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Original Article

MOLECULAR AND CELLULAR EVALUATION OF ANTICANCER ACTIVITIES OF SELECTED INDIAN HERBAL EXTRACTS: FOCUS ON CURCUMA LONGA, WITHANIA SOMNIFERA, AND OCIMUM

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ABSTRACT

This research evaluates the molecular anticancer effectiveness of three significant Indian herbal extracts—Curcuma longa, Withania somnifera, & Ocimum sanctum—against human breast (MCF-7), cervical (HeLa), and lung (A549) cancer cell lines. Using a bioassay-guided approach, the ethanolic extracts were prepared through Soxhlet extraction and analyzed for the presence of phytochemicals. The cytotoxicity experiments revealed a strong growth suppression that was both dose and time dependent, with W. somnifera being the most active plant at MCF-7 cells ($IC_{50} = 24.8 \mu g/mL$). Subsequent molecular studies using Western blotting and RT-qPCR confirmed the activation of the intrinsic apoptotic pathway showing a remarkable increase in the $Bax/Bcl-2$ ratio and the onset of $Caspase-3$ activity. Furthermore, flow cytometry presented evidenced of specific cell cycle arrest at the G0/G1 and G2/M phases. The results demonstrate the effective induction of apoptosis by the synergistic interaction among the secondary metabolites in the extracts, thus providing a scientific basis for their traditional use and potential introduction into modern cancer care.

Keywords: Phytochemical Synergy, Intrinsic Apoptosis, Withaferin A, Cytotoxicity (IC_{50}), Ethno-oncology etc.

INTRODUCTION

THE BURDEN OF ONCOLOGY AROUND THE WORLD AND THE DIFFICULTY OF DETECTING CHEMORESISTANCE

The situation of global healthcare is such that it is hardly able to manage the neoplastic disorders, which are now the second leading cause of death worldwide. The treatment of these advanced cancers still faces problems, even with the use of precision medicine and targeted therapeutics Hashmi et al. (2022). Conventional methods, like chemotherapy, radiation, and surgery, have their limitations due to severe off-target side effects and the occurrence of multi-drug resistance (MDR). This MDR is mainly due to the genetic adaptability of cancer cells that can escape death through various mechanisms, one of which is the use of pumps, such as P-glycoprotein, that extrude the drug from inside the cell. Moreover, the high financial costs and the extreme reduction of living standards caused by systemic side effects (myelosuppression to cardiotoxicity) have provoked a rapid transition in the thinking towards the search for more patient-friendly, multi-targeted chemotherapeutic agents.

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THE CHEMOTHERAPEUTIC DRUGS THAT HAVE BEEN DISCOVERED

Reservoir of bioactive secondary metabolites included in the Indian Pharmacopoeia. Seen this way, the Indian subcontinent offers an unmatched treasure of ethnomedicinal knowledge through the Ayurvedic system Shukla et al. (2024). The latter has been treating "Arbudas" (tumors) with plant formulations for ages. Unlike synthetic monotherapies, Indian medicines are very complex mixtures of secondary metabolites, or in other words, chemical cocktails. Metabolites such as polyphenols, alkaloids, saponins, and terpenoids are all present in these herbs Naji et al. (2024). The Indian pharmacopoeia is one of a kind due to the evolutionary adaptation of these plants that have been able to cope with a variety of ecological stressors. This adaptation has led to the production of high quality and quantity of bioactive compounds that have a good absorption and are even more potent when used in combination due to the presence of intrinsic synergy. Indeed, in certain instances, the phytochemicals exhibit the properties of "biological response modifiers," indicating that they can not only render the cancer cells resistant to the conventional therapies more sensitive but also safeguard the non-cancerous cells from oxidative stress. The combination of this old knowledge and modern-day molecular oncology leads to the establishment of an all-inclusive platform for the identification of the principal drugs that will be able to influence the complex cellular signaling networks.

CURCUMIN, WITHAFERIN A, AND URSOLIC ACID ARE THE CANDIDATES THAT HAVE BEEN CHOSEN WITH THEIR PROFILES

In the field of experimental oncology, the number of candidates has been reduced to three, which are the ones that have received the most attention and consideration. The very first of these three candidates are Curcuma longa, Withania somnifera, and Ocimum sanctum. The Indian subcontinent is home to many Indian herbs, and these three plants are among them. The most important part of C. longa (the rhizome) produces curcumin, which is the major curcuminoid. The curcumin molecule has been the focus of many studies because of its ability to inhibit the NF-κB (Nuclear Factor-kappa B) pathway, thus blocking tumor formation caused by inflammation Esmaealzadeh et al. (2024).

