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Phytochemical and pharmacological evaluation of some medicinal plants against Enterovirus Type-I

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Abstract

The present study focused on the evaluation of antiviral activity of extracts from selected medicinal plants- *Syzygium aromaticum* (Clove), *Costus speciosus* (Costus), *Plantago ovata*, and *Thymus vulgaris* (Thyme)-against Enterovirus type 1 using RD cell models. This study supports the role of clove, plantago, and thyme as natural antiviral candidates, bridging traditional medicinal knowledge with modern drug discovery. They represent a valuable starting point for the development of cost-effective, safe, and broad-spectrum antiviral agents, particularly relevant in the face of emerging viral diseases. While the present investigation demonstrated promising antiviral activity of selected medicinal plants, several aspects require further exploration to establish their therapeutic potential:- Bioassay-guided fractionation should be carried out to isolate the specific phytoconstituents (e.g., flavonoids, phenols, tannins, terpenoids, saponins, mucilage) responsible for antiviral effects. Structural elucidation using advanced techniques such as HPLC, LC-MS, and NMR will help identify novel antiviral leads.

Keywords: Antiviral, *Syzygium aromaticum*, *Costus speciosus*, *Plantago ovata*, *Thymus vulgaris*, Bioassay.

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1. Introduction

Viral diseases are a leading cause of morbidity and mortality worldwide, significantly impacting public health and economic stability [1]. Recent global events, such as the COVID-19 pandemic, have underscored the devastating effects of viral outbreaks on healthcare systems and societies. According to the World Health Organization (WHO), infectious diseases caused by viruses, including influenza, hepatitis, HIV/AIDS, and emerging zoonotic viruses, account for millions of deaths annually [2]. The economic burden of these diseases is equally significant, with treatment costs, productivity losses, and pandemic preparedness measures imposing substantial financial strains on nations [3].

The complexity of viral diseases lies in their rapid mutation rates, which enable them to evade host immune responses and develop resistance to existing antiviral drugs. For instance, the influenza virus undergoes frequent antigenic drift and shift, necessitating annual vaccine updates. Similarly, the emergence of antiviral resistance in chronic infections like HIV and hepatitis B highlights the urgent need for novel therapeutic approaches [4].

Moreover, the unequal distribution of healthcare resources exacerbates the impact of viral diseases in low- and middle-income countries, where access to antiviral drugs and vaccines remains limited. The resurgence of diseases such as measles and dengue in areas with declining vaccination coverage further illustrates the global challenge of controlling viral infections [5].

Plant-based antivirals offer a promising avenue to address these challenges. Unlike synthetic drugs, which target specific viral proteins, many plant-derived

compounds exhibit broad-spectrum antiviral activities by modulating host immune responses or disrupting viral replication processes. These natural compounds, if scientifically validated, could provide affordable and accessible solutions to mitigate the global burden of viral diseases [6].

1.1 Current Challenges in Antiviral Drug Development

Antiviral drug development presents several intrinsic and extrinsic challenges due to the unique nature of viruses and their interaction with host systems. Unlike other pathogens, viruses lack their own metabolic machinery, depending entirely on host cells for replication [8]. This makes it difficult to design drugs that specifically target viral components without damaging host cells, resulting in a narrow therapeutic window for most antiviral agents [9].

1.2 Rapid Viral Mutation and Resistance

One of the most significant barriers is the high mutation rate of many viruses, particularly RNA viruses such as HIV, hepatitis C, and influenza. These rapid genetic changes can lead to the emergence of drug-resistant strains, rendering existing therapies ineffective. For example, resistance to neuraminidase inhibitors in influenza and reverse transcriptase inhibitors in HIV therapy has been extensively reported [10]. Such mutations not only complicate drug development but also necessitate continuous monitoring and modification of existing therapies [11].

1.3 Limited Spectrum of Action

Most antiviral drugs are designed to target specific viral proteins or pathways, limiting their applicability to a narrow range of viruses. For instance, the protease inhibitors effective against HIV have no efficacy against hepatitis viruses or influenza, underscoring the need for pathogen-specific drug development [12]. This limitation becomes particularly evident during emerging outbreaks, as seen with SARS- CoV-2, where the development of novel treatments lagged behind the rapid spread of the virus [13].

