RESEARCH ARTICLE

Editorial Process: Submission:12/04/2024 Acceptance:05/09/2025

Evaluation of *In Vitro* Anticancer Activity and Apoptotic Potential of Wrightia Tinctoria Bark Extracts on Oral Cancer Cell Lines

Deepa Anchalayil Gopalakrishnan[1]*, Beena Valappil Thannikunnath[2], Rajesh Ramachandran[3], Sudha Sivasankar[4]

Abstract

Background and objectives: Bioprospecting of indigenous plants for biological/cytotoxic properties has become an area of growing interest in cancer research. Wrightia tinctoria is a lesser-studied plant widely distributed in India which is commonly used in traditional medicinal systems like Ayurveda and Siddha and holds potential as a source for novel therapeutic agents. In this study, the cytotoxic effects of Wrightia tinctoria bark extracts on oral cancer (KB) cell lines were evaluated, focusing on three different extraction solvents ethanol, ethyl acetate, and water. The present study aimed to evaluate and compare the cytotoxic effects of Wrightia tinctoria bark extracts, while also investigating their potential to induce apoptosis which can contribute into exploration of new plant based anticancer agents. **Methods:** Cytotoxic effects of ethanol, ethyl acetate, and aqueous extracts of Wrightia tinctoria bark were evaluated on human oral cancer cells (KB cell line). Cell viability was assessed by 3-(4,5-dimethylthiazoline- 2-yl)-2,5-diphenyltetrazolium bromide (MTT assay). Apoptosis was evaluated by acridine orange (AO) and ethidium bromide (EB) double staining method and expression of apoptotic enzymes Caspase 3 and Caspase 7 were measured by quantitative indirect ELISA technique. **Results:** Ethanol extract of Wrightia tinctoria bark exhibited highest cytotoxic activity towards oral cancer cell lines. The half-maximum inhibitory concentration (IC_{50}) values of the ethanol, ethyl acetate, aqueous extracts were 75.084µg/ml, 179.743µg/ml and 115.258 µg/ml respectively. IC 50 concentration of ethanol and ethyl acetate extracts stimulated significant apoptosis in KB cells. Indirect ELISA showed increased expression of caspase 3 and caspase 7 among the cells on treatment with ethanol and ethyl acetate extracts. **Conclusion:** Ethanol extract of Wrightia tinctoria bark exhibited highest cytotoxic activity towards oral cancer cell lines and induced apoptotic changes. Further research may help us to identify newer, better anti cancer agents against oral cancer from Wrightia tinctoria bark.

Keywords: Oral cancer- apoptosis- cell line

Asian Pac J Cancer Prev, **26 (5)**, 1753-1759

Introduction

Oral and oropharyngeal cancers are among the most common cancer types globally. As per WHO IARC Oral cancer prevention handbook, volume 19, in 2020, there were 377,713 new cases of oral cancers and 177,757 oral cancer related deaths reported worldwide [1]. Surgery, radiation, chemotherapy, antibody blocking therapy, or a combination of therapies are the mainstays of treatment for oral cancer [2]. Many commonly used anticancer agents like paclitaxel, vincristine, epipodophyllotoxin are derived from plants [3]. Testing plant extracts for potential anticancer activity is the first step in developing a safe and effective phytochemical-based anticancer therapy, followed by purification of phytochemicals and isolation of active compounds via bioassay-guided fractionation, and testing them for in vitro and in vivo effects [3].

Wrightia tinctoria R. Br. belongs to family Apocynaceae commonly called as Sweet Indrajao, Pala Indigo, Dyer's Oleander and Jaundice Curative Tree in south India. It is a small, deciduous tree with a light gray, scaly smooth bark and white fragrant flowers. The juice of the tender leaves is used efficaciously in jaundice. Crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it is used for psoriasis and other skin diseases. Oil 777 prepared out of the fresh leaves of the plant is used effectively in the treatment of psoriasis. Its bark is also used to treat psoriasis, pyrexia,

*[1]Department of Oral and Maxillofacial Pathology, Government Dental College, Kottayam, Kerala, India. [2]Joint Director of Medical Education, Government of Kerala, Thiruvananthapuram, Kerala, India. [3]Director and Research Head Center For Research on Molecular Biology and Applied Science, Thiruvananthapuram, Kerala, India. [4]Department of Oral and Maxillofacial Pathology, Government Dental College, Kozhikode, Kerala, India. *For Correspondence: deepakrishna4@gmail.com*

dysentery, diarrhea, hemorrhage, and as an antidote for snake poison [4] .