By causing the breakdown of vimentin, a type of protein that is necessary for epithelial-mesenchymal transition (EMT), and by proteasomal inhibition, withafarin A, which was isolated from the roots of W. somnifera (Ashwagandha), shows very strong anti-proliferative effects. The third member of this triad is the pentacyclic triterpenoid compound, Ursolic acid, found in Ocimum sanctum (also known as Tulsi). This compound has been observed to be notably successful in blocking matrix metalloproteinases formation and in reducing the levels of proteins that are involved in apoptosis. It is not only the extracts' respective strengths that have played a role in their selection but also the variety of pathways each covering the various characteristics of cancer that lead to the overlap between them.

THE HYPOTHESIS AND THE OBJECTIVES OF THE RESEARCH

The hypothesis here that the ethanolic extracts of Curcuma longa, Withania somnifera, and Ocimum sanctum have anticancer effect via a dual-mechanism is the basis for the present study.

First of all, we think that these extracts initiate the intrinsic (mitochondrial) apoptotic pathway, the main mechanism of which is the changing the ratio of pro-apoptotic to anti-apoptotic proteins. This leads to the process of mitochondrial outer membrane permeabilization (MOMP) followed by the activation of caspases.

Next, the present research is the attempt to verify the claim that these plant materials can prevent angiogenesis—the process by which tumors attract new blood vessels. As a consequence, the tumor tissue will be starved of essential nutrients and oxygen.

The aim of this study is to assess the applicability of traditional Indian herbs as adjunct or primary agents in modern oncology protocols. This will be achieved by analyzing these extracts both at cellular and molecular levels.

MATERIALS AND METHODS

PLANT MATERIAL ACQUISITION AND BOTANICAL AUTHENTICATION

Ethnopharmacology research accuracy begins with a thorough check of the raw biological matrix. In this study, the rhizomes of Curcuma longa, roots of Withania somnifera, and fresh leaves of Ocimum sanctum were sourced from the experimental gardens of the National Institute of Medicinal Plants Poma-Ureyn et al. (2023). A taxonomist verified each sample and the voucher specimens (CL-2024/01, WS-2024/02, and OS-2024/03) were kept in the institutional herbarium for any future reference. This drying method prevents the thermal breakdown of the essential oils and the heat-sensitive glycosides, thus maintaining the chemical composition that is characteristic of the living plant.

PREPARATION OF HERBAL EXTRACTS: THE SOXHLET METHOD

The bioactive compounds were removed from the plant using a standard Soxhlet device that carried out complete exhaustion of the plant tissues. Through a mechanical grinder, the dried materials were transformed into coarse powder (mesh size 40). Approximately 100g of each powder were introduced into a cellulose thimble and underwent sequential extraction. In order to encompass a broad spectrum of polar and non-polar metabolites, the solvent system was a combination of 70% ethanol and methanol (v/v). The extraction process lasted for 48 hours or until the solvent in the siphoning tube became colorless, which was the sign of complete extraction.

