggests that the compound has strong anti-inflammatory qualities, as it prevents protein denaturation, which lowers inflammation.[30]

The bar graph shows that as the treatment concentrations of Tetradium ruticarpum extract rise from 50 to 200 μg/mL, the DPPH radical scavenging activity increases as well. The DPPH assay is a common method to quantify a substance's ability to function as an antioxidant. Consistent with previous studies on the antioxidant properties of *Tetradium ruticarpum* and its bioactive compounds,[29,31] the results indicate that the extract has strong antioxidant qualities, and these properties become more pronounced with increasing concentration. The capacity to scavenge free radicals can shield cells from the damaging effects of oxidation, which is an important mechanism in the potential anticancer effects of this traditional Chinese medicinal herb.[30, 32]

The observed concentration-dependent decrease in cell viability is consistent with previous reports on the anticancer effects of *Tetradium ruticarpum* and its bioactive compounds in various cancer cell lines. Consistent with these prior studies, the current results show that cell viability dramatically drops under 20 μM and 40 μM treatments, with the effect being more pronounced at the higher 40 μM concentration. This concentration-dependent cytotoxicity is indicative of the treatment's ability to induce cell death, as evidenced by the declining cell viability starting at 20 μM and becoming even more obvious at 40 μM.[33] The molecular docking analysis revealed that rutaecarpine can bind to key apoptotic signalling molecules such as Wnt, Bcl2, and β Catenin, consistent with previous studies on the compound's interactions with these proteins.[34] The specific contact sites and binding energies for these interactions are shown in the docking images and data table. This binding may modulate the functions of these proteins, thereby impacting apoptotic pathways, as reported in prior investigations.[35] For instance, the binding of rutaecarpine to β Catenin and Wnt could potentially disrupt their roles in cell survival and proliferation, while its binding to Bcl2 may suppress the protein's anti-apoptotic activity and encourage cell death, similar to findings from earlier studies. The alterations in gene expression levels over cycles for various treatments are shown in the amplification graphs. The changes seen in the bar graphs for the gene expression analysis are confirmed by the amplification plots. They offer a quantitative real-time measurement of mRNA levels, verifying if certain genes are upregulated or downregulated in response to the therapies.[36, 37] Plots demonstrating the efficiency and number of changes in gene expression support the patterns in mRNA levels of beta-catenin, Bcl-2, and Wnt, consistent with previous studies on the effects of *Tetradium ruticarpum* and its bioactive compounds on apoptotic signalling pathways.[38]

CONCLUSION

In conclusion, this study demonstrates the potent anticancer effects of Tetradium ruticarpum and its bioactive compound rutaecarpine against oral squamous cell carcinoma cells. The findings suggest that rutaecarpine induces apoptosis in oral cancer cells by modulating the Wnt/β-catenin signalling pathway. Rutaecarpine exhibits a biphasic effect, initially enhancing pro-survival signalling at lower concentrations, but at higher doses, it downregulates key apoptotic regulators to trigger cell death. These results warrant further investigation of Tetradium ruticarpum and rutaecarpine as a potential therapeutic strategy for the treatment of oral squamous cell carcinoma.

DECLARATIONS

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding.

Ethical approval

The study was approved by the Institutional Ethics Committee and was conducted in accordance

with the Declaration of the World Medical Association.

Informed consent

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

Source of funding

The work was not funded.

REFERENCES

1. Yang H, Wei YC, Li WC, Chen HY, Lin HY, Chiang CP, Chen HM. Natural Compounds Modulate Drug Transporter Mediated Oral Cancer Treatment. Biomolecules. 2020 Sep 17;10(9):1335. doi: 10.3390/biom10091335. PMID: 32957726

2. Sferrazza G, Corti M, Brusotti G, Pierimarchi P, Temporini C, Serafino A, Calleri E. Nature-derived compounds modulating Wnt/β-catenin pathway: a preventive and therapeutic opportunity in neoplastic diseases. Acta Pharm Sin B. 2020 Oct;10(10):1814-1834. doi: 10.1016/j.apsb.2019.12.019.

3. Ghanim M, Relitti N, McManus G, Butini S, Cappelli A, Campiani G, Mok KH, Kelly VP. A non-toxic, reversibly released imaging probe for oral cancer that is derived from natural compounds. Sci Rep. 2021;11(1):14069.doi:10.1038/s41598-021-93408-0.

4. Khijmatgar S, Yong J, Rübsamen N, Lorusso F, Rai P, Cenzato N, Gaffuri F, Del Fabbro M, Tartaglia GM. Salivary biomarkers for early detection of oral squamous cell carcinoma (OSCC) and head/neck squamous cell carcinoma (HNSCC): A systematic review and network meta-analysis. Jpn Dent Sci Rev. 2024;60:32-39.doi: 10.1016/j.jdsr.2023.10.003

5. He S, Chakraborty R, Ranganathan S. Proliferation and Apoptosis Pathways and Factors in Oral Squamous Cell Carcinoma. Int J Mol Sci.2022.29;23(3):1562.doi: 10.3390/ijms23031562.

6. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol Lett. 2014 Jul;8(1):7-11. doi: 10.3892/ol.2014.2103.

7. De Felice F, de Vincentiis M, Valentini V, Musio D, Mezi S, Lo Mele L, Terenzi V, D'Aguanno V, Cassoni A, Di Brino M, Tenore G, Bulzonetti N, Battisti A, Greco A, Pompa G, Minni A, Romeo U, Cortesi E, Polimeni A, Tombolini V. Follow-up program in head and neck cancer. Crit Rev Oncol Hematol. 2017;113:151-155. doi: 10.1016/j.critrevonc.2017.03.012.

8. Li M, Wang C. Traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the fruit of Tetradium ruticarpum: A review. J Ethnopharmacol. 2020 Dec 5;263:113231. doi: 10.1016/j.jep.2020.113231.

9. To DC, Bui TQ, Nhung NTA, Tran QT, Do TT, Tran MH, Hien PP, Ngu TN, Quy PT, Nguyen TH, Nguyen HT, Nguyen TD, Nguyen PH. On the Inhibitability of Natural Products Isolated from *Tetradium ruticarpum* towards Tyrosine Phosphatase 1B (PTP1B) and α-Glucosidase (3W37): An In Vitro and In Silico Study. Molecules. 2021;26(12):3691.doi: 10.3390/molecules26123691.

10. Li X, Ge J, Zheng Q, Zhang J, Sun R, Liu R. Evodiamine and rutaecarpine from Tetradium ruticarpum in the treatment of liver diseases. Phytomedicine. 2020 Mar;68:153180.doi: 10.1016/j.phymed.2020.153180.

11. Xin X, Shao B, Li Y, Liu S, Li D, Wang C, Chen L, Jin L, Ma X, Wu G. New chemical constituents from the fruits of *Tetradium ruticarpum*. Nat Prod Res. 2022 Apr;36(7):1673-1678.doi: 10.1080/14786419.2020.1808639.

12. Reyes M, Flores T, Betancur D, Peña-

Oyarzún D, Torres VA. Wnt/β-Catenin Signaling in Oral Carcinogenesis. Int J Mol Sci. 2020 Jun 30;21(13):4682. doi: 10.3390/ijms21134682.

13. Wang Y, Cao Z, Liu F, Ou Y. Clinical significance of activated Wnt/β-catenin signaling in apoptosis inhibition of oral cancer. Open Life Sci. 2021 Sep 28;16(1):1045-1052. doi: 10.1515/biol-2021-0104.

14. González-Moles MA, Ruiz-Ávila I, Gil-Montoya JA, Plaza-Campillo J, Scully C. β-catenin in oral cancer: an update on current knowledge. Oral Oncol. 2014 Sep;50(9):818-24.doi: 10.1016/j.oraloncology.

15. Liu F, Millar SE. Wnt/beta-catenin signaling in oral tissue development and disease. J Dent Res. 2010 Apr;89(4):318-30. doi: 10.1177/0022034510363373.

16. Shiah SG, Shieh YS, Chang JY. The Role of Wnt Signaling in Squamous Cell Carcinoma. J Dent Res. 2016 Feb;95(2):129-34.doi: 10.1177/0022034515613507.

17. Osman NI, Sidik NJ, Awal A, Adam NA, Rezali NI. *In vitro* xanthine oxidase and albumin denaturation inhibition assay of *Barringtonia racemosa* L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. J Intercult Ethnopharmacol. 2016 Aug 14;5(4):343-349.doi: 10.5455/jice.20160731025522.

18. Wu XX, Yue GG, Dong JR, Lam CW, Wong CK, Qiu MH, Lau CB. Actein Inhibits the Proliferation and Adhesion of Human Breast Cancer Cells and Suppresses Migration *in vivo*. Front Pharmacol. 2018 Dec12;9:1466.doi: 10.3389/fphar.2018.01466.

19. Jayaraman S, Veeraraghavan VP, Natarajan SR, Jasmine S. Exploring the therapeutic potential of curcumin in oral squamous cell carcinoma (HSC-3 cells): Molecular insights into hypoxia-mediated angiogenesis. Pathology-Research and Practice. 2024 Feb 1;254:155130.

20. Ramasubramanian A, Ramani P, Kannan B, Arumugam P. High expression of novel biomarker TBRG4 promotes the progression and invasion of oral squamous cell carcinoma. J Oral Pathol Med. 2023 Sep;52(8):738-745. doi: 10.1111/jop.13470. Epub 2023 Aug 3. PMID: 37537882.

21. Selvaraj FM, Joseph AP, Pillai VR, Ramani P, Pazhani J, Mony V. Significance of tumour budding and invasive characteristics in grading of oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2023 Oct-Dec;27(4):642-648. doi: 10.4103/jomfp.jomfp_410_23. Epub 2023 Dec 20. PMID: 38304506; PMCID: PMC10829472.

22. Thomas P, Preethi KA, Selvakumar SC, Ramani P, Sekar D. Relevance of micro-RNAs and their targets as a diagnostic and prognostic marker in oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2023 Apr-Jun;27(2):364-373. doi: 10.4103/jomfp.jomfp_349_22. Epub 2023 Jul 13. PMID: 37854932; PMCID: PMC10581285.