Phytoconstituents present in Wrightia tinctoria (WT) include glycosides,steroids, triterpenoids, saponins, tannins, phenolics, and flavonoids. Lupeol, stigmasterol, campetosterol, indigotin, indirubin, tryptanthrin, isatin, anthranillate, rutin, triacontanol, wrightial, cycloartenone, cycloeucalenol, β-amyrin, alpha-Amyrin, β-sitosterol, 3,4-Seco-lup-20 (29)-en-3-oic acid and 14α-methylzymosterol have been isolated from WT [5].

WT has been shown to be effective against cancer cell lines of skin, HeLa (cervical cancer cells) and breast cancer cells [6]. However, there is limited research on the effects of WT on oral cancer cells. Hence, a comparative analysis was performed on the anti proliferative and apoptotic effects of ethanol, ethyl acetate and aqueous extracts of WT bark on oral cancer cell lines (KB cell lines).

Materials and Methods

This is an in vitro experimental study which does not involve human subjects or animals. Ethical approval was obtained from Institutional Ethics Committee, Government Dental College, Kottayam, Kerala, India(IEC/M26/2023/R501/DCK).

Collection and identification of plant

Bark of WT was collected from the medicinal garden of St. Antony's Ayurvedic Hospital, Kottayam, Kerala, India in December and was sent for authentication. The plant specimen was identified and authenticated by a scientist from the Department of Botany, Baselius College, Kottayam. It was washed thoroughly, shade dried for three weeks and powdered without contamination.

Extraction

100 g of Wrightia tinctoria(Bark) was extracted with 70% ethanol, 80% ethyl acetate, and water using cold percolation method for 24 hours. The extracts were recovered by filtration using muslin cloth and the filtrate kept for air drying. It was then collected in sterile eppendorf tube and was used for further studies. The yield of the samples obtained were 6.33%, 6.15% and 3.49% respectively.

In vitro anticancer effect determination

Cell line

Authenticated KB (Human oral cancer) cell line was provided by National Centre for Cell Sciences (NCCS), Pune, India and was tested mycoplasma free. They were maintained in Dulbecco's modified Eagles medium(DMEM) (Sigma Aldrich, USA) with 10% Fetal Bovine Serum, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml) at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany) as per standard procedures [7] .

Morphological changes

Serially diluted samples (100µg, 50µg, 25µg, 12.5µg, 6.25µg per µl of DMEM) were added in triplicates to the 96- well plate containing monolayer of cells and incubated at 37ºC in a humidified 5% CO_2 incubator. Non treated control cells were also maintained. After 24hours, morphological changes of cells were evaluated by inverted phase contrast microscope (Olympus CKX41 with Optika Pro5 CCD camera).

Anticancer Assay by MTT Method

Fifteen mg of MTT (Sigma, MA, USA, M-5655) was dissolved in 3 ml of phosphate-buffered saline and sterilized through filtration. After 24-hour incubation, 30 µl of the MTT solution was added to both test and control wells. The plate was gently shaken and incubated at 37ºC in a 5% CO_2 humidified environment for 4 hours. Post-incubation, the supernatant was removed, and 100 µl of dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA) was added to solubilize the formazan crystals. The assay was performed in triplicate, and cell viability was determined by comparing the absorbance of treated cells to untreated controls. Absorbance was measured at 540 nm using a microplate reader [7] .

Determination of apoptosis by acridine orange (AO) and ethidium bromide (EB) double staining

KB cells and normal control cells were treated with IC 50 concentrations of ethanol, ethyl acetate and aqueous extracts of WT (75.084µg/ml, 179.743µg/ml and 115.258µg/ml respectively) for 24 hours, followed by staining with a mixture of DNA-binding dyes AO and EB in 1:1 ratio (Sigma Aldrich, USA) for 10 minutes. The morphology of apoptotic cells was examined with a fluorescence microscope (Olympus CKX41 with Optika Pro5 camera, India). The cells were categorized as living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange-stained cell nuclei).

