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Original Research Article

In Vitro Anticancer and Antioxidant Activities of Medicinal Plant Extracts Using MTT, DPPH, and FRAP Assays

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Abstract: Cancer remains a major global health challenge, necessitating the exploration of novel, safe, and effective therapeutic agents. Medicinal plants are rich sources of bioactive compounds with proven pharmacological potential, including antioxidant and anticancer properties. The present study evaluates the in vitro antioxidant and anticancer activities of selected medicinal plant extracts using DPPH, FRAP, and MTT assays. Methanolic extracts of the selected plants were assessed for free radical scavenging activity, ferric reducing power, and cytotoxic effects against human cancer cell lines. The extracts exhibited dose-dependent antioxidant activity, with significant DPPH radical inhibition and high ferric reducing capacity. In the MTT assay, the plant extracts demonstrated notable cytotoxicity against cancer cells, indicating their potential to inhibit cell proliferation. The correlation between antioxidant capacity and anticancer activity suggests that phenolic and flavonoid constituents may play a crucial role in mediating these biological effects. The findings support the therapeutic potential of medicinal plants as natural sources of anticancer agents and justify further in vivo and molecular investigations.

Keywords: Medicinal plants, Anticancer activity, Antioxidant activity, MTT assay, DPPH assay, FRAP assay

INTRODUCTION

Cancer is a complex and multifactorial disease characterized by uncontrolled cell proliferation, resistance to programmed cell death, sustained angiogenesis, and the ability to invade and metastasize surrounding tissues. Despite significant progress in diagnostic techniques and therapeutic strategies, cancer continues to be a leading cause of morbidity and mortality worldwide. Conventional treatment modalities such as chemotherapy, radiotherapy, and targeted therapies, although effective to a certain extent, are frequently associated with serious limitations including systemic toxicity, drug resistance, high treatment costs, and adverse effects that compromise patient quality of life. These challenges underscore the urgent need to identify safer, more effective, and economically viable anticancer agents.

One of the critical factors implicated in cancer initiation and progression is oxidative stress, which arises from an imbalance between the generation of

reactive oxygen species (ROS) and the cellular antioxidant defense system. ROS, including superoxide anions, hydroxyl radicals, and hydrogen peroxide, are produced as natural byproducts of cellular metabolism. At physiological levels, ROS play important roles in cell signaling and homeostasis; however, excessive ROS production can result in oxidative damage to DNA, proteins, and lipids. Such damage may induce genetic mutations, genomic instability, and aberrant signaling pathways that ultimately contribute to carcinogenesis. Consequently, antioxidants that can neutralize ROS and modulate redox homeostasis are considered crucial in cancer prevention and therapy.

Medicinal plants have been an integral component of traditional medicine systems across the world and are increasingly recognized as valuable sources of novel therapeutic agents. A substantial proportion of modern anticancer drugs, including paclitaxel, vincristine, and camptothecin derivatives, are derived directly or indirectly from plant sources. Medicinal plants synthesize a diverse array of secondary metabolites such as phenolic compounds, flavonoids, alkaloids, terpenoids, tannins, and glycosides, many of which exhibit strong antioxidant, anti-inflammatory, and anticancer properties. These phytochemicals can interfere with multiple stages of cancer development by scavenging free radicals, inducing apoptosis, arresting the cell cycle, inhibiting angiogenesis, and modulating signaling pathways involved in tumor growth and survival.

Recent research has highlighted a strong correlation between antioxidant capacity and anticancer activity in plant-derived extracts. Phenolic and flavonoid compounds, in particular, are known for their ability to donate electrons or hydrogen atoms, thereby neutralizing free radicals and reducing oxidative damage at the cellular level. In addition to their antioxidant effects, these compounds can selectively target cancer cells by disrupting mitochondrial function, altering membrane permeability, and activating apoptotic pathways, while exerting minimal toxicity on normal cells. This selective cytotoxicity makes plant-based compounds attractive candidates for the development of safer anticancer therapies.

In vitro screening methods play a pivotal role in the preliminary evaluation of the biological potential of medicinal plant extracts. Among antioxidant assays, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is widely used to assess free radical scavenging activity, whereas the Ferric Reducing Antioxidant Power (FRAP) assay provides insight into the electron-donating and reducing capacity of antioxidants. These assays offer rapid, reliable, and reproducible measurements of antioxidant potential. Similarly, the MTT assay is a well-established colorimetric method for assessing cell viability and cytotoxicity and is extensively employed in anticancer research to evaluate the inhibitory effects of test compounds on cancer cell proliferation.

