Exploring the Anticancer Properties of *Ziziphus rugosa*: A Detailed Phytochemical and Cytotoxicity Study

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Abstract

The current study presents an in-depth investigation into the phytochemical composition and anticancer potential of solvent extract derived from the bark of *Ziziphus rugosa* bark, Recognizing the traditional medicinal value of *Z. rugosa* and the growing need for novel anticancer agents from natural sources, we employed a sequential extraction protocol to maximize the yield of diverse secondary metabolites. Comprehensive qualitative screening revealed that the extracts contain a complex array of bioactive compounds including alkaloids, glycosides, flavonoids, tannins, carbohydrates, saponins, terpenoids, steroids, and phenols. Detailed GC-MS analysis of the ethanolic extract further confirmed the presence of multiple compounds with potential pharmacological activities, such as terpenoids and phenolic constituents, which are known to exhibit antioxidant and apoptosis inducing effects. The anticancer efficacy of the extracts evaluated using the MTT assay on MCF-7 human breast cancer and A-547 ovarian cancer cell lines. Notably, the aqueous extract demonstrated a dose-dependent inhibition of MCF-7 cell viability with an IC_{50} of 65.64 µg/mL, while the ethanolic extract exhibited significant cytotoxicity against A-547 cells with an IC_{50} of 57.43 µg/mL. These results suggest that the bioactive compounds present in *Z. rugosa* bark extracts may interfere with cellular pathways critical for cancer cell survival and proliferation. The findings underscore the therapeutic potential of *Z. rugosa* as a source of novel anticancer agents and provide a solid foundation for future studies aimed at isolating and elucidating the mechanisms of action of the active constituents.

Keywords: *Ziziphus rugosa*, phytochemical screening, GC-MS, cytotoxicity, MTT assay

1. Introduction

The search for novel anticancer agents continues to be a primary focus in biomedical research, with natural products providing a rich source of potential therapeutic compounds [1]. Medicinal plants have historically contributed to the development of effective anticancer drugs, largely due to their complex mixtures of secondary metabolites [2-9]. *Ziziphus rugosa*, a member of the Rhamnaceae family, has been traditionally used in ethnomedicine for its diverse therapeutic properties [10]. Recent investigations into related *Ziziphus* species have highlighted their potential in mitigating cancer cell proliferation, primarily through the action of alkaloids, flavonoids, and saponins that modulate apoptosis and cell cycle arrest [11-13].

Advancements in extraction techniques and analytical methodologies, such as Soxhlet extraction and GC-MS, have enabled a more comprehensive understanding of the chemical constituents of plant materials [13-15]. This study aims to (i) conduct a detailed phytochemical screening of *Z. rugosa* bark extracts prepared using solvents of varying polarities, (ii) characterize the chemical profile of the most active extract using GC-MS, and (iii) evaluate the cytotoxic efficacy of these extracts against MCF-7 human breast cancer and A-547 ovarian cancer cell lines using the MTT assay [6-7]. Through a correlation between phytochemical content and observed cytotoxicity, we seek to elucidate potential mechanisms underlying the anticancer properties of *Z. rugosa*.

2. Materials and Methods

2.1. Plant Material and Authentication

Fresh bark of *Ziziphus rugosa* was collected from Koppa, Karnataka, India 577126. It was authenticated by a botanist at ALN Rao medical college Koppa. The sample was then washed, air dried at room temperature for 10–14 days, and ground into a fine powder using a mechanical grinder.

2.2. Extraction Procedure

Approximately 100 g of the powdered bark was subjected to sequential Soxhlet extraction using three solvents of increasing polarity: chloroform, ethanol, and water. Each extraction cycle lasted for 8–10 hours to ensure exhaustive extraction of phytoconstituents. The obtained extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4°C in amber colored bottles to prevent photo degradation [19].

2.3. Phytochemical Screening

Qualitative analysis was performed using standard protocols to detect the presence of major classes of secondary metabolites [15]. The tests included:

- **Alkaloids:** Detected using Mayer's and Dragendorff's reagents.
- **Glycosides:** Identified through Keller-Kiliani and Legal's tests.
- **Flavonoids:** Screened using the Shinoda test and Alkaline reagent test.
- **Tannins:** Evaluated by the ferric chloride test.
- **Carbohydrates:** Confirmed by Molisch's and Benedict's tests.
- **Saponins:** Detected by the frothing test.
- **Terpenoids:** Screened using the Salkowski test.
- **Steroids:** Identified using Liebermann-Burchard reaction.
- **Phenols:** Confirmed by ferric chloride test.