Estimation of Caspase-3 and Caspase-7 by quantitative indirect ELISA

Expression of apoptotic markers caspase-3 and caspase-7 were measured by quantitative indirect ELISA technique. KB cells and control cells were exposed with IC_{50} concentration of samples and incubated for 24 hours at 37ºC in a humidified 5% CO_2 incubator. 100µl each of the supernatant was added to the 96-well plate and incubated overnight. Blocking buffer(0.2% gelatin in 0.05% Tween 20: Merck; Germany) was added and incubated for 1 hour to block the remaining protein binding sites in wells. 100µl of primary antibodies Caspase 3, Caspase 7 (1:500, Invitrogen, USA) were added and left for 2 hours. 100µL of secondary antibody (Horse radish peroxidase conjugate, Santacruz, USA) was added and left for 1 hour. All steps were performed at 370 C. The reaction was stopped by adding 50µl 5N hydrochloric acid. Intensity was read at 415 nm in an ELISA reader. The protein content was measured using a colorimetric assay by Bradford method. The levels of caspases were obtained as optical densities values, and normalized to the protein content [8].

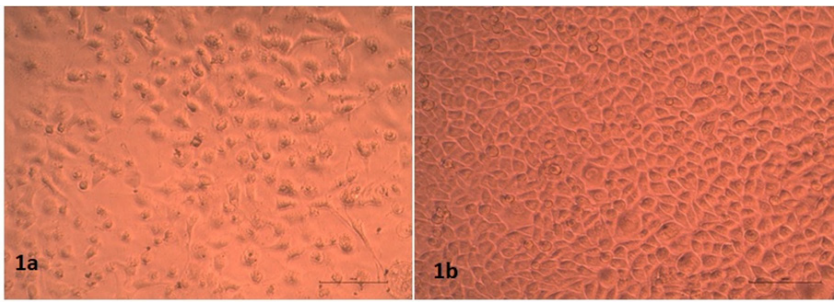

Figure 1. Ethanol Extracts of WT Induces Significant Morphological Changes in Oral Cancer Cells (1a) than Control (1b) as Determined by Microscopic Observation

Statistical analysis

All experiments were done in triplicates and results represented as Mean+/- SE. Prism GraphPad version 10.4.1(GraphPad software, San Diego, CA) for Windows was used for statistical analysis. One-way ANOVA and Dunnets test were performed to analyse data. Four point regression analysis was done to estimate IC_{50}. P value < 0.05 was considered significant compared to control group.

Results

Cell shrinkage and nuclear condensation were the key morphological changes observed, suggesting the involvement of apoptotic pathways (Figure 1). MTT assay showed a dose-dependent cell inhibition with different doses of extracts (Table 1). Ethanol extract of WT bark resulted maximum cell inhibition (42.97%) at the highest extract concentration of 100µg/µl. Ethyl acetate extract of WT bark produced maximum cell inhibition (57.14%) whereas aqueous extract of WT bark exhibited maximum inhibition of 71.31%.

IC_{50} values were calculated using four point regression analysis. IC_{50} value of ethanol extract of bark of WT was found to be 75.084334µg/ml, whereas IC_{50} value of aqueous extract was 179.743µ).g/ml(Figures 2 and 3). Ethyl acetate extracts from the bark showed an IC_{50} value of 115.258µg/ml (Figure 4). Thus ethanol extract of WT showed most potent activity among the three extracts compared.

The AO/EB staining showed no significant apoptosis in the control group. Early stage apoptotic cells, marked by bright green nucleus and condensed chromatin were observed in cells treated with IC_{50} of aqueous extract of WT. Late stage apoptotic cells with concentrated and asymmetrically localized orange nuclear EB staining were detected in ethanol and ethyl acetate extract- treated cells. Ethanol and ethyl acetate extracts stimulated significant apoptosis in the KB cells (Figure 5).