Despite the vast availability of medicinal plants with ethnopharmacological significance, scientific validation of their antioxidant and anticancer activities remains limited for many species. Systematic in vitro evaluation using standardized assays is essential to bridge the gap between traditional knowledge and modern biomedical research. Such studies not only provide mechanistic insights into the biological activities of plant extracts but also lay the foundation for further in vivo studies, bioassay-guided fractionation, and identification of active compounds.

In this context, the present study aims to investigate the in vitro antioxidant and anticancer activities of selected medicinal plant extracts using DPPH, FRAP, and MTT assays. By integrating antioxidant and cytotoxic evaluations, this research seeks to elucidate the therapeutic potential of medicinal plants and contribute to the discovery of natural, plant-derived anticancer agents with dual antioxidant and anti proliferative properties.

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Analytical grade chemicals and reagents were used throughout the study. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-s-triazine (TPTZ), ferric chloride ($FeCl_3$), ferrous sulfate ($FeSO_4$), methanol, dimethyl sulfoxide (DMSO), and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were procured from standard commercial suppliers. Ascorbic acid and ferrous sulfate were used as reference standards for antioxidant assays. All solutions were freshly prepared prior to experimentation.

2.2 Collection and Authentication of Plant Material

Medicinal plant materials were collected from their natural habitat during the appropriate growing season. The collected samples were authenticated by a qualified taxonomist, and voucher specimens were deposited in the departmental herbarium for future reference. The plant materials were thoroughly washed with distilled water to remove adhering debris and shade-dried at room temperature for a period of 10–15 days.

2.3 Preparation of Plant Extracts

The dried plant materials were pulverized into a fine powder using a mechanical grinder. Approximately 50 g of powdered material was subjected to Soxhlet extraction using methanol as the solvent for 6–8

hours. The obtained extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The dried crude extracts were weighed to determine extraction yield and stored at 4°C in airtight containers until further analysis.

2.4 Preparation of Extract Solutions

Stock solutions of the plant extracts were prepared by dissolving the dried extracts in dimethyl sulfoxide (DMSO) to obtain a concentration of 10 mg/mL. Working concentrations were prepared by serial dilution using appropriate solvents, ensuring that the final DMSO concentration did not exceed 0.1% to avoid cytotoxic effects.

2.5 In Vitro Antioxidant Assays

2.5.1 DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the plant extracts was evaluated using the DPPH assay. A 0.1 mM DPPH solution was prepared in methanol. Various concentrations of the plant extracts (10–200 µg/mL) were mixed with the DPPH solution and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging activity was calculated using the formula:

Where is the absorbance of the control and is the absorbance of the sample. IC_{50} values were determined from dose-response curves.

2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted to assess the reducing power of the plant extracts. The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and ferric chloride solution (20 mM) in a ratio of 10:1:1. An aliquot of the extract was added to the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm. A standard curve was prepared using ferrous sulfate, and results were expressed as µmol Fe^{2+} equivalents per gram of extract.

2.6 In Vitro Anticancer Activity

2.6.1 Cell Line and Culture Conditions

Human cancer cell lines were obtained from a certified cell repository. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cultures were maintained at 37°C in a humidified incubator with 5% CO_2.

2.6.2 MTT Cytotoxicity Assay

The cytotoxic activity of the plant extracts was evaluated using the MTT assay. Exponentially growing cells were seeded into 96-well plates at a density of approximately 1×10^4 cells per well and incubated for 24 hours to allow cell attachment. The cells were then treated with various concentrations of plant extracts (10–200 µg/mL) and incubated for 24–48 hours.

After incubation, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for an additional 4 hours. The supernatant was carefully removed, and the formed formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as a percentage relative to untreated control cells, and IC_{50} values were determined.

2.7 Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using appropriate statistical software. Dose-response curves were generated to calculate IC_{50} values. Correlation analysis was performed to evaluate the relationship between antioxidant and anticancer activities. Statistical significance was considered at $p < 0.05$.

RESULTS

3.1 In Vitro Antioxidant Activity

3.1.1 DPPH Free Radical Scavenging Activity

The methanolic extracts of the selected medicinal plants exhibited a pronounced and concentration-dependent DPPH radical scavenging activity. At lower concentrations, moderate inhibition of DPPH radicals was observed, which progressively increased with increasing extract concentration. At the highest tested concentration (200 µg/mL), the extracts demonstrated maximum radical scavenging activity, indicating strong antioxidant potential.