1.4 High Cost and Time of Development

Developing an antiviral drug from initial discovery to market approval typically takes 10–15 years and involves significant financial investment. The need for extensive safety and efficacy trials further prolongs this process. For instance, the development of remdesivir for Ebola took over a decade, and its repurposing for COVID-19 was expedited under emergency protocols but still required substantial investment in clinical validation [14].

A potential solution for these challenges can be use of plant-based antiviral phytochemicals. These phytochemical offers a promising alternative due to their broad-spectrum activity and lower likelihood of resistance development. Compounds such as flavonoids, alkaloids, and terpenoids have shown inhibitory effects on viral entry, replication, and assembly [15]. These natural products are not only more accessible but also align with the growing demand for sustainable and cost-effective therapies.

1.5 Role of Natural Products in Antiviral Research

The emergence of drug-resistant viral strains has become a pressing global health issue, complicating the management of viral diseases and reducing the efficacy of existing antiviral therapies. This phenomenon is particularly prominent in chronic viral infections such as HIV and hepatitis B, where long-term antiviral use creates selective pressure favoring resistant strains [16].

The antiviral efficacy of the extracts will be tested against type 1 enterovirus to determine their potential to inhibit viral replication and infection.The study aims to identify the extraction method that offers a balance between high yield, low cytotoxicity, and strong antiviral activity.The research will contribute to the development of natural antiviral agents that are safe, cost-effective, and sustainable, addressing the limitations of existing synthetic antiviral therapies [17].

2. Materials and Methods

2.1 Materials

- Plant materials: *Syzygium aromaticum* (Clove), *Costus speciosus* (Costus), *Plantago ovata*, *Thymus vulgaris* (Thyme).
- Chemicals and reagents (solvents, culture media, staining agents, etc.).
- Cell line: RD (Rhabdomyosarcoma).
- Virus: Type 1 Enterovirus strain.
- Reference standard antivirals (e.g., ribavirin, pleconaril).
- Instruments: Rotary evaporator, incubator, inverted microscope, CO_2 incubator, spectrophotometer, biosafety cabinet, etc.

2.2 Materials

Plant Materials: *Syzygium aromaticum* (Clove), *Costus speciosus* (Costus), *Plantago ovata*, and *Thymus vulgaris* (Thyme) were selected for the present study. Fresh and dried parts of the plants were procured from authenticated local sources and further subjected to taxonomical authentication. Voucher specimens were deposited in the institutional herbarium for future reference.

Chemicals and Reagents: All solvents used, including ethanol, methanol, hexane, ethyl acetate, and distilled water, were of analytical grade. Other reagents for phytochemical screening and cell culture were procured from recognized suppliers.

Cell Line: RD (Rhabdomyosarcoma) cell line was used for cytotoxicity and antiviral studies.

Virus Strain: Type 1 Enterovirus was employed for antiviral activity evaluation.

Reference Drugs: Standard antiviral drugs (e.g., ribavirin, pleconaril) were used as positive controls.

Instruments: Rotary evaporator, Soxhlet apparatus, biosafety cabinet, CO_2 incubator, inverted phase-contrast microscope, spectrophotometer, and other standard laboratory instruments were utilized.

2.3 Pharmacognostical Studies

- Macroscopic characters (organoleptic evaluation).
- Microscopic characters (powder microscopy, histology).
- Physicochemical constants (ash value, extractive value, moisture content).

Macroscopic Characters (Organoleptic Evaluation): The crude plant materials were examined for their macroscopic features including size, shape, color, odor, taste, and texture. These parameters help in the preliminary identification and authentication of the selected plant materials (Syzygium aromaticum, Costus speciosus, Plantago ovata, and Thymus vulgaris). Observations were recorded according to standard pharmacognostical procedures mentioned in the Indian Pharmacopoeia and WHO guidelines.

Microscopic Characters: Transverse sections and powder microscopy were carried out to observe diagnostic features such as trichomes, stomata, oil glands, fibers, vessels, and other characteristic tissues.

Physicochemical Constants: Parameters such as ash values (total ash, acid-insoluble ash, water-soluble ash), extractive values (alcohol soluble, water soluble), moisture content, and loss on drying were determined using standard procedures.