Quantitative indirect ELISA showed increasing expression of caspase 3 and caspase 7 among cells on treatment with IC_{50} concentration of extracts. A significant increase in levels of capase-3 (up to 3.4-folds of control cells) and caspase 7 (upto 3.0 folds of control cells) were

Table 1. Percentage of Cell Viability in MTT assay after Treatment of KB Cells with Serially Diluted Samples of Ethanol, Ethyl Acetate and Aqueous Extracts of Wrightia Tinctoria

Ethanol extract of Wrightia tinctoria bark	Mean ± S. E
Control	100
6.25	74.67917 ± 0.075847
12.5	66.12195 ± 0.758846
25	52.32159 ± 0.285642
50	47.89308 ± 0.030231
100	36.24791 ± 0.214302
Ethyl acetate extract of Wrightia tinctoria bark	Mean ± S. E
Control	100
6.25	96.54 ± 0.235086
12.5	88.24147 ± 0.312312
25	80.26678 ± 0.136813
50	74.15534 ± 0.031523
100	57.14451± 0.030231
Aqueous extract of Wrightia tinctoria bark	Mean ± S. E
Control	100
6.25	98.07981 ± 0.26273
12.5	95.11683 ± 0.255135
25	91.2045 ± 0.342808
50	87.15285 ± 0.377678
100	71.30986 ± 0.119844

Table 2. Caspase- 3 and Caspase- 7 Levels in KB Cells after 24 hours of Treatment with IC_{50} Concentration of Samples Evaluated by Quantitative Indirect ELISA. Data are represented as optical density normalized to the cell protein content (OD/µg protein).

Caspase 3	Mean± SE
1. Control	0.140973967 ± 0.001776
2. Ethanol extract	0.485300233 ± 0.004351
3. Ethyl acetate extract	0.269275733 ± 0.009231
4. Aqueous extract	0.254081933 ± 0.002926
Caspase 7	Mean± SE
1. Control	0.159474433 ± 0.001754
5. Ethanol extract	0.462028633 ± 0.005601
6. Ethyl acetate extract	0.3106961 ± 0.002971
7. Aqueous extract	0.380134533 ± 0.003617

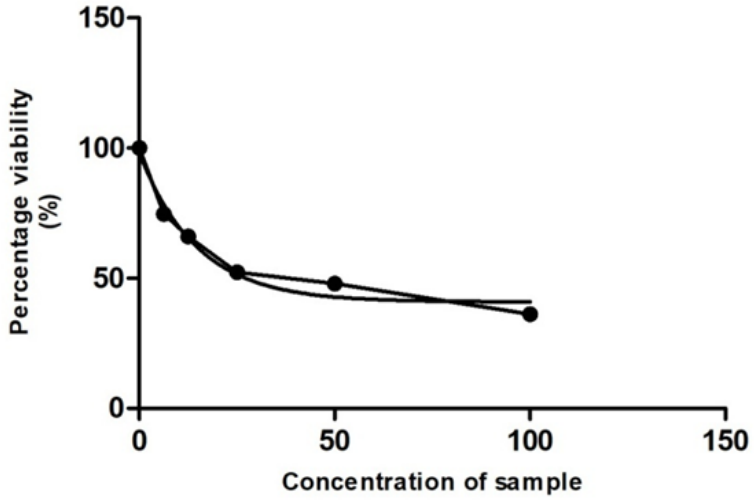

Figure 2. Reduction in Cell Viability with Increase in Concentration of Ethanol Extract of Wrightia Tinctoria

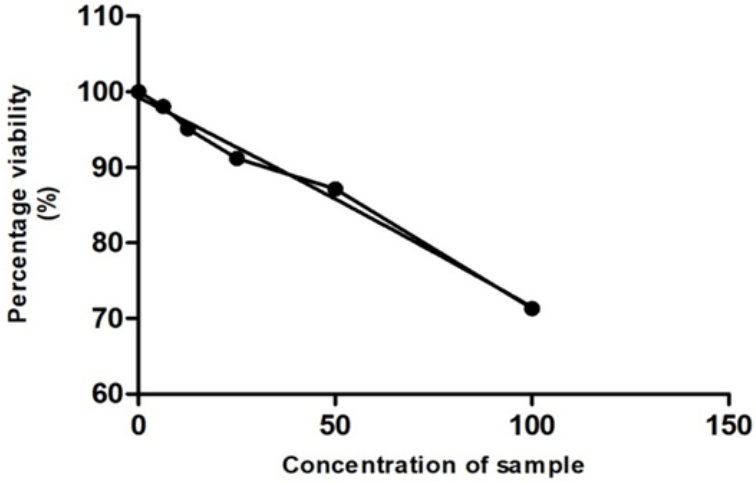

Figure 3. Reduction in Cell Viability with Increase in Concentration of Ethyl Acetate Extract of Wrightia Tinctoria

observed in cells treated with ethanol extracts of WT bark (Table 2).

