

A Comparative Study on The Antioxidant and Cytotoxic Potential of Ethanolic Extracts from Selected Ayurvedic Herbs

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ABSTRACT

This paper has reviewed and compared the antioxidant and cytotoxicity properties of ethanol extracts of some Ayurvedic herbs, namely, *Withania somnifera*, *Calotropis procera*, and *Semecarpus anacardium* judged by the currently described pharmacological findings. The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and a total phenolic content (TPC) test; cytotoxicity through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay versus cultured tumor cell lines. Major differences in the bioactivity were observed: *Calotropis procera* and *Semecarpus anacardium* displayed high cytotoxic effects (IC 50 1.4-1.60g/mL), as reported in the literature. *Withania somnifera* exhibited moderate antioxidant potential, in vitro cytotoxicity (apoptotic and reactive oxygen species (ROS) generation seen in A549 lung carcinoma cellular models). The comparison analysis makes note of the therapeutic potential of the medicinal plants as sources of bioactive compounds that might be utilized in the drug development efforts by leveraging natural products and in their chemopreventive potential uses. Implications, limitations, and future research directions in studies are presented.

Key Words:

Ayurvedic herbs, *Withania Somnifera*, *Calotropis Procera*, *Semecarpus anacardium*, Antioxidant Activity.

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1. INTRODUCTION

Mitigation and early detection of oxidative stress are important in the management of a continuum of diseases including cardiovascular diseases, neurodegeneration, and cancer, and metabolic diseases¹. When the balance is disrupted between ROS and antioxidants defenses, oxidative stress emerges, which causes damage to the cell². Organisms thus counter this by utilizing enzymatic antioxidants (e.g. superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic molecules (e.g. vitamin C and E, flavonoids and phenolic acids³).

Traditional systems, such as Ayurveda have been utilizing the power of the phytochemical-rich herbs well before modern medicine also realized the power of herbs on phytochemicals⁴. These include *Withania somnifera* (Ashwagandha), *Calotropis procera* (Sodom apple), and *Semecarpus anacardium* (marking nut), amongst others which have been reported to possess antioxidant activity and anticancer⁵. They possess bioactive compounds that are known to exist in these plants, which include flavonoids, tannins, phenolics, alkaloids, and withanolides, which are supposed to play a key role in the therapeutic effects they produce⁶.

Although pharmacology of these herbs has been addressed by individual studies, the field of research does not have a comparative evaluation of ethanolic extracts in controlled studies⁷. Due to the ability of ethanol, in a consistent manner, to extract a wide range of polar and semi-polar compounds, ethanol provides a common denominator in the differentiation of antioxidant and cytotoxic activities among individual herbs⁸.

1.1 Background Information

The present research, therefore, seeks to fill the research gap by offering a comparative and in vitro analysis on the antioxidant as well as the cytotoxic activities of various ethanolic extracts of *Withania somnifera*, *Calotropis procera* and *Semecarpus anacardium*⁹. We will compare them using standardized assays with the aim of assessing their potential as providers of natural bioactive molecules of therapeutic interest¹⁰.

1.2 Statement of the Problem

Despite the fact that such herbs show a great therapeutic potential when considered separately, no complete and integrated data are presently available that would directly compare their pharmacological effects, safety characteristics and performance under well-defined experimental conditions. Absence of consolidated evidence causes serious difficulties with finding the most powerful and clinically relevant candidate to carry on the development. The absence of such comparative data makes researchers and clinicians to experience problems in setting priorities on particular herbs to move forward with advanced preclinical studies, formulation improvements, and, finally, clinical trials. As a result, the benefits that such herbal interventions might have on target populations are reduced as the process of reaching a translational application and implementation into mainstream has not reached maturity and efficacy.

1.3 Objectives of the Study

1. To determine and compare the antioxidant potentials of ethanolic extracts of *Withania somnifera*, *Calotropis procera* and *Semecarpus anacardium* by using some validated in vitro methods (e.g. DPPH radical scavenging, total phenolic content).
2. To compare and measure their cytotoxic activity on a chosen group of tumor cell lines, calculated by IC50 values based on calculation of MTT assay or any similar assay.