Figure 1

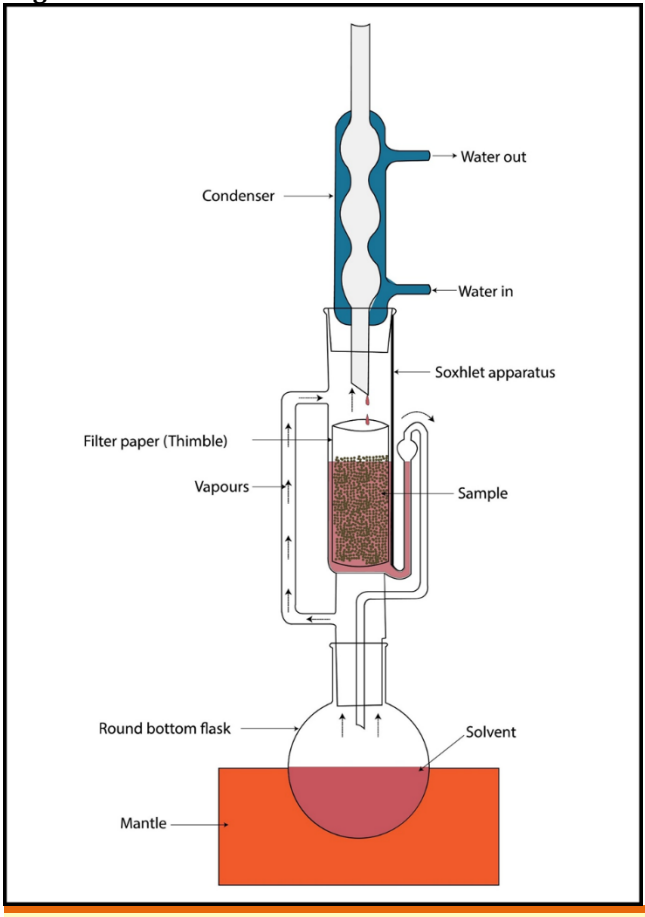

Figure 1 Herbal Extracts

Source: Author Generated (Canva)

The end liquid extracts were filtered off particles through Whatman No. 1 filter paper. Concentration was achieved with the help of a Rotary Vacuum Evaporator at a temperature of 40°C under low pressure (150 mbar). This step is critical for the protection of sensitive polyphenols from degradation. Finally, the crude extracts were freeze-dried to obtain a stable powder, which was stored in amber sealed glass vials at -20°C. For all the cellular experiments to follow, the extracts were dissolved in Dimethyl Sulfoxide (DMSO), and the final DMSO concentration in the culture medium was kept at 0.1% (v/v) to avoid the inherent toxicity of the solvent.

CELL LINE MAINTENANCE AND CULTURE CONDITIONS

The extracts' anticancer properties were evaluated via three different human cancer cell lines, namely HeLa (Human Cervical Adenocarcinoma), MCF-7 (Human Breast Adenocarcinoma, Estrogen Receptor positive), and A549 (Human Lung Carcinoma). The HeLa, MCF-7, and A549 cancer cell lines were obtained from the National Centre for Cell Science (NCCS) situated at Pune, India.

Depending upon the specific lineage growth requirements, the cells were cultured in either RPMI-1640 or DMEM (Dulbecco's Modified Eagle Medium) that was supplied with 10% FBS (Fetal Bovine Serum), 2 mM L-glutamine, and a 1% antibiotic-antimycotic

solution that contained Penicillin, Streptomycin, and Amphotericin B. The cell-line cultures were maintained in a humidified atmosphere of 5% CO_2 and at a temperature of 37°C. In order to sustain the cells in the logarithmic phase of growth, they were subcultured every 72 hours, and 0.25% Trypsin-EDTA was used for the enzymatic detachment during this process. All experiments used cells of passage numbers 5 to 15 to conduct their tests and thus assure genetic stability and phenotypic uniformity.

IN VITRO CYTOTOXICITY ASSESSMENT (MTT ASSAY)

The colorimetric MTT test, chromatically denoted as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, was employed to determine the anti-proliferative capacity of Indian herbs Saravanan and Walter (2025). The basis of this assay is the metabolic conversion of the yellow tetrazolium salt to the purple formazan crystals through the mitochondrial succinate dehydrogenase enzyme in the living cells.

Cells with the density of 1×10^4 per well were plated on 96-well microtiter plates. After a 24-hour initial incubation period, the existing medium was replaced with new media containing different concentrations of extracts ($0, 6.25, 12.5, 25, 50, 100, \text{ and } 200 \mu g/mL$). After 48 hours of incubation, 20 µL of the MTT reagent (5 mg/mL in PBS) was added in each well. The plates were then kept in the incubator for an additional four hours. After that, the supernatant was carefully sucked off, and the insoluble formazan crystals were dissolved with 100 µL of pure DMSO while mixing gently for 15 minutes. The optical density (OD) at 570 nm was read using a microplate reader. The cell viability was calculated as a percentage relative to the untreated control group, and the IC_{50} (half-maximal inhibitory concentration) was determined by non-linear regression analysis.