Discussion

Wrightia tinctoria possess multiple pharmacological activities such as wound healing, anti-inflammatory, antipsoriatic, antinociceptive, antidandruff, antioxidant,

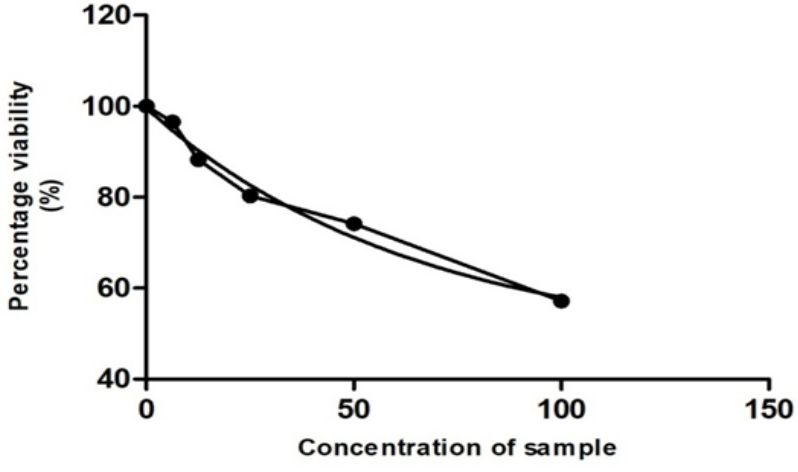

Figure 4. Reduction in Cell Viability with Increase in Concentration of Aqueous Extract of Wrightia Tinctoria

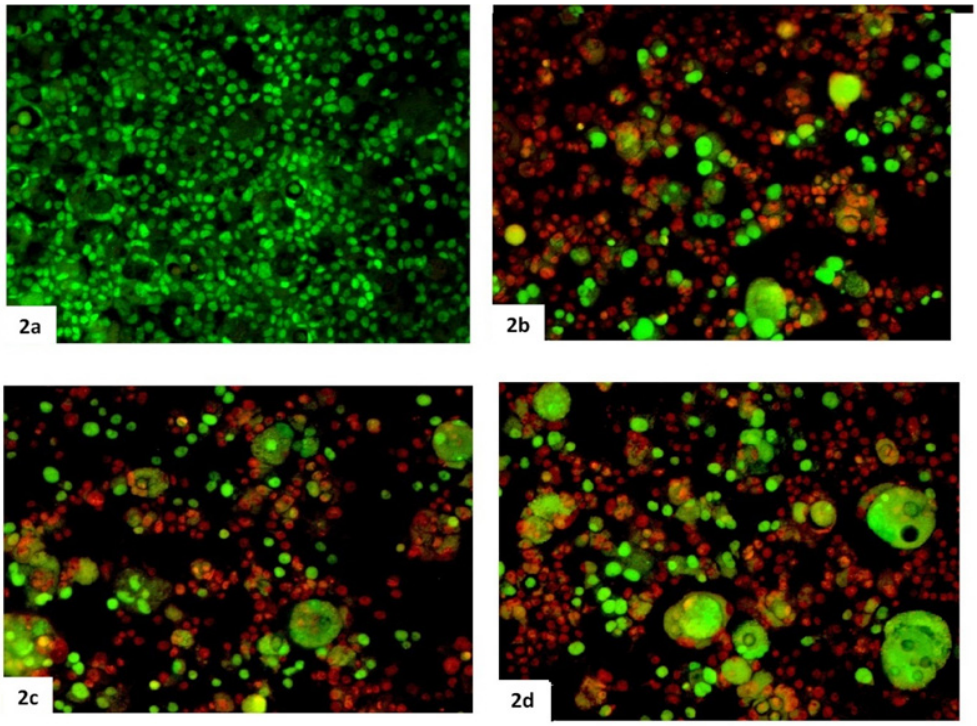

Figure 5. Representative Fluorescence Microscopic Images Showing Morphological Changes of KB Cells after Different Treatments; control (2a), ethanol extract of WT(2b), ethyl acetate extract of WT (2c) and aqueous extract of WT (2d) The cells were subjected to AO- EB dual staining.

clot inducing, diuretic, antiviral, cytotoxic and antiulcer activity [8]. The present study aimed to evaluate and compare the anticancer activity of ethanol, ethyl acetate and aqueous extracts of WT bark on oral cancer cells. Cytotoxicity was assessed and compared using MTT assay. Role of apoptosis was evaluated by AO and EB staining. Activity of caspases was assessed by indirect ELISA.