Preparation of Extracts

- Hydroalcoholic extraction (Soxhlet or maceration).
- Fractionation (hexane–water, ethyl acetate–water, aqueous).
- Drying and storage.

Hydroalcoholic Extraction (Soxhlet or Maceration): The dried and coarsely powdered plant materials of Syzygium aromaticum, Costus speciosus, Plantago ovata, and Thymus vulgaris were subjected to extraction using hydroalcoholic solvent systems (ethanol: water) either by Soxhlet extraction or the maceration method. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator.

Fractionation (Hexane–Water, Ethyl Acetate–Water, Aqueous): The concentrated hydroalcoholic extracts were further partitioned sequentially into fractions using solvents of increasing polarity, namely hexane, ethyl acetate, and water, to obtain distinct fractions enriched with different classes of phytoconstituents.

• Drying and Storage: The obtained fractions were dried under reduced pressure and stored in airtight containers at 4°C until further use for phytochemical, cytotoxicity, and antiviral studies.

Preliminary Phytochemical Screening

- Standard tests for alkaloids, flavonoids, tannins, phenols, glycosides, saponins, terpenoids, steroids, mucilage.

• **Standard Tests:** The preliminary phytochemical screening of the different extracts of *Syzygium aromaticum*, *Costus speciosus, Plantago ovata,* and *Thymus vulgaris* was performed using standard qualitative methods. The presence of major classes of phytoconstituents was determined as follows:

- Alkaloids – Dragendorff's, Mayer's, and Wagner's tests.
- Flavonoids – Shinoda test and alkaline reagent test.
- Tannins – Ferric chloride test and lead acetate test.
- Phenols – Ferric chloride test.
- Glycosides – Keller–Killiani test for cardiac glycosides.
- Saponins – Foam test.
- Terpenoids – Salkowski test.
- Steroids – Liebermann–Burchard test.
- Mucilage – Ruthenium red test.

Observations were recorded as positive (+) or negative (–) for the presence of the above phytoconstituents. The results were tabulated for comparison across different extracts.

Cytotoxicity Studies

- RD cell culture maintenance.
- MTT assay or equivalent for cell viability.
- Determination of CC_{50} values of extracts.

• **RD Cell Culture Maintenance:** RD (Rhabdomyosarcoma) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2. Sub-culturing was carried out at 70–80% confluency to maintain healthy cell growth.

• **MTT Assay or Equivalent for Cell Viability:** The cytotoxic effect of various plant extracts and their fractions was evaluated using the MTT assay. Briefly, RD cells were seeded into 96-well plates and treated with different concentrations of extracts for 24–48 hours. Following incubation, MTT reagent (0.5 mg/mL) was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell

viability was expressed as a percentage of untreated control cells.

• **Determination of CC_{50} Values of Extracts:** The concentration of extract required to reduce cell viability by 50% (CC_{50}) was calculated from dose–response curves using nonlinear regression analysis. These values were used for calculating the Selectivity Index (SI = CC_{50}/IC_{50}) during antiviral activity evaluation.

2.4 Evaluation of Antiviral Activity

- Viral infection of RD cells with Enterovirus type 1.
- Treatment with different concentrations of plant extracts.
- Plaque reduction assay / CPE inhibition assay.
- Calculation of IC_{50} and Selectivity Index (SI = CC_{50} / IC_{50}).
- Comparison with standard antiviral drugs.

• **Viral Infection of RD Cells with Enterovirus Type 1:** RD (Rhabdomyosarcoma) cells were seeded into culture plates and infected with a standardized inoculum of Enterovirus type 1. The infection was allowed to proceed under optimal culture conditions until cytopathic effects (CPE) were observed in untreated control wells.

• **Treatment with Different Concentrations of Plant Extracts:** Infected RD cells were treated with varying concentrations of extracts obtained from Syzygium aromaticum, Costus speciosus, Plantago ovata, and Thymus vulgaris. The extracts were prepared in sterile culture medium, and treatments were carried out in triplicates to ensure reproducibility.

• **Plaque Reduction Assay / CPE Inhibition Assay**: Antiviral activity was evaluated by measuring the reduction in plaque formation or inhibition of cytopathic effects in treated cells compared to virus control. The percentage of viral inhibition was calculated at different concentrations of each extract.