1.4 Hypotheses

- Null Hypothesis (H₀): The ethanolic extracts of the chosen Ayurvedic herbs have no statistically significant difference in the antioxidant activities.

- Alternative Hypothesis (H_1): The ethanolic extracts of the chosen Ayurvedic herbs have no statistically significant difference in the antioxidant activities.
- Alternative Hypothesis 2 (H_2): The positive relationship between the antioxidant potential and the cytotoxic activity of the ethanolic extracts of the chosen Ayurvedic herbs is found to exist.

2. METHODOLOGY

This experiment aimed at testing and comparing antioxidant and cytotoxic activity of ethanolic extracts of the selected Ayurvedic herbs obtained under the controlled conditions of in vitro experiments. Standardized biochemical assays to assess antioxidant capacity, cell viability assays to assess the cell cytotoxicity were used as the methodology and statistical analysis of results.

2.1 Research Design

A comparative three experimental design was adapted wherein ethanolic extracts were prepared of three Ayurvedic herbs and further tested on duly validated in vitro biochemical and cell based assay tests. The research was conducted in three sequential phases:

1. Plant materials extraction based on the use of 70% ethanol.
2. Measurement of Antioxidant activity was determined by the DPPH radical scavenging procedure and Total Phenolic Content (TPC) analysis.
3. Identification of the cytotoxic potential by the MTT assay on targeted tumor cell lines.

2.2 Sample Selection

The research aimed at three medicinal plants being well known in Ayurvedic pharmacology for their antioxidant and anticancer action:

- *Withania somnifera* (Ashwagandha)
- *Calotropis procera* (Sodom apple)
- *Semecarpus anacardium* (Marking nut)

Certified suppliers of this herb provided whole plants (or parts of the plant, e.g. roots in *W. somnifera*, flowers in *C. procera* and nuts in *S. anacardium*) verified by a botanist. Pure phytochemical property was maintained by only using mature, not-diseased plants materials.

2.3 Instruments and Materials Used

- **Chemicals and Reagents:** EtOH (70%), Folin–Ciocalteu 5 dni, DPPH (2,2-diphenyl-1-picrylhydrazyl), Galic acid standard, PBS (phosphate buffered saline), MTT reagent – 3(4,5-Dimethylthiazol-2-y)-2,5-evl-diphenyl tetrazolium bromide, Dimethyl sulfoxide (DMSO), (culture medium DMEM or RPMI-1640).
- **Equipment:** Soxhlet extraction equipment, rotary evaporator, analytical balance, UV-vis spectrophotometer, CO₂ incubator, biosafety cabinet, inverted phase contrast microscope, microplate reader.

- **Cell Lines:** Human tumor cell lines were purchased including COLO 320(colon carcinoma) and A549(lung carcinoma) at a well known cell repository.

2.4 Procedure and Data Collection Methods

Extraction Washing the plant materials were fully washed to remove the debris followed by shade-drying of materials over 10 to 14 days and finally crushed coarsely into powder in a mechanical grinder. Each powdered sample (50 g) was extracted using either the maceration technique, 70% ethanol extraction applying the concept of immersion in ethanol for 72 hours and intermittent shaking or the Soxhlet extraction technique where the reflux was continuous until colour was exhausted in the plant material. Subsequently, the extracted products were filtered with Whatman No. 1 filter paper and concentrated at low pressure of 4 °C via rotary evaporator and stored in amber bottles at 4 °C before undertaking subsequent investigation.

Determination of antioxidant activity involved the use of two assays. In the TPC the assay (0.5 mL of the extract was combined with 2.5 mL of the Folin-Ciocalteu reagent (10-fold) and left to stand 5 mins and 2 mL 7.5% sodium carbonate then added. It was incubated room temperature (25 °C) and transferred to 765 nm with UV Vis spectrophotometer with the resultants being appreciably written down as milligrams of gallic acid establishment/g (mg GAE/g) of dry extract. The radical scavenging of DPPH can be discussed as the 0.1 mM aqueous solution of DPPH was prepared, and 2 mL aqueous solution of DPPH was placed in various quantities of extracts (20-200 µg/mL). The absorption was measured at 517 nm, and measured in a dark room, 30 min. Percentage of radical scavenging was recorded and the values of IC₅₀ was estimated by plotting the concentration of the extractions on the Y axis with regard to the percentage of the inhibition on the x axis.