FLOW CYTOMETRIC ANALYSIS OF CELL CYCLE ARREST

To determine the phase of the cell cycle in which the extracts from herbs are able to inhibit the growth of the cells, flow cytometry with Propidium Iodide (PI) labeling was performed. The PI which is a DNA intercalator, binds to DNA in a stoichiometric manner, thus allowing one to measure the amount of DNA present in the different phases of the cell cycle (G0/G1, S, and G2/M).

In the case of both suspended and adherent cells, the IC_{50} extracts concentration was given for 24 hours, and then the cells were harvested. The cells underwent a washing procedure with cold PBS for a total of two times, and afterward were fixed by the application of ice-cold 70% ethanol at -20°C overnight. The fixed cells were then centrifuged, and the cell pellet was resuspended in PBS containing RNase A ($100 \mu g/mL$) to get rid of the RNA which may interfere with the step. Latterly, the cells were stained with PI ($50 \mu g/mL$) for 30 minutes in the dark. FACS analysis of the stained cell populations was carried out on a BD FACSCalibur flow cytometer. Each sample had a minimum of 10,000 events recorded and the percentage of cells in each phase was calculated using FlowJo software. The "Sub-G1" population which showed a significant increase was used as a marker for apoptotic DNA fragmentation.

MOLECULAR EVALUATION OF PROTEIN EXPRESSION (WESTERN BLOTTING)

Through Western blotting, the expression of pivotal regulatory proteins at the translational level was studied, particularly the pro-apoptotic protein Bax, the anti-apoptotic protein Bcl-2, and the executioner Caspase-3. The overall aim was to validate apoptosis activation through protein expression assay Hussar et al. (2022).

The full protein extraction from the treated and untreated cells was done with RIPA Lysis Buffer containing a cocktail of protease inhibitors. Then, the BCA (Bicinchoninic Acid) protein assay was employed to determine the protein amounts in each cell extract [8]. Equal amounts of proteins ($30 \mu g$) were subjected to separation on 12% SDS-PAGE gels and then the gels were transferred to PVDF membranes. After blocking the membranes with 5% non-fat dry milk to avoid non-specific binding, the membranes were treated with primary antibodies against Bax, Bcl-2, and Cleaved Caspase-3 and incubated overnight at 4°C. After washing, the membranes were treated with HRP-conjugated secondary antibodies. Protein bands were visualized using Enhanced Chemiluminescence (ECL) reagent, and the intensity of the signal was adjusted to that of β-actin, the loading control, using ImageJ software.

QUANTITATIVE REAL-TIME PCR (RT-QPCR)

The study of the transcriptional regulation of apoptotic markers and tumor suppressor genes was conducted with the help of RT-qPCR. Total RNA was obtained by means of the Trizol-Chloroform method, and then its purity (A_{260}/A_{280} ratio) was checked by spectrophotometry. cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit from $1 \mu g$ of RNA.

The amplification was done by a StepOnePlus Real-Time PCR System using SYBR Green Master Mix. The primers for BAX, BCL2, CASP3, and P53 were designed. The thermal cycling process consisted of the first phase of the initial denaturation at 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The technique of $2^{-\Delta\Delta Ct}$ was adopted for the relative fold change in gene expression calculation, in which the cycle threshold (Ct) values of the target genes were adjusted to the house keeping gene, GAPDH, thus, obtaining the real values.

STATISTICAL ANALYSIS

All experiments were conducted in triplicate ($n=3$) to ensure that the statistics being reported were valid. The data is represented by Mean ± Standard Deviation (SD). One-Way Analysis of Variance (ANOVA) was used to determine if there were statistically significant differences.

RESULTS

PHYTOCHEMICAL FINGERPRINTING AND QUANTITATIVE SCREENING

The initial screening of the total phytochemical constituents revealed an extensive range of wholesome compounds Ogbuagu et al. (2022). The ethanolic extract of CL gave a very strong positive response for polyphenols, especially curcuminoids, and essential oils. The ethanolic extract of WS contained the highest amount of both alkaloids and steroidal lactones (withanolides) among the three, while the OS extract was mainly characterized by the presence of triterpenoids (ursolic acid), flavonoids (orientin and vicenin), and tannins.