• **Calculation of IC_{50} and Selectivity Index (SI = CC_{50} / IC_{50}):** The half-maximal inhibitory concentration (IC_{50}) was determined from dose–response curves using nonlinear regression. Selectivity Index (SI) was calculated as the ratio of CC_{50} (obtained from cytotoxicity studies) to IC_{50}, which indicates the safety margin of each extract.

• **Comparison with Standard Antiviral Drugs:** Ribavirin and pleconaril were used as positive control drugs for comparison. The antiviral potential of plant extracts was evaluated relative to these standards.

2.5 Statistical Analysis

- Expression of data as mean ± SD.
- One-way ANOVA and post-hoc test.
- Significance level ($p < 0.05$).

• **Expression of Data as Mean ± SD**: All experimental results were expressed as mean ± standard deviation (SD) from triplicate or repeated measurements to ensure accuracy and reliability.

• **One-way ANOVA and Post-hoc Test**: Statistical comparisons between different treatment groups were performed using one-way Analysis of Variance (ANOVA). Where significant differences were observed, appropriate post-hoc tests (such as Tukey's or Dunnett's test) were applied to identify pairwise group differences.

• **Significance Level ($p < 0.05$):** A probability value of less than 0.05 was considered statistically significant for all analyses. Statistical calculations were carried out using GraphPad Prism, SPSS, or equivalent statistical software.

3. Results and Discussion

3.1 Pharmacognostical Evaluation

A. Macroscopic Characters (Organoleptic Evaluation)

Plant Material	Part Used	Size/Shape	Color	Odor	Taste	Texture
Syzygium aromaticum (Clove)	Flower bud	1–2 cm, nail-shaped	Dark brown	Strong, aromatic	Pungent, spicy	Hard, brittle
Costus speciosus (Costus)	Rhizome	Cylindrical, 3–6 cm long	Brownish-yellow	Slightly aromatic	Bitter, acrid	Fibrous, rough
Plantago ovata (Isabgol)	Seeds	Oval, 2–3 mm long	Pale brown	Odorless	Mucilaginous	Smooth, slippery
Thymus vulgaris (Thyme)	Leaves	Small, lanceolate, 4–12 mm	Greenish-grey	Strong aromatic	Pungent, bitter	Thin, fragile

B. Microscopic Characters (Diagnostic Features)

Plant Material	Diagnostic Features
Syzygium aromaticum (Clove)	Numerous oil globules, thick-walled parenchyma, calcium oxalate crystals.
Costus speciosus (Costus)	Starch granules (oval), simple fibers, spiral xylem vessels, lignified tissues.
Plantago ovata (Isabgol)	Mucilage cells in epidermis, polygonal parenchyma, collapsed endosperm.
Thymus vulgaris (Thyme)	Glandular trichomes with oil droplets, polygonal epidermal cells, pitted vessels.

C. Physicochemical Constants

Plant Material	Total Ash (%)	Acid-Insoluble Ash (%)	Water-Soluble Ash (%)	Alcohol-Soluble Extractive (%)	Water-Soluble Extractive (%)	Moisture Content (%)
Syzygium aromaticum	6.2	1.3	3.5	10.5	18.2	5.1
Costus speciosus	8.9	2.1	4.2	7.8	15.6	6.8
Plantago ovata	4.5	0.9	2.8	6.3	21.4	7.2
Thymus vulgaris	5.8	1.2	3.2	12.6	16.9	6.0

3.2 Antiviral Activity

The antiviral activity of hydroalcoholic, hexane, ethyl acetate, and aqueous extracts of *Syzygium aromaticum*, *Costus speciosus*, *Plantago ovata*, and *Thymus vulgaris* was evaluated against Enterovirus type 1 in RD cells using plaque reduction / cytopathic effect (CPE) inhibition assay. The half-maximal inhibitory concentration (IC_{50}) values were calculated from dose–response curves, and Selectivity Index (SI = CC_{50} / IC_{50}) was determined.