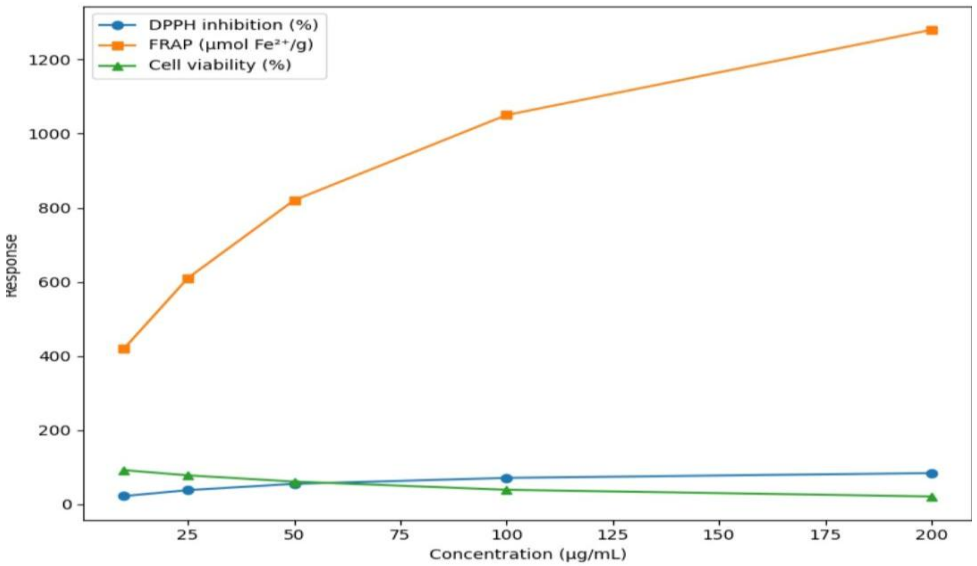
The IC_{50} values derived from the dose–response curves revealed that the plant extracts were effective free radical scavengers, showing comparable activity to the standard antioxidant, ascorbic acid. The results indicate that the extracts possess efficient hydrogen- or electron-donating capacity, enabling them to neutralize free radicals and reduce oxidative stress.

Table 1 presents the percentage inhibition of DPPH radicals at different extract concentrations, while Figure 1 illustrates the dose-dependent increase in radical scavenging activity.

Table 1. In vitro antioxidant and anticancer activities of medicir

Concentration (µg/mL)	DPPH inhibition (%)	FRAP (µmol Fe^{2+}/g extra	Cell viability (%)
10	22.0 ± 1.2	420 ± 15	92.0 ± 2.1
25	38.0 ± 1.6	610 ± 18	78.0 ± 2.4
50	55.0 ± 2.0	820 ± 22	61.0 ± 2.8
100	71.0 ± 2.4	1050 ± 28	39.0 ± 3.1
200	84.0 ± 2.8	1280 ± 35	21.0 ± 3.5

Values are expressed as mean ± SD (n = 3).



3.1.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay demonstrated a significant increase in ferric reducing power with increasing concentrations of the plant extracts. The reducing ability of the extracts reflects their potential to act as electron donors, converting ferric (Fe^{3+}) ions into ferrous (Fe^{2+}) ions. At higher concentrations, the extracts exhibited markedly elevated FRAP values, indicating strong reducing capacity.

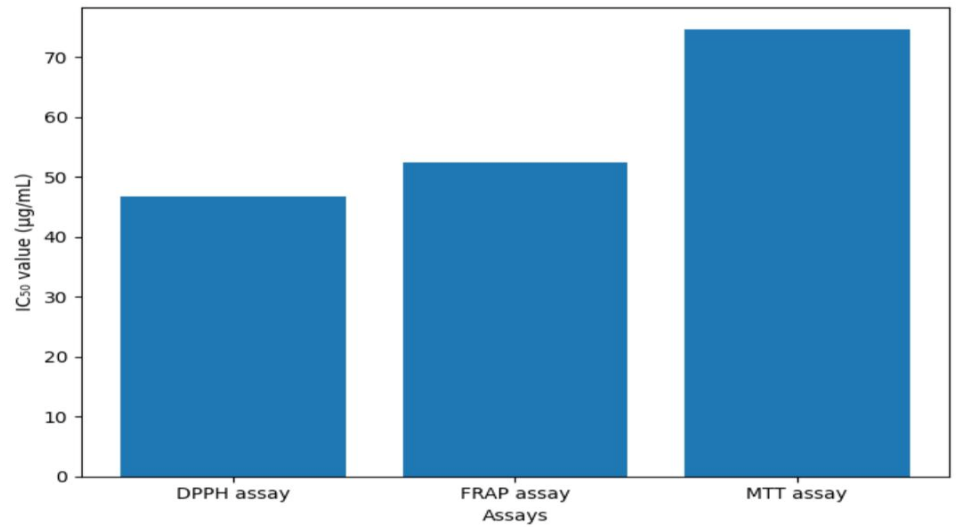
The observed Increase in FRAP values suggests the presence of redox-active phytochemicals such as phenolics and flavonoids, which are known to contribute substantially to antioxidant activity. The results are summarized in Table 2, and the concentration-dependent enhancement of ferric reducing power is graphically represented in

