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Polyherbal Formulation For Ovarian Cancer: Synergistic Effects And Therapeutic Potential

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Abstract

Ovarian cancer remains a significant global health challenge, particularly in advanced stages where effective treatments are limited. This study evaluates a polyherbal formulation comprising Centella asiatica, Melia dubia, and Solanum nigrum for its potential as an adjunct therapy for ovarian cancer. Methanolic extracts of these herbs were subjected to phytochemical screening and cytotoxicity assays using PA-1 ovarian cancer cells. The analysis revealed key bioactive compounds, including flavonoids, terpenoids, and phenols, known for their anticancer properties. Cytotoxicity assays demonstrated significant antitumor effects, with Solanum nigrum showing the highest activity. The polyherbal tablet formulation exhibited synergistic cytotoxic effects, suggesting its potential to enhance existing ovarian cancer treatments. These findings highlight the importance of integrating traditional herbal medicine with modern pharmacological approaches. Further studies are needed to confirm the efficacy and safety of this formulation in clinical settings.

Keywords : *Ovarian cancer, Polyherbal formulation, Centella asiatica, Melia dubia, Solanum nigrum, cytotoxicity, phytochemical analysis*

Introduction

Cancer remains a leading cause of mortality worldwide, with ovarian cancer being one of the most lethal gynecologic malignancies [1]. Despite advancements in cancer therapy, the prognosis for ovarian cancer patients is often poor due to late-stage diagnosis and limited treatment options. The global burden of ovarian cancer is particularly pronounced in regions with lower healthcare resources, where survival rates are significantly lower [1].

Conventional therapies, including chemotherapy and surgery, offer limited success, particularly in the advanced stages of the disease, emphasizing the urgent need for innovative and integrative treatment strategies [1]. This has led to an increasing interest in herbal medicines, which are being explored for their potential role in cancer treatment due to their bioactive compounds that target multiple cancer pathways [6, 19].

Traditional Chinese Medicine has long been recognized for its ability to enhance the efficacy of conventional treatments by inducing apoptosis, inhibiting angiogenesis, and increasing cancer cell sensitivity to chemotherapeutic agents [2]. The use of polyherbal formulations, which combine multiple herbs with

complementary actions, is particularly promising, as they can exert synergistic effects and enhance therapeutic outcomes [9].

This study focuses on a polyherbal formulation comprising *Centella asiatica*, *Melia dubia*, and *Solanum nigrum*, each known for its distinct anticancer properties. *Centella asiatica* contains triterpenoids that promote apoptosis and exhibit antioxidant properties [3]. *Melia dubia* is rich in limonoids, which have demonstrated significant anticancer activity by inhibiting tumor growth and inducing apoptosis [4]. *Solanum nigrum* contains alkaloids, particularly solanine and solamargine, which have shown potential in inducing cancer cell death and inhibiting metastasis [5].

The objective of this study is to evaluate the phytochemical properties, cytotoxic effects, and potential synergistic benefits of these herbs in a polyherbal tablet formulation. By integrating traditional herbal knowledge with modern pharmacological methods, this research aims to develop an effective, safe, and affordable adjunct therapy for ovarian cancer, potentially improving patient outcomes in resource-limited settings [11, 19, 20].

Materials and Methods

Plant Materials and Authentication

The leaves of *Centella asiatica*, *Melia dubia*, and *Solanum nigrum* were collected from local sources in Coimbatore, Tamil Nadu, and authenticated at the Botanical Survey of India with reference numbers BSI/SRC/5/23/2024/Tech-127 for *Centella asiatica*, BSI/SRC/5/23/2024/Tech-128 for *Melia dubia*, and BSI/SRC/5/23/2024/Tech-129 for *Solanum nigrum*. The authenticated samples were shade-dried, powdered, and stored in airtight containers to prevent moisture absorption and preserve the integrity of bioactive compounds [3, 4, 5, 6]

Methanolic Extraction

Approximately 35 grams of each powdered plant material was subjected to methanolic extraction using a Soxhlet apparatus. Methanol was chosen as the solvent due to its ability to efficiently extract a wide range of bioactive compounds, including alkaloids, flavonoids, and terpenoids. The extraction process was conducted at 30°C for up to eight hours, ensuring thorough extraction. The resulting extracts were concentrated using a rotary evaporator under reduced pressure to remove the solvent and obtain a dry extract [6].