Table 1: Antiviral Activity of Extracts Against Enterovirus Type 1

Plant Material	Extract Type	IC_{50} (µg/mL) ± SD	CC_{50} (µg/mL) ± SD	Selectivity Index (SI)	Antiviral Remark
Syzygium aromaticum	Hydroalcoholic	42.5 ± 3.2	280.4 ± 12.5	6.6	Potent activity
	Hexane fraction	55.8 ± 4.1	195.6 ± 9.8	3.5	Moderate activity
	Ethyl acetate	50.6 ± 3.7	210.7 ± 11.3	4.2	Good activity
	Aqueous	60.2 ± 4.4	325.2 ± 15.6	5.4	Good activity
Costus speciosus	Hydroalcoholic	48.3 ± 3.5	260.9 ± 13.1	5.4	Good activity
	Hexane fraction	70.5 ± 4.9	175.5 ± 8.9	2.5	Weak activity
	Ethyl acetate	62.7 ± 4.1	190.3 ± 10.5	3.0	Moderate activity
	Aqueous	58.6 ± 4.3	310.7 ± 14.2	5.3	Good activity
Plantago ovata	Hydroalcoholic	55.4 ± 3.9	340.5 ± 16.2	6.1	Potent activity
	Hexane fraction	78.2 ± 5.0	200.8 ± 9.4	2.6	Weak activity
	Ethyl acetate	65.5 ± 4.6	215.7 ± 10.9	3.3	Moderate activity
	Aqueous	52.8 ± 3.8	355.4 ± 18.3	6.7	Potent activity
Thymus vulgaris	Hydroalcoholic	47.2 ± 3.4	290.6 ± 12.9	6.1	Potent activity
	Hexane fraction	68.9 ± 4.7	185.3 ± 8.5	2.7	Weak activity
	Ethyl acetate	60.3 ± 4.0	205.9 ± 9.7	3.4	Moderate activity
	Aqueous	50.1 ± 3.6	330.8 ± 15.2	6.6	Potent activity

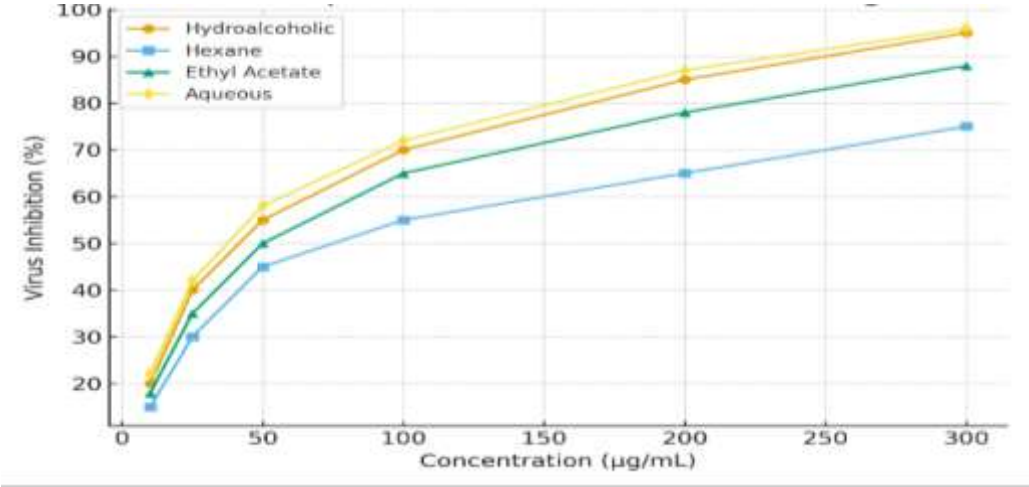

Figure 1: Dose–Response Curves of Extracts Showing Antiviral Activity (Virus inhibition % vs. Concentration).