Table 1. Phytochemical Constituents of *Z. rugosa* Bark Extracts

Phytochemicals	Chloroform Extract	Ethanolic Extract	Water Extract
Alkaloids	++	++	++
Glycosides	+	+	++
Flavonoids	+	++	+
Tannins	–	++	+
Carbohydrates	+	++	++
Saponins	+	++	++
Terpenoids	+	++	–
Steroids	+	++	–
Phenols	–	++	++

Results were interpreted as "+" (presence), "++" (strongly confirmed), or "–" (absence).

2.4.GC-MS Analysis

The ethanolic extract, which exhibited a broad spectrum of phytoconstituents, was selected for GC-MS analysis. Approximately 1 mg of the dried extract was dissolved in 1 mL of HPLC-grade methanol and filtered through a 0.45 µm membrane filter. Analysis was carried out on a GC-MS system equipped with a 15m Alltech EC-5 column (30 m × 0.25 mm, 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed to increase from 60°C (held for 2 min) to 280°C at a rate of 10°C/min, with a final hold of 10 min. The injector temperature was maintained at 250°C and the ion source at 230°C. Peaks were identified by comparing their mass spectra with the NIST library [6,9]. A representative chromatogram is shown in Figure 1.

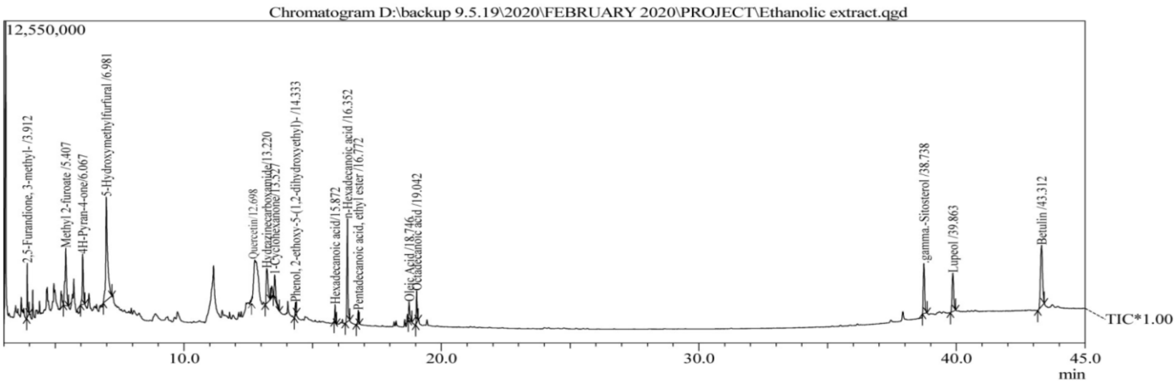

Figure 1. GC-MS Chromatogram of the Active Ethanolic Extract of *Ziziphus rugosa* Bark

2.5. Cytotoxicity Evaluation by MTT Assay

2.5.1. Cell Culture

MCF-7 human breast cancer and A-547 ovarian cancer cell lines were obtained from National Centre for Cell Science (NCCS) in Pune. Cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and maintained at 37°C in a humidified atmosphere with 5% CO_2. Cell viability and confluence were monitored using an inverted phase-contrast microscope.

2.5.2. MTT Assay Procedure

Cells were seeded at a density of 1×10^5 cells/mL in 96-well microtiter plates and allowed to adhere for 24 hours. Treatments were applied with different concentrations (10, 50, and 100 µg/mL) of the respective extracts, with each concentration tested in triplicate. After 48 hours of treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for an additional 4 hours at 37°C. The medium was then removed, and the insoluble formazan crystals were solubilized with 150 µL of DMSO. Absorbance was measured at 570 nm using a microplate reader. The percentage inhibition of cell viability was calculated by the formula:

$$\% \text{ Inhibition} = (1 - [\text{Absorbance of Test} / \text{Absorbance of Control}]) \times 100$$

IC_{50} values were determined by plotting the percentage inhibition against the logarithm of the extract concentration and applying a non-linear regression analysis [7].

2.5.3. Representative Microscopy

Post-treatment cell morphology was documented using an optical microscope (at 40× magnification) to observe cytotoxic effects, including cell shrinkage, membrane blebbing, and reduced cell density.