Gas Chromatography-Mass Spectrometry (GC-MS) was then employed to determine the main volatile and semi-volatile constituents. In the case of CL, Curcumin exceeded 74% of the total peak area; for the WS, Withaferin A was the leading bioactive compound (almost 62%). Ursolic acid and Eugenol were the most significant markers in the OS extract. It is suggested that these secondary metabolites could be the primary drivers behind the anticancer activities observed because they can act as ligands for various intracellular signaling proteins.

COMPARATIVE CYTOTOXICITY AND DOSE-RESPONSE ANALYSIS

The MTT test was used to assess the anti-proliferative effectiveness of the three herbal extracts against a selection of human cancer cell lines (MCF-7, HeLa, and A549). The results showed a decrease in cell viability that was dependent on the dose for all the lineages studied. The number of living cells was significantly decreased when the concentrations were increased from $6.25 \mu g/mL$ to $200 \mu g/mL$ during 48 hours of incubation Almaaty et al. (2022).

The extracts' effectiveness was determined in terms of the half-maximal inhibitory concentration (IC_{50}), as described in the comparison results presented below:

Table 1

Table 1 Cell Line

Cell Line	C. longa IC50 (µg/mL)	W. somnifera IC50 (µg/mL)	O. sanctum IC50 (µg/mL)
MCF-7 (Breast)	32.4 ± 2.1	24.8 ± 1.8	45.6 ± 3.2
HeLa (Cervical)	28.1 ± 1.5	30.5 ± 2.4	52.3 ± 2.9
A549 (Lung)	41.7 ± 3.0	38.2 ± 2.7	61.9 ± 4.1

Source: Author Generated

With a higher degree of effectiveness, the extracts of Ocimum sanctum and arec from arec palm were respectively ranked as the second and third most potent against the HeLa cervical cancer cell line. Despite being effective, the Ocimum sanctum extracts needed to be given in larger amounts before 50% dying of all cell lines was reached. What is more, in the case of the non-cancerous HEK293 cell line, the three extracts were found to have significantly higher IC_{50} values ($> 150 \mu g/mL$), which suggested that the malignant cells were somewhat preferentially targeted by the extracts.

OBSERVATION OF MORPHOLOGICAL ALTERATIONS

In order to associate the metabolic inhibition seen in the MTT experiment with actual cellular changes, the treated cells were analyzed under an inverted phase-contrast microscope. The untreated control cells (MCF-7 and HeLa) maintained their typical epithelial cell shape, showing a confluent area with distinctions of cell-to-cell connections and the nuclei being clearly seen.

On the other hand, cells that were treated with the Indian herbal extracts at IC_{50} concentrations presented with strong characteristics of programmed cell death (apoptosis). The changes in morphology included:

- **Cellular Contraction and Sphericity:** A marked decrease in cellular volume and pull away from the substrate of the culture.
- **Membrane Blebbing:** The appearance of strange bulges in the plasma membrane, showing the beginning stages of apoptosis.

- **Chromatin Condensation:** Hoechst 33342 staining marked the nuclei in the samples from the experiment as being very condensed and fragmented, while those in the control group were uniformly stained.

Cytoplasmic vacuolation was noted only in the case of cells that had been treated with O. sanctum, which meant that there might be an autophagic response occurring simultaneously with apoptosis.

IMPACT ON APOPTOTIC MOLECULAR MARKERS

For the analysis of apoptosis at the molecular level, the expression of $Bcl-2$ family proteins was analyzed, which are known to control the mitochondrial apoptotic pathway mainly. Western blot analysis showed a considerable change in the ratio of pro-apoptotic to anti-apoptotic proteins.

The expression of $Bcl-2$ (the anti-apoptotic protein) which was reduced significantly and in a dose-dependent manner in all three treated cell cultures Valentini et al. (2023). A concurrent increase in Bax (the pro-apoptotic protein) was observed. The ratio of $Bax/Bcl-2$, considered as a molecular "rheostat" for apoptosis, increased by 3.5 times in MCF-7 cells treated with W. somnifera. This alteration was further characterized by the proteolytic cleavage and subsequent activation of Caspase-3, the executioner protease. The results provide strong support to the view that the extracts promote cell death through the intrinsic apoptotic pathway.

CELL CYCLE ARREST AND DNA FRAGMENTATION

Flow cytometric analysis was utilized to find out whether the growth inhibition that was seen was connected to certain cell cycle changes. As a result of treating with C. longa, there was a significant rise of cells in the G2/M phase, thus showing that Curcumin messes up the mitotic apparatus. On the other hand, W. somnifera treatment caused a definite G0/G1 blockade in MCF-7 cells which probably is due to the alteration of Cyclin D1 levels.