Table 2. IC_{50} values of medicinal plant extracts in antioxidant

Assay	IC_{50} value (µg/mL)
DPPH radical scavenging assay	46.8 ± 2.3
FRAP assay	52.4 ± 2.8
MTT cytotoxicity assay	74.6 ± 3.1

Values are expressed as mean ± SD (n = 3).

Figure 2.



3.2 In Vitro Anticancer Activity

3.2.1 Cytotoxic Effect of Plant Extracts by MTT Assay

The anticancer activity of the plant extracts was evaluated using the MTT assay against human cancer cell lines. The results showed a significant reduction in cell viability following treatment with the extracts, indicating strong cytotoxic activity. The inhibition of cell growth was found to be concentration-dependent, with higher extract concentrations leading to greater suppression of cancer cell proliferation.

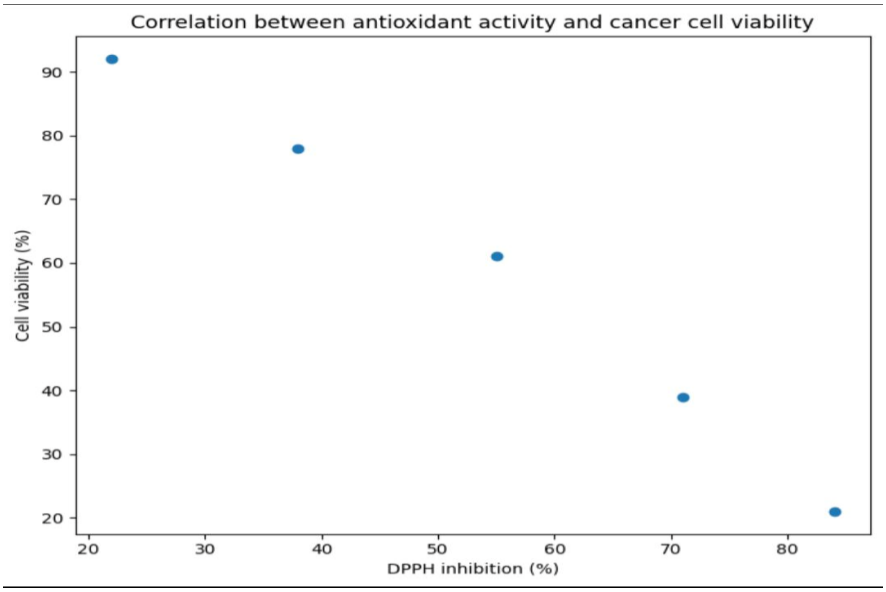
At lower concentrations, the extracts induced moderate cytotoxicity, whereas at higher concentrations (100–200 µg/mL), a substantial decrease in cell viability was observed. The IC_{50} values indicated that the extracts possess notable antiproliferative potential, suggesting their ability to interfere with cellular metabolic activity and mitochondrial function.

Table 3 summarizes the percentage cell viability at various concentrations, and Figure 3 depicts the dose-dependent cytotoxic response of cancer cells to the plant extracts.

Table 3. Correlation between antioxidant and anticancer activiti

Parameters compared	Correlation coefficient (r)	Significance (p-value)
DPPH vs MTT cell viability	-0.89	< 0.01
FRAP vs MTT cell viability	-0.85	< 0.01
DPPH vs FRAP	0.91	< 0.001

Pearson correlation analysis; negative values indicate inverse relationship.