Cell shrinkage and nuclear condensation were the key morphological changes observed, suggesting the involvement of apoptotic pathways. Fatima N et al observed similar morphological alterations along with formation of round apoptotic bodies in both MD Anderson- Metastasis Breast Cancer (MDA-MB-231) and Michigan Cancer Foundation (MCF-7) breast cancer cell lines after exposure with methanol extract of WT bark [9]. Jegadeeshwari et al. noticed a shift in the shape of MCF-7 carcinoma cells when incubated with silver nanoparticles coated with WT seed extract (AgNPs) at concentrations of 50µg/ml and 100 µg/ml [10].

In MTT assay, a decrease in the number of viable cells indicates the cytotoxicity of the extract. Here, highest IC_{50} at the minimum concentration of 75µg/ml was exhibited by ethanol extract confirming to be the most potent agent among them. Chaudhari S et al reported that the ethanol extract and subsequent fractions of WT bark inhibited the viability of HeLa (human epithelial cervical carcinoma) cells in a concentration dependent manner in MTT assay. They demonstrated the selective cytotoxicity of WT extracts to cancer cells when compared to nontumor Chinese hamster normal fibroblasts. HPTLC confirmed the presence of two anti-cancer triterpenoids, lupeol, and β-sitosterol in active fractions [6] .

Antony J et al conducted MTT assay to compare the effects of hexane, dichloromethane, ethyl acetate, and methanol extract of Wrightia tinctoria leaves on melanoma cell line A375, which revealed dichloromethane extract to be the most active. Successive silica gel column chromatography of dichloromethane extract led to the isolation of fraction DW-F5, which exhibited antimelanoma activities, preventing metastasis and angiogenesis in Non-obese diabetic (NOD)-severe combined immunodeficient(SCID) mice, while being nontoxic in vivo [11] .

Double staining by AO and EB is an economic and convenient method to detect apoptosis in tumor cells. DNA-binding dyes AO and EB were used for the morphological detection of apoptotic and necrotic cells. AO is a vital dye that diffuses into all cells and stains both living and dead cells; whereas EB stains cells that have lost membrane integrity ie. those in the late apoptotic stage. Live and active cells emit uniform green fluorescence. On the contrary, due to DNA fragmentation and chromosomal condensation, the early apoptotic cells fluoresce with orange patches. The late apoptotic cells will show fragmented and condensed nuclei. Necrotic cells emit total red-orange fluorescence evenly. However, the cells will resemble viable cells in nuclear morphology without condensation of chromatin. Results observed in the fluorescent AO-EB staining confirmed the apoptotic efficacy of WT bark extracts [12].

Many plant extracts and phytochemicals have shown to activate apoptotic pathways in cancer cells. Evaluation of caspases, the pro-apoptotic enzymes indicate apoptosis inducing property of a plant extract. The ethanol and ethyl acetate extracts of WT bark produced three fold increase in

the levels of caspases 3 and 7, indicating that both internal and external mechanisms were significantly impacted.

Gas chromatography-mass spectroscopy evaluation of methanol extract of WT leaves revealed phytochemicals predominantly vitamin E, steroids , fatty acid esters and terpenes, which could be responsible for the reported free radical scavenging capacity [13] .

Tryptanthrin , an indole quinazoline alkaloid has been isolated and characterized from WT. Shabna A et al. [4] demonstrated its remarkable anti-tumor activity towards human melanoma cells through down-regulation of Microphthalmia-associated transcription factor (MITF-M), the key transcription factor and oncogene aiding their survival [14]. Shankar G M et al. [15] demonstrated topical application of tryptanthrin suppressed skin carcinogenesis in mice .

WT extract has been shown to be safer on normal cells. Chaudhari S et al. have demonstrated its safety towards V79 (non tumor Chinese hamster normal fibroblast) cells. MTT assay with ethanol, petroleum ether and ethyl acetate fractions of WT bark on V79 cells showed higher IC 50(400µg/ml, 267µg/ml and 170µg/ml respectively) indicating their relative safety to a nontumor cell line. Also, this plant has been widely used in India for the treatment of various disorders without any observed toxicity. Jain and Bari observed no mortalities or adverse effects in mice, following acute oral administration at the highest dose of 2500 mg/ kg of crude extracts of WT bark [16] .