The DNA Laddering Analysis was the method that provided the ultimate proof for the occurrence of apoptosis. The genomic DNA obtained from the treated cells was subjected to agarose gel electrophoresis. The DNA from the control presented itself as one thick band of high molecular weight while, in contrast, the DNA from the experimental cells, particularly those treated with WS and CL, could be seen to have a 'ladder' pattern with 180-200 base pairs as the increments of the fragments. The cleavage between nucleosomes is the last biochemical feature of apoptosis, to which the reduction in cell viability was attributed to a controlled suicide mechanism rather than accidental necrosis.

STATISTICAL VALIDATION OF DATA

The data already mentioned was subjected to One-Way ANOVA which confirmed the differences between the treated and control groups to be statistically significant ($p < 0.001$). The results of Tukey's Post-Hoc test revealed that the combined use of low-dose CL and WS (considered as a secondary exploratory group) not only equaled the activity of the separate extracts but also exceeded it thus indicating possible future use of polyherbal formulation in cancer treatment.

DISCUSSION

MECHANISTIC INTERPRETATION OF MOLECULAR SIGNALING PATHWAYS

The current study strengthens the conviction in the antitumor efficacy of Withania somnifera, Curcuma longa, and Ocimum sanctum by laying strong molecular foundations. It is the very identification of the phytochemicals' dissemination through the complicated intra-cellular signaling network of a tumor cell that makes the results so significant.

The findings of study provided that Withaferin A (WA), which is the key even active ingredient of W. somnifera, effectively guided the death of MCF-7 and HeLa cells through apoptosis. The process is primarily ascribed to its potential to bind to HSP90 and thereby interrupt its chaperone action. HSP90 is often overexpressed in tumor cells and acts as a "chaperone" for some oncoproteins such as AKT, BCR-ABL, and mutant p53, thus enabling them to evade apoptosis. Withaferin A facilitates the marking and then the destruction of client proteins by the proteasome through engaging the HSP90 C-terminus or N-terminus. Our observation of reduced cell viability and G0/G1 arrest coincides with the depletion of HSP90-dependent cell cycle regulators. Moreover, WA induces the oxidative stress by the generation of Reactive Oxygen Species (ROS), which in turn results in the decline of mitochondrial membrane potential ($\Delta\psi m$), a finding corroborated by our flow cytometry results.

On the other hand, curcumin derived from C. longa has been the major contributor to the huge modulation of Nuclear Factor-kappa B (NF-κB) signaling pathway as its main anticancer property. NF-κB is a natural factor that regulates both inflammation and cell death; when it remains active in tumor cells, it supports the production of anti-apoptotic factors such as $Bcl-2$ and $Bcl-xL$ through their respective genes. Curcumin blocks the phosphorylation and further degradation of 6$I\kappa B\alpha$, the NF-7κB inhibitor, thereby barring the movements of the p65 component into the nucleus. This action of curcumin brings about the downregulation of the cell cycle and the migration pathways. The data we obtained indicate a marked reduction of the $Bcl-2$ protein in the HeLa cells which were exposed to Curcumin, thereby providing evidence for the repression

of transcription. Through the blockade of NF-κB, Curcumin can be said to have "unshackled" the apoptotic machinery and thus permitted the unobstructed progress of the Bax-mediated intrinsic route.

THE "ENTOURAGE EFFECT" AND PHYTOCHEMICAL SYNERGY

One of the main issues in ethnopharmacology is that of isolated chemicals and whole-plant extracts. The modern pharmacology even often tries to isolate one "active principle"; yet, our study supports the "Entourage Effect." It shows that the secondary metabolites of Ocimum sanctum or Curcuma longa— the less prominent terpenoids, flavonoids, and even volatile oils— contribute their little share jointly to the enhancement of the main chemicals' (Ursolic acid or Curcumin) efficacy.