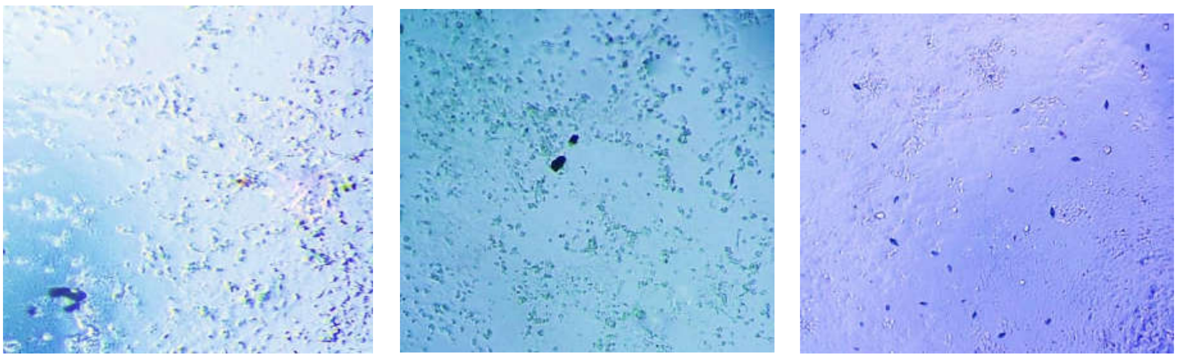

Figure 2. Effect of Aqueous Extract on MCF-7 Cell Morphology
Panel A: Cells treated with 10 µg/mL; Panel B: Cells treated with 50 µg/mL; Panel C: Cells treated with 100 µg/mL. Progressive morphological changes and reduced cell density were observed with increasing concentration.

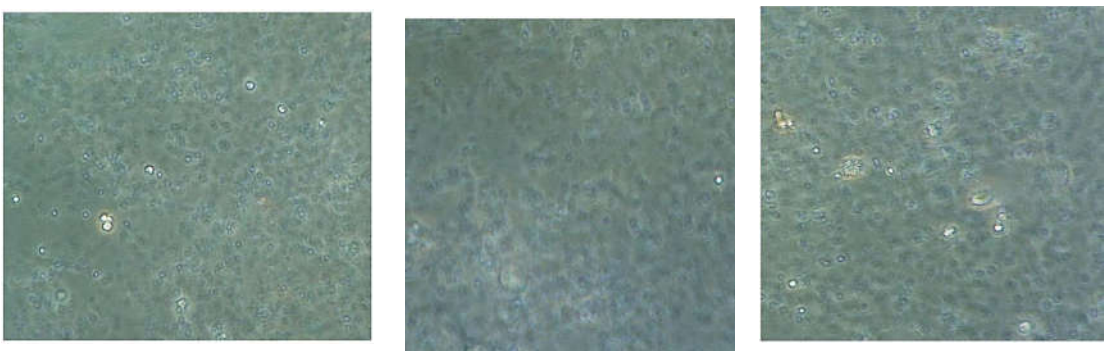

Figure 3. Effect of Ethanolic Extract on A-547 Cell Morphology
Panel A: Cells treated with 10 µg/mL; Panel B: Cells treated with 50 µg/mL; Panel C: Cells treated with 100 µg/mL. A clear dose-dependent cytotoxic effect was observed.

Table 2. MTT Assay Results for Aqueous Extract on MCF-7 Cells

Concentration (µg/mL)	Absorbance (Test)	Percentage Inhibition (%)
10	0.796	24.43
50	0.611	46.58
100	0.472	63.23
Control	0.835	–

Table 3. MTT Assay Results for Ethanolic Extract on A-547 Cells

Concentration (µg/mL)	Absorbance (Test)	Percentage Inhibition (%)
10	0.722	37.16
50	0.649	46.92
100	0.533	62.43
Control	0.748	–

The IC_{50} values were calculated as 65.64 µg/mL for the aqueous extract (MCF-7 cells) and 57.43 µg/mL for the ethanolic extract (A-547 cells).

3. Results

3.1. Phytochemical Analysis

The qualitative screening (Table 1) revealed that all extracts contain significant amounts of alkaloids, glycosides, flavonoids, carbohydrates, and saponins. Notably, the ethanolic extract exhibited a broader spectrum of phytoconstituents, with strong positive results for most classes, suggesting that ethanol is an effective solvent for extracting a wide range of bioactive compounds from *Z. rugosa* bark [1,5].

3.2. GC-MS Profiling

The GC-MS analysis of the ethanolic extract (Figure 1) identified several peaks corresponding to bioactive constituents, including various terpenoids and phenolic compounds. Although the complete identification of all compounds requires further analysis, the presence of these constituents supports the observed biological activities and aligns with findings in similar studies [18].

3.3. Cytotoxic Activity

The MTT assay demonstrated a dose-dependent inhibition of cell viability in both MCF-7 and A-547 cell lines. The aqueous extract exhibited significant cytotoxicity against MCF-7 cells (IC_{50} = 65.64 µg/mL), while the ethanolic extract was more potent against A-547 cells (IC_{50} = 57.43 µg/mL) (Tables 2 and 3; Figures 2 and 3). The observed morphological changes (Figures 2 and 3) corroborated the quantitative data, showing reduced cell density and morphological alterations typical of apoptosis or necrosis.