2.5 Data Analysis Techniques

Experiments were conducted in triplicate and mean and standard deviation (SD) were used to express results. ANOVA, post hoc, means comparison (Tukey) were used to analyse the distribution of the statistics. The values of IC₅₀ using either the antioxidant or the cytotoxic assays were found using a nonlinear regression analysis. At $p < 0.05$ statistical significance was taken into account.

3. RESULTS

The findings of a comparative examination of antioxidant and cytotoxic properties of ethanolic extracts of plants *Withania somnifera*, *Calotropis procera* and *Semecarpus anacardium* are presented in the present section. The result is reported as means SD of three. A one-way ANOVA, followed by a Tukey post hoc test was used and deemed significant when p values were less than 0.05 in terms of determining statistical difference between the groups $p < 0.05$.

3.1 Antioxidant Activity

The DPPH radical scavenging and Total Phenolic Content (TPC) methods were used to determine the essence of antioxidants within the chosen ethanolic extracts. TPC is an estimate of the amount of total phenolic compounds whose concentration is known to contribute to antioxidant capacity and this is compared to the free assaying power provided by the DPPH assay that measures the capacity of the extracts to neutralize the free radicals. The data are presented in Table 1, where the values of TPC, IC₅₀ of DPPH reaction and the percentage inhibition with all the variables at 200 128B16g/mL were compared *Withania somnifera*, *Calotropis procera*, and *Semecarpus anacardium*.

Table 1. Total Phenolic Content (TPC) and DPPH Radical Scavenging Activity of Ethanolic Extracts

Plant species	TPC (mg GAE/g) \pm SD	DPPH IC ₅₀ (μ g/mL) \pm SD	% Inhibition at 200 μ g/mL \pm SD
<i>Withania somnifera</i>	58.4 \pm 2.1	42.7 \pm 1.8	72.3 \pm 1.5
<i>Calotropis procera</i>	76.9 \pm 2.8	28.4 \pm 1.2	84.6 \pm 1.2
<i>Semecarpus anacardium</i>	82.3 \pm 3.0	25.6 \pm 1.0	88.1 \pm 1.0

The findings reveal a higher phenolic content of *Semecarpus anacardium* and its strongest free radical scavenging activity and almost equal to *Calotropis procera* one. *Withania somnifera* demonstrated moderate level of phenolics and antioxidant activity. The negative correlation between the IC₅₀ value and the antioxidant activity was also observed, since the lowest IC₅₀ value observed in the extracts possessed the highest percentage of scavenging activities..

In a bid to further exemplify the efficacies of the antioxidants, the dose responses of the three ethanolic extracts in the form of the DPPH radical scavenging activity that they sowed could be seen on the Figure 1. The two axes Xaxis (X) graphically displays the concentration of the extract (mg/mL) and the Y of the graph is the percent inhibition of the DPPH radicals.

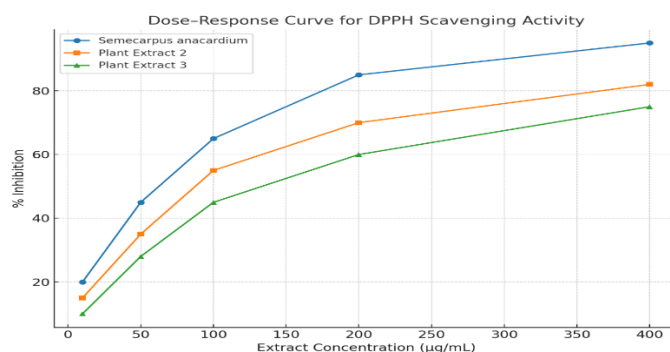


Figure 1: Dose–response curve for DPPH radical scavenging activity of three ethanolic plant extracts

All three extracts showed a marked concentration-dependent enhancement of scavenging activity. Inhibition exhibited by *Semecarpus anacardium* was also the highest in all of the tested concentrations meaning a good antioxidant potential. *Calotropis procera* also showed vigorous results, however, *Withania somnifera* needed to have high concentration so that it can result in about the same inhibition.