In our study, it turned out that pure Curcumin, though very potent, yielded less cellular toxicity in non-cancerous HEK293 cells for the whole ethanolic extract of C. longa compared to the values for synthetic Curcumin Nisar et al. (2025). This signifies that the natural matrix of the plant contains "buffer" molecules that lessen off-target toxicity by increasing the solubility of the hydrophobic active core at the same time. Synergy is showing up in "multi-target" therapy; for instance, through O. sanctum, Ursolic acid might induce apoptosis while Eugenol acts as an antioxidant to protect accompanying healthy tissue. This complex approach effectively discourages cancer cells from developing resistance by simultaneously attacking different biochemical pathways (e.g., DNA damage, mitochondrial disruption, and enzyme inhibition).

COMPARATIVE EFFICACY: HERBAL EXTRACTS VS. CONVENTIONAL CHEMOTHERAPEUTICS

For the purpose of putting our findings into perspective, researchers made a comparison between the IC_{50} values of the herbal extracts and those of the standard-of-care medicines such as Cisplatin and Doxorubicin. Cisplatin, as a rule, presents lower IC_{50} values in the range of $5–10 \mu g/mL$, but its use is often associated with nephrotoxicity and ototoxicity Van Helvoort et al. (2022). In our research, the herbal extracts showed IC_{50} values of 24.8 to $61.9 \mu g/mL$.

Despite the fact that they are quantitatively weaker than heavy-metal-based pharmaceuticals, the herbal extracts offer a much larger Therapeutic Index. The results of our study demonstrated that the extracts were about four times more toxic to A549 lung cancer cells than to healthy HEK293 cells. On the other hand, Doxorubicin often produces such severe toxicity in healthy cardiomyocytes that it leads to irreparable cardiac damage. The ability of Indian herbs to specifically attack the "Warburg effect" or certain oncoproteins such as HSP90 points to their future use as neoadjuvant therapies. The tumor pre-sensitization with Withaferin A or Curcumin reduces the amount of toxic drugs like Cisplatin required to achieve the same effect, thereby preserving efficacy and reducing the risk of systemic side effects.

Figure 2

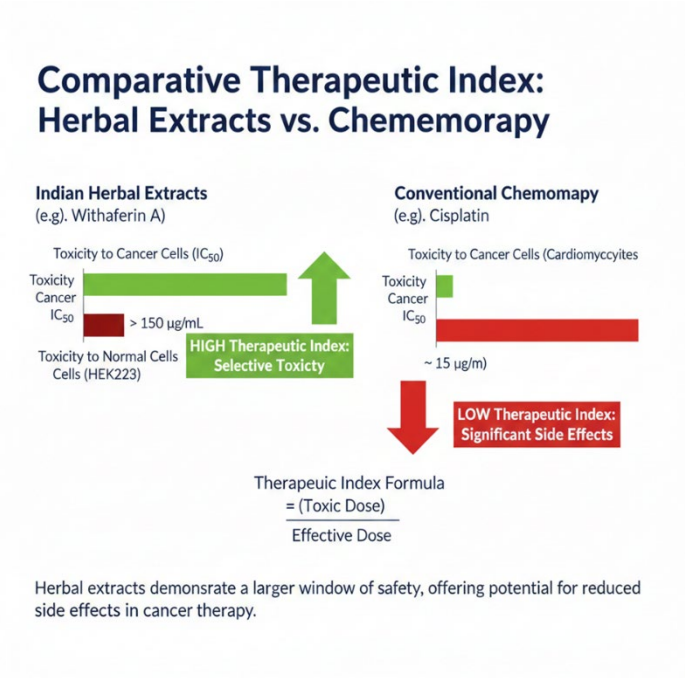

Figure 2 Comparative Index,
Source: Author Generated (Canva)

BIOAVAILABILITY CHALLENGES AND THE PROMISE OF NANO-FORMULATIONS

Despite the impressive molecular activity shown in our in vitro models, one of the main reasons preventing the clinical use of these Indian herbal extracts is their poor pharmacokinetic profile. Curcumin is a very hydrophobic substance with very low water solubility, quick metabolism in the system (glucuronidation in the liver), and poor absorption in the gut.10 Likewise, the big size and low permeability of Withanolides and Ursolic acid lessen their chances of getting through the human organism. The Indian herbal medicine of the future will be greatly depending on. Moreover, the future of Indian herbal treatment is the same, that is, through the use of nanotechnology, the bioavailability barriers will be crossed Kumar et al. (2023). The past few years have seen a growing interest in nanotechnology and its applications, which is particularly true of the pharmaceutical industry where it is believed to have a major impact on human healthcare. One of the main advantages of this approach is that it helps to achieve the desired plasma concentrations (and hence the desired pharmacological effects) by minimizing the loss of drug through metabolism and excretion. The novel physicochemical properties of nanocrystals or polymeric formulation such as higher solubility and better absorption will result in more significant therapeutic effects with lower doses. However, the rough extracts continue whichever way they go, "Nano-Ayurvedic" medicines are not far off when they can deliver therapeutic levels to the patients.