Thiagarajan MK et al recently reported the cytotoxicity of ethanol extract of WT leaves on KB cell lines. They obtained an IC 50 of 48 µg/ml in MTT assay, a ladder pattern in DNA fragmentation assay and presence of cleaved caspase 3 in western blot analysis, indicating the apoptotic potential of the extract [17]. Molecular docking analysis was conducted with chemicals derived from the plant against target oncogenic proteins EGFR and MAPK. They concluded that 3-O-methyl-d-glucose and squalene found in W. tinctoria leaves can inhibit the activity EGFR and MAPK in oral cancer [18] .

Results of the present study indicate that extract of WT bark is a cytotoxic agent against oral cancer cells. AO-EB double staining and indirect ELISA for caspases 3 and 7 prove its role in apoptosis as well. Also, it is evident that the polarity of solvents used in the extraction process plays a crucial role in determining the chemical composition of the bioactive extract and its potential to modulate apoptosis.

To our best knowledge, this study gives the first evidence of WT bark extract's anticancer activity against oral cancer cells. Further in depth researches are being conducted to extract and identify specific anticancer compounds from WT bark and to elucidate the molecular pathways responsible for its anticancer activity against oral cancer cells. The AMIRDA-Standard-Reporting-for-Anticancer Activity of Natural-Compounds-Tool was followed in preparation of this manuscript [19].

In conclusion, wrightia tinctoria has been used extensively in traditional medicine to treat various ailments such as psoriasis, jaundice, dysentery etc. Present research throws light on the cytotoxicity and apoptosis inducing effects of WT bark on oral cancer cells. Further investigations can draw new insights on the mechanism of apoptotic induction by chemical compounds extracted from W. tinctoria bark.

Author Contribution Statement

Deepa A G- conceptualization, investigation, data curation, writing original draft. Beena VT- conceptualization, review and editing of draft, project administration. Rajesh Ramachandran- conceptualization, investigation, resources, methodology. Sudha S - conceptualization, methodology, review and editing of draft.

Acknowledgements

Approval

The research protocol was approved by the Scientific Review Committee of Government Dental College, Kottayam, Kerala, India.

Ethical Declaration

The research protocol was approved by the Institutional Ethics committee of Government Dental College, Kottayam, Kerala, India (IEC/M26/2023/R501/DCK).

References

1. Bouvard V, Nethan ST, Singh D, Warnakulasuriya S, Mehrotra R, Chaturvedi AK, et al. IARC perspective on oral cancer prevention. N Engl J Med. 2022;387(21):1999-2005. https://doi.org/10.1056/NEJMsr2210097.
2. Huang SH, O'Sullivan B. Oral cancer: Current role of radiotherapy and chemotherapy. Med Oral Patol Oral Cir Bucal. 2013;18(2):e233-40. https://doi.org/10.4317/medoral.18772.
3. Ahmed MB, Islam SU, Alghamdi AAA, Kamran M, Ahsan H, Lee YS. Phytochemicals as chemo-preventive agents and signaling molecule modulators: Current role in cancer therapeutics and inflammation. Int J Mol Sci. 2022;23(24). https://doi.org/10.3390/ijms232415765.
4. Srivastava R. A review on phytochemical, pharmacological, and pharmacognostical profile of Wrightia tinctoria: Adulterant of kurchi. Pharmacogn Rev. 2014;8(15):36-44. https://doi.org/10.4103/0973-7847.125528.
5. Fatima N, Ahmad MK, Ansari JA, Ali Z, Khan AR, Mahdi AA. Anticancer, antioxidant potential and profiling of polyphenolic compounds of wrightia tinctoria roxb. (r.Br.) bark. J Adv Pharm Technol Res. 2016;7(4):159-65. https://doi.org/10.4103/2231-4040.191428.
6. Chaudhary S, Devkar RA, Bhere D, Setty MM, Pai KS. Selective cytotoxicity and pro-apoptotic activity of stem bark of Wrightia tinctoria (roxb.) r. Br. In cancerous cells. Pharmacogn Mag. 2015;11(Suppl 3):S481-7. https://doi.org/10.4103/0973-1296.168976.
7. Jerard C, Michael BP, Chenicheri S, Vijayakumar N, Ramachandran R. Rosmarinic Acid-Rich Fraction from Mentha arvensis Synchronizes Bcl/Bax Expression and Induces Go/G1 Arrest in Hepatocarcinoma Cells. Proc Natl Acad Sci India Sect B Biol Sci. 2020 Sep 1;90(3):515–22.
8. El-Hamid ESA, Gamal-Eldeen AM, Sharaf Eldeen AM. Liposome-coated nano doxorubicin induces apoptosis on oral squamous cell carcinoma CAL-27 cells. Arch