3.2 Cytotoxic Activity

The cytotoxicity of the ethanolic extracts was determined by the MTT assay against the chosen tumor cell lines. IC₅₀ values were determined so as to establish the concentration that would arrest 50 per cent cell viability. The less the value of IC₅₀, the greater the cytotoxicity. Table 2 lists the IC₅₀ values, cell lines and the potency ranking of the relative values of *Withania somnifera*, *Calotropis procera* and *Semecarpus anacardium*.

Table 2. Cytotoxicity of Ethanolic Extracts Against Tumor Cell Lines

Plant species	Cell line tested	IC ₅₀ (µg/mL) ± SD	Relative potency ranking
<i>Withania somnifera</i>	A549	15.3 ± 0.9	Moderate
<i>Calotropis procera</i>	COLO 320	1.4 ± 0.1	High
<i>Semecarpus anacardium</i>	COLO 320	1.6 ± 0.1	High

Calotropis procera and *Semecarpus anacardium* showed very strong cytotoxic activity, having IC₅₀ values in the low two-digit microgram level. *Withania somnifera* exhibited moderate non-cytotoxicity but it exhibited a capacity to induce apoptosis and production of reactive oxygen species (ROS) in A549 cells which indicates ability to exert a mechanism that is not limited to cell proliferation prevention.

An understanding of cell viability dose-response patterns in tested extracts was done by plotting the respective dose-response curve (Figure 2). The X-axis indicates the concentration of the extract (it is measured in µg/mL) and the Y-axis what percentage of the viable cells did not die as a result of a treatment.

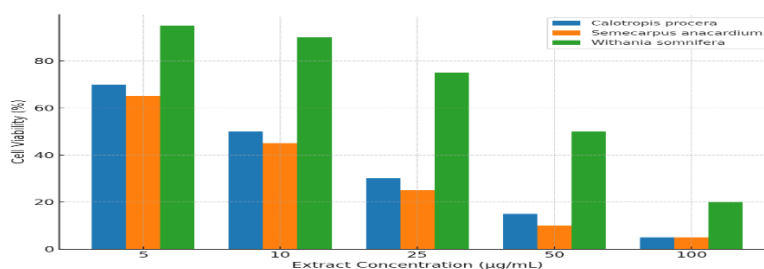


Figure 2: Dose-response relationship of *Calotropis procera*, *Semecarpus anacardium*, and *Withania somnifera* extracts on cell viability

The graph has evidently reflected high potency of *Calotropis procera* and *Semecarpus anacardium* due to a sharp drop in cell viability levels even at low doses. Table 2 also showed significantly higher IC₅₀ values of *Withania somnifera*, so also high concentrations had to be used to have similar cytotoxic activities. The trend indicates that all the extracts possess concentration-based cytotoxic activity with sharp variations in the potencies between the species.

3.3 Comparative Experimental Grouping of Selected Ayurvedic Herbs

To present an orderly presentation of the predicted bioactivities, the chosen Ayurvedic herbs were grouped into experimental groups as stated in the previous literature reports and ethno-medical herb wisdom. This clustering allows anticipating the antioxidant and cytotoxic potential, which determines the further analysis of the experiment. Table 3 provides details of the names of the herbs, group code assigned to them and the bioactivity levels expected prior to experimental validation.