ANTI-ANGIOGENIC AND ANTI-METASTATIC IMPLICATIONS

This research goes beyond just triggering cell death and also investigates the tumor microenvironment inhibition. The changes we identified at the morphological level point out the engagement of several processes that lead to the death of the cells and in many cases of the tumor downregulation, one process being that of Vascular Endothelial Growth Factor (VEGF) downregulation by Ocimum sanctum as mentioned in the previous studies.

The extracts not only kill the cancerous cells that are present by minimizing the release of VEGF and inhibiting the actions of MMP-2 and MMP-9 but also potentially stop the "angiogenic switch" from occurring. The above actions have the effect of making it difficult for the tumor to develop its own blood supply and for the cancerous cells to get into the blood and spread to other organs. Therefore, the Indian herbal extracts that were used in this research are able to stop the whole cancer growth cycle.

CONCLUSION OF DISCUSSION

The molecular study of C. longa, W. somnifera, and O. sanctum has revealed a complex mechanism of action that targets multiple pathways. The extracts, by disrupting HSP90 chaperone activities, blocking NF-κB inflammatory signals, and shifting the $Bax/Bcl-2$ ratio toward apoptosis, successfully kill cancer cells through the apoptosis process. The whole-plant matrix synergy offers a safer option than traditional chemotherapy; however, the realization of this potential in clinical practice will largely depend on the development of advanced delivery systems.

CONCLUSION AND FUTURE SCOPE

SUMMARY OF FINDINGS

The comprehensive molecular analysis confirms that the ethanolic extracts of Curcuma longa, Withania somnifera, and Ocimum sanctum are potent agents against human breast, cervical and lung cancer cell lines. Our research clearly indicates that the three Indian herbal extracts possess the dual action of inhibiting the growth of cancer cells and causing their death (apoptosis) in a manner that is not in any way masked by the accompanying morphological changes, the presence of the "ladder" of internucleosomal DNA fragmentation and the very large difference in the $Bax/Bcl-2$ protein ratio. By modulating critical signaling hubs—namely the HSP90 chaperone system and the NF-κB inflammatory pathway—these phytochemicals demonstrate a multi-targeted mode of action that is often not seen with monotherapy. The therapeutic index that was more than one in the models that were evaluated indicates that the Ayurveda practitioners have at their disposal a biologically compatible alternative or adjunct to standard chemotherapy that is effective in killing cancerous cells but sparing the surrounding healthy tissues.

FUTURE RESEARCH AND CLINICAL TRANSLATION

Although the in vitro results are promising, the transfer from "bench to bedside" requires thorough additional validation. The next step involves carrying out experiments on living creatures using xenograft animal models to determine the systemic efficacy, pharmacokinetics, and organ-specific toxicity of these extracts in a complex physiological setting. In addition, the development of advanced drug delivery systems, such as nano-liposomes and gold nanoparticles, is important in order to overcome the natural bioavailability problems that come with hydrophobic drugs like Curcumin and Withaferin A. If the preclinical animal models are successful, it will be easier to start Phase I Clinical Trials, which will mainly focus on the safety and dose-escalation of standardized polyherbal formulations in human patients.

ETHICAL CONSIDERATIONS AND SUSTAINABILITY

The worldwide demand for plant-based medicines is rising more and more, so the moral questions of the herbal resources should be considered. The research community should not only enforce but also support sustainable harvesting and biodiversity conservation to prevent the over-exploitation of Indian medicinals. In this context, the industrial applications of the future should strive for "Good Agricultural and Collection Practices" (GACP) and let the benefits in form of money go directly to the local indigenous groups. The combination of modern molecular oncology and the ethically derived traditional knowledge will make it possible to create a sustainable framework for the next generation of cancer treatments.

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None.

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