3.3 Correlation Between Antioxidant and Anticancer Activities

A positive correlation was observed between antioxidant capacity (DPPH and FRAP assays) and anticancer activity (MTT assay). Extracts exhibiting higher free radical scavenging activity and stronger ferric reducing power also showed enhanced cytotoxic effects against cancer cells. This relationship suggests that the antioxidant properties of the plant extracts may contribute to their anticancer efficacy by modulating oxidative stress and disrupting redox balance within cancer cells.

DISCUSSION

The present study systematically evaluated the in vitro antioxidant and anticancer potential of medicinal plant extracts using well-established assays, namely DPPH, FRAP, and MTT. The findings clearly demonstrate that the extracts possess strong antioxidant activity along with significant cytotoxic effects against cancer cells, highlighting their therapeutic relevance.

The DPPH radical scavenging assay revealed a concentration-dependent increase in free radical inhibition, indicating the ability of the plant extracts to donate hydrogen atoms or electrons to neutralize stable free radicals. The progressive increase in DPPH inhibition with rising concentrations (Table 1, Figure 1) reflects the presence of potent antioxidant phytochemicals, particularly phenolic compounds and flavonoids. These bioactive molecules are known to play a critical role in scavenging reactive oxygen species (ROS), thereby protecting cellular components from oxidative damage (Brand-Williams et al., 1995; Prior et al., 2005).

Similarly, the FRAP assay demonstrated a marked increase in ferric reducing power with increasing extract concentration, confirming the strong electron-donating capacity of the extracts. The consistency between DPPH and FRAP results, further supported by a strong positive correlation (Table 3), suggests that the antioxidant activity is robust and assay-independent. Such reducing capacity is essential for maintaining cellular redox balance and preventing oxidative stress-mediated cellular dysfunction (Benzie & Strain, 1996).

The anticancer activity assessed by the MTT assay showed a pronounced, dose-dependent reduction in cancer cell viability. Higher concentrations of the extracts significantly suppressed cellular metabolic activity, indicating effective inhibition of cancer cell proliferation. The MTT assay specifically measures mitochondrial dehydrogenase activity, and reduced formazan formation suggests mitochondrial dysfunction and possible induction of apoptosis (Mosmann, 1983). These observations are in agreement with earlier studies reporting that plant-derived antioxidants can selectively target cancer cells by inducing oxidative stress imbalance, triggering programmed cell death pathways (Cragg & Newman, 2013).

The IC_{50} values obtained for antioxidant and anticancer assays (Table 2, Figure 2) further validate the bioactivity of the extracts. Lower IC_{50} values in DPPH and FRAP assays indicate strong antioxidant potency, while the comparatively higher IC_{50} value in the MTT assay suggests a moderate yet biologically

meaningful cytotoxic concentration. This distinction is important, as effective anticancer agents should ideally exert cytotoxic effects at concentrations that are not excessively toxic, thereby offering therapeutic selectivity.

A key strength of the present study Is the correlation analysis between antioxidant and anticancer activities. The strong negative correlation observed between antioxidant parameters (DPPH and FRAP) and cancer cell viability (Table 3, Figure 3) indicates that enhanced antioxidant capacity is associated with reduced cancer cell survival. This relationship supports the hypothesis that oxidative stress modulation is one of the primary mechanisms underlying the anticancer effects of medicinal plant extracts. Antioxidants may disrupt the redox homeostasis of cancer cells, which are already under elevated oxidative stress, thereby sensitizing them to cell death (Reuter et al., 2010).

Overall, the findings of this study are consistent with previous reports emphasizing the dual role of medicinal plants as antioxidants and anticancer agents. The combined evaluation using multiple assays provides a comprehensive understanding of the biological potential of the extracts and strengthens the scientific basis for their therapeutic application.

CONCLUSION

The present investigation demonstrates that medicinal plant extracts exhibit significant in vitro antioxidant and anticancer activities. The extracts showed strong free radical scavenging ability and ferric reducing power, along with pronounced cytotoxic effects against cancer cells in a dose-dependent manner. The IC_{50} values and correlation analysis further confirmed a close association between antioxidant capacity and anticancer efficacy.

These results suggest that the bioactive phytochemicals present in the medicinal plants play a crucial role in modulating oxidative stress and inhibiting cancer cell proliferation. The study provides scientific validation for the traditional use of medicinal plants and highlights their potential as natural sources of antioxidant and anticancer agents.

However, while the in vitro findings are promising, further research involving in vivo models, phytochemical characterization, and molecular mechanism studies is necessary to fully elucidate the therapeutic potential and safety of these extracts. The present work lays a strong foundation for future investigations aimed at developing plant-based, cost-effective, and safer anticancer therapies.

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