4. Discussion

The comprehensive phytochemical screening and GC-MS profiling of *Ziziphus rugosa* bark extracts reveal a rich and diverse chemical composition that likely underpins their cytotoxic effects. The strong presence of alkaloids, flavonoids, and saponins in all extracts is consistent with previous studies, which have documented the anticancer properties of these compounds through mechanisms such as the induction of apoptosis, inhibition of angiogenesis, and disruption of cell cycle regulation [20]. The enhanced extraction efficiency observed with ethanol suggests that intermediate polarity solvents can extract a broader range of bioactive compounds compared to non-polar or highly polar solvents [19].

GC-MS analysis provided additional insight into the chemical complexity of the ethanolic extract. The detection of multiple terpenoids and phenolic compounds aligns with reports that these molecules can act as antioxidants and apoptosis inducers [20]. These compounds have been shown to modulate intracellular signaling pathways, leading to reduced proliferation and increased cell death in cancer cells [20].

The cytotoxicity data from the MTT assay further support the potential anticancer applications of *Z. rugosa* extracts. The significant inhibition of cell viability in both MCF-7 and A-547 cells, along with the corresponding morphological changes observed under microscopy, indicates that these extracts may exert their effects through the induction of programmed cell death or cell cycle arrest. These findings are in agreement with previous literature highlighting the anticancer potential of natural products [17].

Future investigations should focus on the isolation and structural characterization of the active compounds within these extracts. Moreover, mechanistic studies at the molecular level (e.g., analysis of apoptotic markers, cell cycle regulatory proteins, and signal transduction pathways) are essential to fully elucidate the anticancer mechanisms of these phytochemicals. *In vivo* studies and clinical evaluations will be crucial to validate these in vitro findings and to assess the safety and efficacy of *Z. rugosa* derived compounds for therapeutic use.

5. Conclusion

This detailed study demonstrates that *Ziziphus rugosa* bark extracts possess a rich phytochemical profile and significant cytotoxic activity against human breast and ovarian cancer cell lines. The aqueous and ethanolic extracts, in particular, show promising anticancer potential with IC_{50} values of 65.64 µg/mL and 57.43 µg/mL, respectively. These preliminary results provide a strong rationale for further isolation, characterization, and mechanistic studies of the bioactive compounds in *Z. rugosa*, with the ultimate goal of developing novel anticancer therapies.

6. References

1. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Springer; 1998.
2. Olatunde A, Nigam M, Singh RK, Panwar AS, Lasisi A, Alhumaydhi FA, et al. Cancer and diabetes: The interlinking metabolic pathways and repurposing actions of antidiabetic drugs. Cancer Cell Int. 2021;21:499.
3. Ferlay J, Colombet M, Soerjomataram I. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144:1941–53.
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71:209–49.
5. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359–E386.
6. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.

7. Manjunatha E, Vedigounder M, Geetha KM, Nandeesh R, Palaksha MN. Review on a wild medicinal plant: Ziziphus rugosa. Int J Pharm Sci Rev Res. 2020;62(2):40–4.
8. Manjunatha E, Vedigounder M, Geetha KM, Nandeesh R, Ahmed SM. Antioxidant and antiulcer activity of Ziziphus rugosa Lam. bark. Res J Pharm Technol. 2020;13(10):4720–4.
9. Manjunatha E, Nandeesh R, Ahmed SM. Antiulcer and ulcer healing potential of some medicinal plants: A review. Res J Pharmacogn Phytochem. 2022;14(1):37–42.
10. Sarker SD, Nahar L. Chemistry for Pharmacy Students: General, Organic and Natural Product Chemistry. Wiley; 2012.
11. Sharma S. The potential role of phytochemicals in cancer therapy. J Ethnopharmacol. 2016;194:114–28.
12. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82.
13. Singh A, et al. Anticancer properties of medicinal plants: The case of Ziziphus species. Phytother Res. 2017;31(8):1175–84.
14. Ali I, et al. GC-MS analysis of bioactive compounds in plant extracts: A comprehensive review. Anal Methods. 2020;12(7):982–93.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55–63.
16. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 2016;79(3):629–61.
17. Stahl E, et al. GC-MS methods in phytochemical research. Anal Chem. 2018;90(3):1853–62.
18. Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol. 2000;88(2):308–16.
19. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta. 2005;1746(1):1–14.
20. Boukhris I, et al. Traditional uses and phytochemical properties of *Ziziphus* species. J Ethnopharmacol. 2017;207:45–57.