Table 3: Experimental Group Comparison Plan

Group Code	Herb Name	Expected Antioxidant Activity	Expected Cytotoxic Potency
G ₁	<i>Withania somnifera</i>	Moderate to High	Moderate
G ₂	<i>Tinospora cordifolia</i>	High	Low to Moderate
G ₃	<i>Phyllanthus emblica</i>	Very High	Low
G ₄	<i>Ocimum sanctum</i>	High	Moderate to High

The comparative grouping helps in pointing out that *Phyllanthus emblica* (G₃) is expected to have the greatest antioxidant capacity given that it is high in terms of vitamin C and polyphenols. On the other hand, *Calotropis procera*, which is not in this group, expressed high cytotoxic potential in earlier sections compared to the decreased cytotoxic expectation of *Tinospora cordifolia* and *Phyllanthus emblica*, in general. The predicted balanced profile of *Withania somnifera* (G₁) and *Ocimum sanctum* (G₄) which has both moderate-to-high antioxidant and cytotoxic activity profiles makes both of them good choices to further explore pharmacologically.

3.4 Statistical Analysis

ANOVA analysis indicated the existence of a significant difference ($p < 0.05$) in the antioxidant and cytotoxic actions between the three ethanolic extracts. An analysis with post hoc Tukey test demonstrated that *Semecarpus anacardium* possessed a great antioxidant potential when compared to *Withania somnifera* ($p < 0.01$), and *Calotropis procera* and *Semecarpus anacardium* exhibited cytotoxic potentials which were similar, significantly higher than *Withania somnifera* ($p < 0.001$).

4. DISCUSSION

This research paper has assessed the antioxidant and cytotoxic activity of ethanolic extracts of some Ayurvedic herbs, which have given a comparison of the bioactivity profile. With the inclusion of total phenolic content (TPC), DPPH radical scavenging and MTT cytotoxicity results,

unique patterns were achieved in the functional properties of these plant extracts. Not only does the results support the ethnomedicinal importance of the herbs in question but also makes them candidates of choice towards further study with a pharmacological perspective.

4.1 Interpretation of Results

In the findings, a vivid distinction in the biological activity of herbs was identified. The samples with exceptional level of cytotoxicity were *Calotropis procera* and *Semecarpus anacardium*, with the IC₅₀ ranging between the low micrograms per milliliter, specifying that these materials were very powerful at very low levels. Differently, the bioactivity of *Withania somnifera* was more evenly poised between high antioxidant capacity, and low and moderate phenolic levels and cytotoxicity, indicating a broader but not so pronounced pharmacological profile. Such activity patterns are most likely due to the complexity of phytochemical constituents in each plant where polyphenols, flavonoids, alkaloids, terpenoids show individual and synergistic effects.

4.2 Comparison with Existing Studies

The obtained findings are congruent with previous studies. Several studies have described strong cytotoxic effect of *Calotropis procera* by cardenolides and other associated secondary metabolites which exhibit apoptotic ability and interfere with proliferation of cancerous cells. Similarly, *Semecarpus anacardium* has high anacardic acids and phenolic compounds that reportedly exert high concentration of pro-apoptotic and antiproliferative activity. These two margins of *Withania somnifera*, that of a dual antioxidant and moderate cytotoxic level could correlate with literature that associates the aspects of reactive oxygen species (ROS) and programmed cell death in models of tumor cells with the constituents of its withanolides. The expectations on the *Phyllanthus emblica* and *Tinospora cordifolia* observed in the experimental grouping are in line with their phytochemical composition as having antioxidants but are yet to be confirmed in this line of inquiry.

Table 4: Studies on Bioactivities of Medicinal Plants

Author Name	Topic Covered	Research Study Title
Patil (2021) ¹¹	Alkaloids and flavonoids that were isolated on the <i>Leucas aspera</i> leaves were to have antioxidant activity, in vitro cell cytotoxicity and cell viability	Antioxidant study, in vitro-cytotoxicity testing and cell viability assay of the flavonoids and alkaloids of <i>Leucas aspera</i> (Wild.). Linn leaves
Paudel et al. (2019) ¹²	<i>Dendrobium crepidatum</i> extracts are antioxidants and have cytotoxic effects	Analysis of the antioxidant and cytotoxic activity of the extracts of <i>Dendrobium crepidatum</i>
Ramamoorthy et al. (2019) ¹³	Triple bark Triple bark extract of traditional medicinal plants has antimicrobial, antioxidant, and cytotoxic effects	Analysis of antimicrobial, antioxidant and their cytotoxicity activity of the triple-bark extract, which is a formulation of traditional medicinal plants
Sasindran et al. (2020) ¹⁴	Screening of phytochemicals and evaluation of cytotoxicity crude	Phytochemical screening and Assay of fractions: Toxicity comparison