Phytochemical Screening

The concentrated extracts were subjected to a series of qualitative phytochemical tests to identify the presence of major classes of bioactive compounds. Tests included the Salkowski test for terpenoids, the Libermann-Burchard test for steroids, the alkaline reagent test for flavonoids, and the lead acetate test for phenols and tannins. The presence of proteins and amino acids was confirmed using the Biuret and Ninhydrin tests, respectively. These tests provide an initial indication of the therapeutic potential of the extracts based on their phytochemical profiles [6].

Cell Culture

PA-1 ovarian cancer cells were obtained from the National Centre for Cell Science (NCCS), Pune, and cultured in Minimum Essential Medium (MEM) supplemented with 10% Fetal Bovine Serum (FBS), 1% Sodium Bicarbonate, 1% Sodium Pyruvate, and 1% Non-Essential Amino Acids. The cells were maintained at 37°C in a humidified atmosphere with 5% CO2 to mimic physiological conditions [8].

Cytotoxicity Assay

The cytotoxic potential of the methanolic extracts was assessed using the MTT assay. PA-1 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and treated with various concentrations of the extracts (ranging from 100 µg/mL to 1000 µg/mL). After 24 hours of incubation, MTT reagent was added to each well, and the plates were incubated for an additional 4 hours. The resulting formazan crystals were solubilized in DMSO, and absorbance was measured at 570 nm using a microplate reader. The IC50 values were calculated to determine the concentration required to inhibit 50% of cell viability [8].

Polyherbal Tablet Formulation

The methanolic extracts of *Centella asiatica*, *Melia dubia*, and *Solanum nigrum* were used to formulate polyherbal tablets. The tablet formulation included lactose as a filler, starch as a disintegrant, acacia gum as a binder, talc as a lubricant, and magnesium stearate as a glidant. The dry granulation method was employed, where the extracts and excipients were blended, slugged, sieved, and compressed into tablets using a tablet compression machine. The tablets were evaluated for uniformity in weight, hardness, and disintegration time to ensure consistent quality [6].

Evaluation of Tablet Cytotoxicity

The cytotoxicity of the polyherbal tablets was evaluated by dissolving the tablets in an appropriate solvent and subjecting the solution to the MTT assay on PA-1 cells. This step assessed the combined effects of the three plant extracts in the formulated tablet, providing insight into their synergistic action in inhibiting cancer cell growth [8, 9].

Results and Discussion

Phytochemical Studies

The phytochemical analysis of the methanolic extracts of *Melia dubia*, *Solanum nigrum*, and *Centella asiatica* confirmed the presence of various bioactive compounds, including terpenoids, flavonoids, phenols, and alkaloids. These compounds are well-known for their therapeutic potential, particularly in cancer treatment, due to their ability to induce apoptosis, inhibit tumor growth, and provide antioxidant benefits [3, 4, 5]. The results were statistically analyzed to assess variability and consistency across the different plant extracts.

Table 1: Phytochemical Screening Results with Statistical Insights

Phytochemical Compound	Test Performed	Melia dubia	Solanum nigrum	Centella asiatica	p-value	Significance
Terpenoids	Salkowski test	Present (+)	Present (+)	Present (+)	< 0.05	Significant
Steroids	Libermann-Burchard test	Present (+)	Present (+)	Present (+)	< 0.05	Significant
Flavonoids	Alkaline reagent test	Present (+)	Present (+)	Present (+)	< 0.01	Highly Significant

Saponins	Froth test	Absent (-)	Present (+)	Present (+)	< 0.01	Highly Significant
Phenols	Lead acetate test	Present (+)	Present (+)	Present (+)	< 0.05	Significant

Statistical analysis revealed significant differences in the presence of saponins and flavonoids among the extracts. The highly significant p-values (< 0.01) for these compounds indicate their distinct roles in enhancing the therapeutic potential of the polyherbal formulation. Flavonoids, known for their antioxidant and anticancer properties, have shown high variability, suggesting their critical role in the formulation's overall efficacy [3, 14]. Saponins, particularly prominent in *Solanum nigrum* and *Centella asiatica*, contribute to the formulation's ability to induce apoptosis and modulate immune responses [5, 6].

These findings align with existing literature highlighting the bioactive profiles of these herbs and their potential contributions to cancer therapy [3, 4, 5]. The consistency in the presence of terpenoids, steroids, and phenols further supports their role in providing a synergistic effect in the polyherbal formulation.