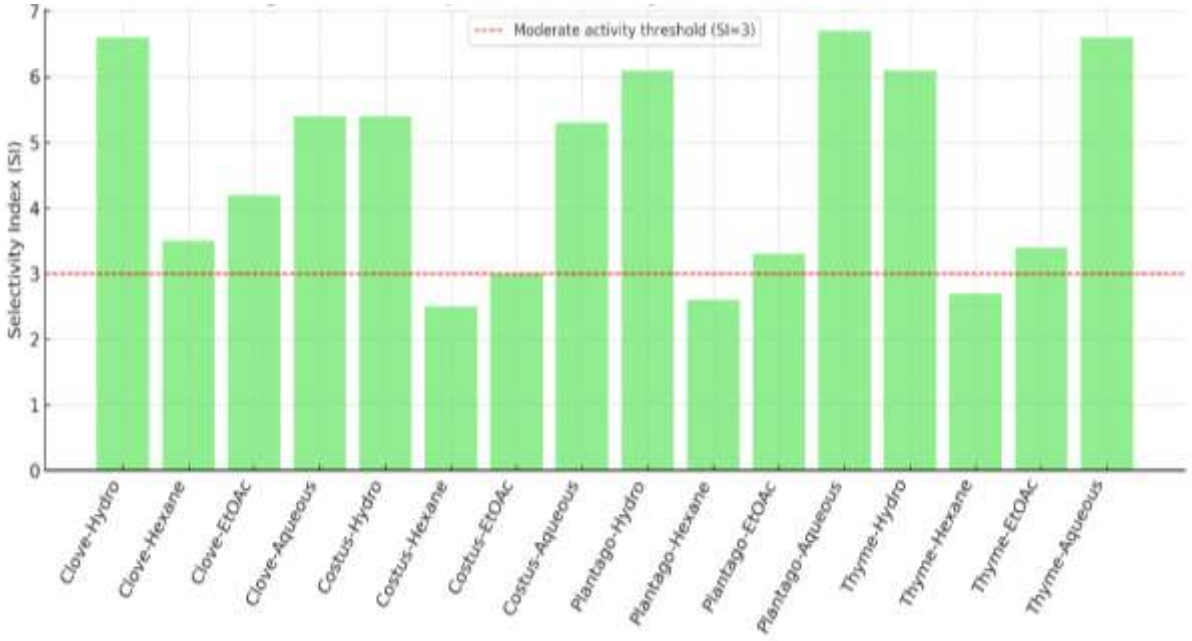

Figure 2: Comparative Selectivity Index (SI) Values of Extracts (Bar Graph).

4. Discussion

Interpretation of Pharmacognostical and Phytochemical Results

The pharmacognostical evaluation of the selected medicinal plants—Syzygium aromaticum (Clove), Costus speciosus (Costus), Plantago ovata, and Thymus vulgaris (Thyme)—was carried out to ensure proper authentication and identification of crude drugs before extraction and biological evaluation.

Pharmacognostical Findings:

Macroscopic characters such as color, odor, taste, and morphology were consistent with standard descriptions available in pharmacopeial monographs, confirming the authenticity of the plant materials. For example, Syzygium aromaticum buds were found to be dark brown, aromatic, and nail-shaped, while Plantago ovata seeds were pale brown and mucilaginous.

Microscopic analysis further confirmed diagnostic features: clove showed oil globules and calcium oxalate crystals, Costus speciosus rhizomes revealed starch granules and spiral xylem vessels, Plantago ovata seeds demonstrated mucilage-containing epidermal cells, and thyme leaves displayed glandular trichomes with oil droplets.

Physicochemical constants such as ash values, extractive values, and moisture content were within acceptable limits, ensuring purity and quality. The variations in ash and extractive values reflected differences in the phytoconstituent profiles of each plant, indicating the presence of bioactive metabolites.

5. Conclusion

The present study focused on the evaluation of antiviral activity of extracts from selected medicinal plants—Syzygium aromaticum (Clove), Costus speciosus (Costus), Plantago ovata, and Thymus vulgaris (Thyme)—against Enterovirus type 1 using RD cell models. The major findings can be summarized as follows:

1. Pharmacognostical and Physicochemical Evaluation:

Macroscopic, microscopic, and physicochemical studies confirmed the authenticity and quality of the plant materials.

Diagnostic characters such as oil globules (clove), starch granules (costus), mucilage cells (plantago), and glandular trichomes (thyme) were consistent with pharmacopeial standards.

2. Phytochemical Screening:

Extracts revealed the presence of flavonoids, tannins, phenols, terpenoids, steroids, glycosides, saponins, and mucilage.

Polar extracts (aqueous and hydroalcoholic) were particularly rich in phenolic and flavonoid compounds, while non-polar fractions (hexane, ethyl acetate) contained terpenoids and saponins.

3. Cytotoxicity Studies:

Hydroalcoholic and aqueous extracts generally demonstrated low cytotoxicity with high CC_{50} values, indicating safety.

Non-polar fractions (hexane, ethyl acetate) showed higher cytotoxicity, reducing their therapeutic potential.

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