Oral Biol. 2019;103:47-54. https://doi.org/10.1016/j.archoralbio.2019.05.011.
9. Hari A, Pattam S, Nihal P, Athira A. An Updated Review on Wrightia tinctoria (Roxb). R Br.J Pharm Res Int. 2021;33(56A):234-44.
10. Jegadeeshwari A, Seelam NR, Myneni VR. Evaluation of antibacterial and anticancer characteristics of silver nanoparticles synthesized from plant extracts of wrightia tinctoria and Acacia chundra. Int J Anal Chem. 2023;2023:6352503. https://doi.org/10.1155/2023/6352503.
11. Antony J, Saikia M, Vinod V, Nath LR, Katiki MR, Murty MS, et al. Dw-f5: A novel formulation against malignant melanoma from Wrightia tinctoria. Sci Rep. 2015;5:11107. https://doi.org/10.1038/srep11107.
12. Prabaharan R, Rengan R, Thangavel SK, Małecki JG. Exploration of antiproliferative activity and apoptosis induction of new nickel(ii) complexes encompassing carbazole ligands. ACS Omega. 2023;8(13):12584-91. https://doi.org/10.1021/acsomega.3c01252.
13. Khan N, Ali A, Qadir A, Ali A, Warsi MH, Tahir A, et al. Gc-ms analysis and antioxidant activity of Wrightia tinctoria r.Br. Leaf extract. J AOAC Int. 2021;104(5):1415-9. https://doi.org/10.1093/jaoacint/qsab054.
14. Shabna A, Antony J, Vijayakurup V, Saikia M, Liju VB, Retnakumari AP, et al. Pharmacological attenuation of melanoma by tryptanthrin pertains to the suppression of MITF-M through MEK/ERK signaling axis. Cell Mol Life Sci CMLS. 2022 Aug 10;79(9):478. https://doi.org/10.1007/s00018-022-04476-y.
15. Shankar GM, Alex VV, Nisthul AA, Bava SV, Sundaram S, Retnakumari AP, et al. Pre-clinical evidences for the efficacy of tryptanthrin as a potent suppressor of skin cancer. Cell Prolif. 2020;53(1):e12710. https://doi.org/10.1111/cpr.12710.
16. Jain PS, Bari SB, Surana SJ. Acute oral toxicity of abelmoschus manihot and Wrightia tinctoria in mice. Pharmacogn J. 2011;3(25):78-81. https://doi.org/https://doi.org/10.5530/pj.2011.25.14.
17. Thiagarajan MK, Chandrasekharan D, Mahalingam R, Ravi A. Evaluation of in vitro anti-cancer potential and apoptotic profile of ethanolic plant extract of wrightia tinctoria against oral cancer cell line. J Oral Maxillofac Pathol. 2024;28(2):211-5. https://doi.org/10.4103/jomfp.jomfp_32_24.
18. Thiagarajan MK, Chandrasekharan D. Novel therapeutic targets for the treatment of oral cancer with compounds identified in Wrightia tinctoria: An in silico docking study with oncogenic pathways. J Orofac Sci. 2023;15(2):152-5. https://doi.org/10.4103/jofs.jofs_252_23.
19. Ahmad R, Riaz M, Aldholmi M, Qureshi MA, Uddin S, Bhat AA, et al. Development of a critical appraisal tool (AIMRDA) for the peer-review of studies assessing the anticancer activity of natural products: A step towards reproducibility. Asian Pac J Cancer Prev. 2022;22(12):3735-40. https://doi.org/10.31557/apjcp.2021.22.12.3735.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.