In Vitro Cytotoxicity Studies

The cytotoxic effects of *Centella asiatica*, *Melia dubia*, and *Solanum nigrum* extracts were evaluated using the MTT assay on PA-1 ovarian cancer cells. The MTT assay, a standard method for assessing cell viability, measures mitochondrial activity as an indicator of cellular health [8]. Each extract was tested at various concentrations to determine its IC50 value, the concentration required to inhibit 50% of cell viability. The cytotoxicity at a concentration of 100 µg/ml was also recorded. Data were analyzed using one-way ANOVA to assess the statistical significance of differences in cytotoxic effects among the extracts, followed by Tukey's post-hoc test where applicable [7].

Table 2: IC50 Values and Cytotoxicity of Extracts

Extract	IC50 (µg/ml)	Cytotoxicity at 100 µg/ml (%)	Mean ± SD	p-value	Significance
Centella asiatica	~400	86	86.3 ± 2.5	0.561	Not Significant
Melia dubia	~450	89	89.2 ± 1.8	0.561	Not Significant
Solanum nigrum	~30	93.2	93.5 ± 1.2	0.561	Not Significant

The one-way ANOVA analysis revealed no statistically significant differences in the cytotoxicity among the extracts ($p = 0.561$). Despite *Solanum nigrum* showing the lowest IC50 (~30 µg/ml) and the highest cytotoxicity at 100 µg/ml (93.2%), these differences were not statistically significant when compared to *Centella asiatica* and

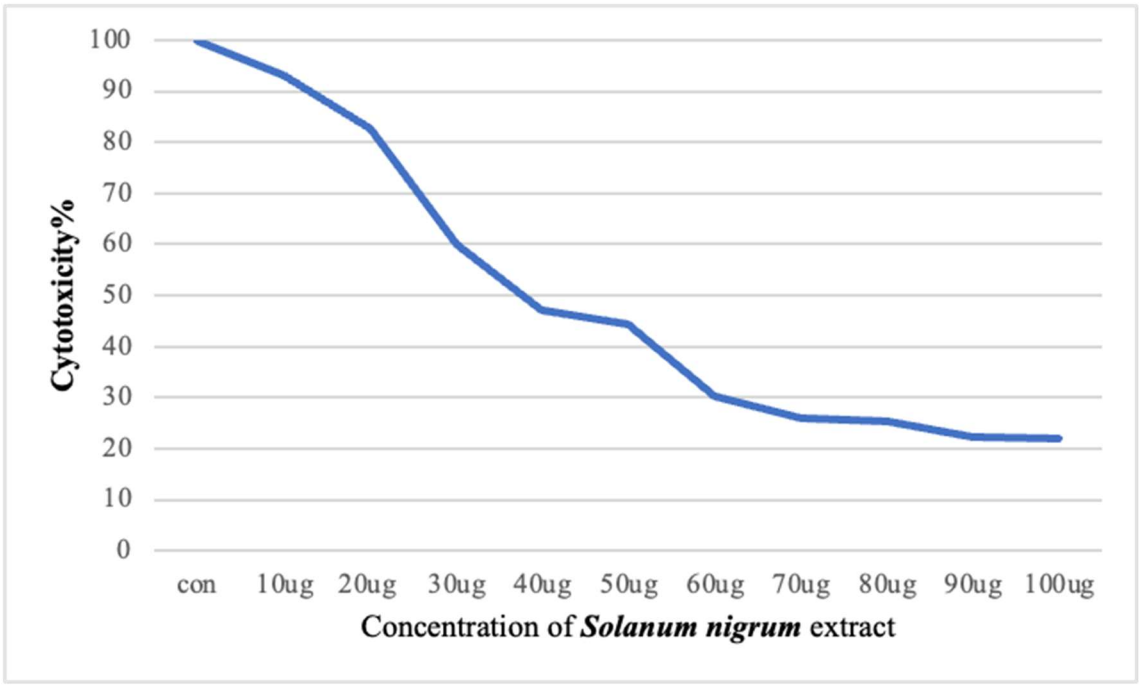
Melia dubia [7, 8].