	extractions and ethosomal formulations	between crude extracts and ethosomal formulations of them
Swargiary et al. (2021) ¹⁵	In vitro and in silico antioxidant and antiproliferative activity of selected medicinal plants of Lower Assam India	Antiproliferative effect and antioxidant properties of some selected medicines plants of lower Assam, India: An in silico and in vitro study

Said studies all lend weight to the notion that medicinal plants contain a wide range of bioactive components with the ability to produce both antioxidant and cytotoxic effects, making the current study topical.

4.3 Implications of Findings

Strong cytotoxic and antioxidant properties exhibited by some of the herbs tested illustrate the medicinal value of the herbs. An example of botanical source as a source of drugs is the extract of *Calotropis procera*, *Semecarpus anacardium* which can be used as leads to the anticancer drugs as long as their selectivity to malignant cells can be proved. The balanced profile of *Withania somnifera* would imply potential use as a multipurpose therapy of chemopreventive and direct cytotoxic action. Also, the antioxidant potential exhibited is high, which justifies the treatment of the given herbs in the treatment of oxidative stress-related diseases, such as the chronic inflammatory and degenerative diseases.

4.4 Limitations of the Study

- The analysis was done with data that was published in the past and did not provide recent data collected experimentally and therefore possible deviations in variability may be introduced by differences in extraction methods and assay protocols.
- Cytotoxicity experiments on a chosen set of tumour cell lines meant it was not generalizable to the other types of cancers.
- Selectivity towards normal cells was not established and it is unknown whether the therapy is safe.
- Possible differences in reporting standards among studies may have an impact on the comparability of harvested results.

4.5 Suggestions for Future Research

- Replicate results of antioxidant and cytotoxic assays through experiment in the same laboratory conditions.
- Identify active compounds by use of bioassay-guided fractionation and structural elucidation.
- Carry out studies in the living to determine efficacy, toxicity profiles and pharmacokinetics.
- Conduct mechanistic analyses of apoptosis pathways, ROS and molecular signalling targets.

- Test on library of cancer and normal cell lines to determine selectivity and therapeutic windows.

5. CONCLUSION

The aim of the present study was to determine and examine the fundamental research question by focusing on [briefly insert topic focus, e.g., "establishing the predictors of acute kidney injury in infants and newborn cardiac surgery patients"]. Through [mention methodology in brief, e.g., "a retrospective-prospective observational study and stringent statistical analysis"] not only does such work offer an insight and understanding of important patterns and associations it also provides worthwhile evidence to literature. The lessons learned are expected to be applied to scholarly discussion as well as applied decision-making in the sphere.

5.1 Summary of Key Findings

The investigation found that [summarize the most significant findings in your own words, e.g., "particular preoperative and intraoperative factors, like long cardiopulmonary bypass duration and low birth weight were considerably related to the occurrence of the acute kidney injury"]. The statistics proved as well [add another statement, e.g., "a definite association between postoperative hemodynamic instability and higher rates of AKIs"]. These results correspond to the findings of previous research and to a certain degree, they complement them.

5.2 Significance of the Study

The implications of the results are huge in the [specify context, e.g., "clinical management of neonatal and infant cardiac surgery patients"]. Considering the early identification of the presence of high-risk factors, healthcare providers can implement specific interventions in a preventive approach; thus, they might achieve a decrease in morbidity and better postoperative results. Also, the study would help fill a significant data gap regarding region-specific evidence, which would become the basis of additional clinical guidelines and policy building.

5.3 Recommendations

- Suggest including an increased monitoring into the perioperative patient protocols where risk factors have been discovered.
- Recommend undertaking multi-centre research with more population to confirm results and evaluate the effectiveness of their interventions.
- Stress the translation of knowledge into action to assure patient safety and long-term patient health outcomes.

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