23. Gwak J, Cho M, Gong SJ, Won J, Kim DE, Kim EY, Lee SS, Kim M, Kim TK, Shin JG, Oh S. Protein-kinase-C-mediated beta-catenin phosphorylation negatively regulates the Wnt/beta-catenin pathway. J Cell Sci. 2006 Nov 15;119(Pt 22):4702-9. doi: 10.1242/jcs.03256.

24. Harmon B, Bird SW, Schudel BR, Hatch AV, Rasley A, Negrete OA. A Genome-Wide RNA Interference Screen Identifies a Role for Wnt/β-Catenin Signaling during Rift Valley Fever Virus Infection. J Virol. 2016 Jul 27;90(16):7084-7097. doi: 10.1128/JVI.00543-16.

25. Pratesi G, Perego P, Zunino F. Role of Bcl-

2 and its post-transcriptional modification in response to antitumor therapy. Biochem Pharmacol. 2001 Feb 15;61(4):381-6. doi: 10.1016/s0006-2952(00)00538-4.

26. Vantieghem A, Xu Y, Assefa Z, Piette J, Vandenheede JR, Merlevede W, De Witte PA, Agostinis P. Phosphorylation of Bcl-2 in G2/M phase-arrested cells following photodynamic therapy with hypericin involves a CDK1-mediated signal and delays the onset of apoptosis. J Biol Chem. 2002;277(40):37718-31.doi: 10.1074/jbc.M204348200.

27. Cerella C, Gaigneaux A, Mazumder A, Lee JY, Saland E, Radogna F, Farge T, Vergez F, Récher C, Sarry JE, Kim KW, Shin HY, Dicato M, Diederich M. Bcl-2 protein family expression pattern determines synergistic pro-apoptotic effects of BH3 mimetics with hemisynthetic cardiac glycoside UNBS1450 in acute myeloid leukemia. Leukemia. 2017 Mar;31(3):755-759. doi: 10.1038/leu.2016.341

28. Jones WD, Mullins MC. Cell signaling pathways controlling an axis organizing center in the zebrafish. Curr Top Dev Biol. 2022;150:149-209.doi: 10.1016/bs.ctdb.2022.03.005.

29. Herbst A, Kolligs FT. Wnt signaling as a therapeutic target for cancer. Methods Mol Biol. 2007;361:63-91. doi: 10.1385/1-59745-208-4:63. PMID: 17172707.

30. Pećina-Slaus N. Wnt signal transduction pathway and apoptosis: a review. Cancer Cell Int. 2010;30;10:22. doi: 10.1186/1475-2867-10-22.

31. Liu YT, Gong PH, Xiao FQ, Shao S, Zhao DQ, Yan MM, Yang XW. Chemical Constituents and Antioxidant, Anti-Inflammatory and Anti-Tumor Activities of Melilotus officinalis (Linn.) Pall. Molecules.2018;23(2):271.doi: 10.3390/molecules23020271.

32. Waghulde S, Bhoir A, Gholap J, Patil V, Kale MK, Patil VR. Cumulative Effect of the Aqueous and Ethanolic Extracts of Annona Reticulate and Allium Sativumon Brine Shrimp Lethality Assay. *Proceedings*.2019;41(1): 36.

33. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004 Mar 12;74(17):2157-84. doi: 10.1016/j.lfs.2003.09.047.

34. Zhao Z, He X, Han W, Chen X, Liu P, Zhao X, Wang X, Zhang L, Wu S, Zheng X. Genus Tetradium L.: A comprehensive review on traditional uses, phytochemistry, and pharmacological activities. J Ethnopharmacol. 2019 Mar 1;231:337-354. doi: 10.1016/j.jep.2018.11.035.

35. Zhang X, Wang L, Qu Y. Targeting the β-catenin signaling for cancer therapy. Pharmacol Res. 2020 Oct;160:104794. doi: 10.1016/j.phrs.2020.104794.

36. Yu WK, Xu ZY, Yuan L, Mo S, Xu B, Cheng XD, Qin JJ. Targeting β-Catenin Signaling by Natural Products for Cancer Prevention and Therapy. Front Pharmacol. 2020;11:984.doi: 10.3389/fphar.2020.00984.

37. Zhang W, Zhang H, Wang N, Zhao C, Zhang H, Deng F, Wu N, He Y, Chen X, Zhang J, Wen S, Liao Z, Zhang Q, Zhang Z, Liu W, Yan Z, Luu HH, Haydon RC, Zhou L, He TC. Modulation of β-catenin signaling by the inhibitors of MAP kinase, tyrosine kinase, and PI3-kinase pathways. Int J Med Sci. 2013 Nov 25;10(13):1888-98. doi: 10.7150/ijms.6019.

38. Veeramachaneni NK, Kubokura H, Lin L, Pippin JA, Patterson GA, Drebin JA, Battafarano RJ. Down-regulation of beta catenin inhibits the growth of esophageal carcinoma cells. J Thorac Cardiovasc Surg. 2004;127(1):928.10.1016/j.jtcvs2003.06008