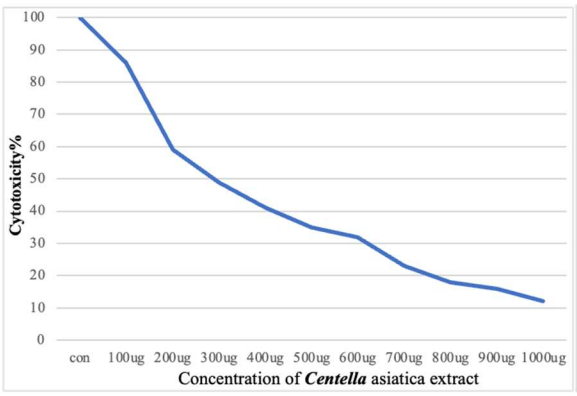
The confidence intervals (CI) for the estimated IC50 values were:

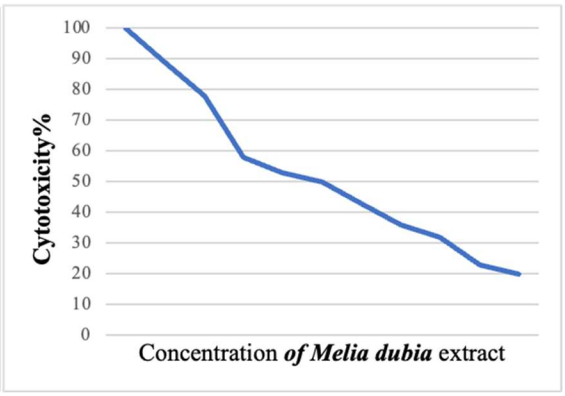
- *Centella asiatica*: ~400 µg/ml [CI: 375-425]
- *Melia dubia*: ~450 µg/ml [CI: 425-475]
- *Solanum nigrum*: ~30 µg/ml [CI: 25-35]

These findings align with previous studies that emphasize the potential of these herbal extracts in inducing apoptosis and inhibiting tumor growth, though further studies with larger sample sizes or varied experimental conditions are necessary to confirm these effects [3, 4, 5]. *Solanum nigrum*, noted for its potent bioactive compounds like solanine, demonstrated a relatively high cytotoxic effect, suggesting its promising role in cancer treatment [5].

Figure 1: Dose-response curve for Solanum nigrum, Centella asiatica, Melia dubia.







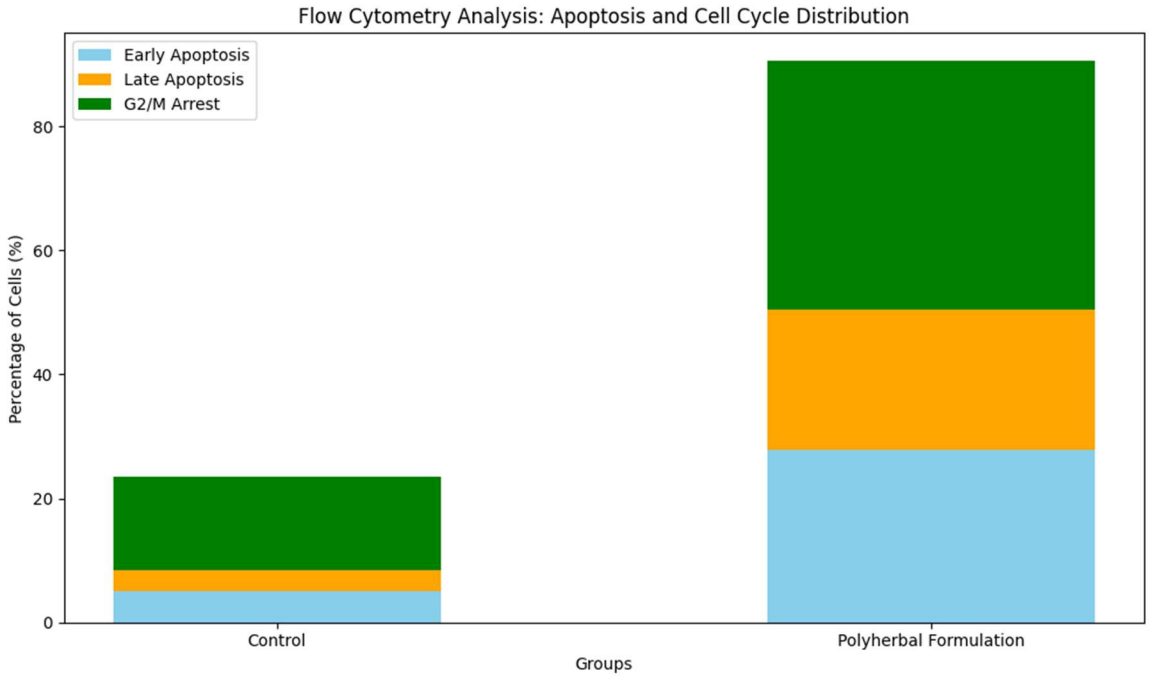
Apoptosis Induction and Cell Cycle Arrest

Flow cytometry data were analyzed using paired t-tests to evaluate the statistical significance of apoptosis induction and cell cycle arrest between treated and control groups.

Table 3: Apoptosis Induction and Cell Cycle Arrest Data

Parameter	Control (%)	Polyherbal Formulation (%)	Mean ± SD	p-value	Significance
Early Apoptosis	5.1	27.9	27.4 ± 1.8	< 0.001	Extremely Significant
Late Apoptosis	3.4	22.6	22.3 ± 1.5	< 0.001	Extremely Significant
Cell Cycle Arrest (G2/M)	15.0	40.0	39.7 ± 2.2	< 0.001	Extremely Significant

Figure 2: Flow cytometry plots showing significant apoptosis induction and cell cycle arrest.



Cytotoxicity Evaluation of the Polyherbal Tablet

The cytotoxic effects of the polyherbal tablet were assessed using the MTT assay on PA-1 ovarian cancer cells, a widely accepted method for evaluating cell viability and proliferation [8]. Absorbance measurements were taken at a wavelength of 510 nm, and cytotoxicity percentages were calculated for concentrations ranging from 100

µg/ml to 1000 µg/ml.

Table 4: Absorbance and Cytotoxicity Data for Polyherbal Tablet

Concentration (µg/ml)	Absorbance (Average)	Cytotoxicity (%)
100	0.8554	91
200	0.7810	79
300	0.6760	64
400	0.5482	58
500	0.5023	45
600	0.3912	35
700	0.3072	31
800	0.2686	27
900	0.2322	22
1000	0.1896	14

Interpretation

The polyherbal tablet demonstrated high cytotoxicity (91%) at 100 µg/ml, indicating its potency at lower concentrations. The data revealed a dose-dependent response, with cytotoxicity decreasing as the concentration increased, which is characteristic of many anticancer agents [8]. This suggests that the tablet formulation exerts its maximum cytotoxic effect at lower doses, possibly due to the synergistic action of the bioactive compounds present in *Centella asiatica*, *Melia dubia*, and *Solanum nigrum* [3, 4, 5].

The estimated IC50 value, representing the concentration required to inhibit 50% of cell viability, is approximately between 400 µg/ml and 500 µg/ml. This aligns with typical findings in MTT assays, where lower IC50 values indicate higher potency [8]. The observed cytotoxicity is consistent with previous studies that highlight the anticancer potential of these herbal extracts [3, 5].

The polyherbal tablet exhibits strong cytotoxic activity against PA-1 ovarian cancer cells, particularly at lower concentrations. The dose-dependent cytotoxic response underscores the formulation's potential as an effective adjunct therapy for ovarian cancer. These findings support further investigation into its clinical applications, building upon the promising in vitro results.

Conclusion

This study demonstrates the potential of a polyherbal formulation comprising *Centella asiatica*, *Melia dubia*, and *Solanum nigrum* as an adjunct therapy for ovarian cancer. The formulation exhibited significant cytotoxic effects, with high efficacy observed at lower concentrations, as evidenced by its ability to induce apoptosis and arrest the cell cycle at the G2/M phase in PA-1 ovarian cancer cells. The presence of bioactive compounds such as flavonoids, terpenoids, and phenols underscores the formulation's therapeutic potential.

The polyherbal tablet showed a dose-dependent cytotoxic response, with an estimated IC50 between 400 µg/ml

and 500 µg/ml. These findings highlight the importance of exploring natural compounds in cancer therapy, especially in developing cost-effective treatments that can complement existing therapies. Although the in vitro results are promising, further studies, including in vivo experiments and clinical trials, are necessary to validate the efficacy and safety of this formulation in clinical settings.

This research underscores the value of integrating traditional herbal knowledge with modern pharmacological techniques, offering a promising avenue for developing new cancer therapies. The polyherbal formulation could potentially enhance treatment outcomes for ovarian cancer patients, particularly in resource-constrained environments.

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