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COMPARATIVE EXTRACTION, MCF-7 CYTOTOXICITY SCREENING, AND BIOASSAY-GUIDED FRACTIONATION OF TURRAEA VILLOSA ROOT EXTRACTS

Neha Patil[1]*, John Disouza[2], Mansingraj Nimbalkar[3], Popat Kumbhar[4]

[1,4]Department of Pharmaceutical Sciences, Tatyasaheb Kore College of Pharmacy, Warananagar, Maharashtra, India;
[2]Bombay Institute of Pharmacy & Research, Dombivli, Maharashtra, India;
[3]Department of Botany, Shivaji University, Kolhapur, Maharashtra, India.
*Corresponding Author: Neha Patil, Email: nehajpatil1994@gmail.com

ABSTRACT

Background:*Turraea villosa,*an underexplored member of the Meliaceae family, may contain cytotoxic secondary metabolites. Extraction methods can markedly affect yield, phytochemical enrichment, and biological activity**. Objective:** This study compared seven root extract preparations of *Turraea villosa* and used MCF-7 breast cancer cell viability reduction to guide selection and fractionation of the superior extract.

Methods: Authenticated *Turraeavillosa*plant material collected from Gaganbavada forest, Kolhapur, Maharashtra, India, was extracted by Soxhlet extraction, ultrasonication-assisted extraction, accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE). Methanol and water were used for solvent-based methods, while SFE was performed using supercritical CO2 at 350 bar and 55°C for 90 min with a CO2 flow rate of 30 g/min and ethanol as a co-solvent. Extract yields were calculated as % w/w. MCF-7 cells were treated with extracts and SFE fractions, and cell viability was determined using the MTT assay.

Results: SFE produced the highest yield (12.15% w/w) and showed the greatest extract-level reduction in MCF-7 cell viability, with 54.04% viability at 10 µg/mL and the lowest estimated IC_{50} (11.02 µg/mL). ASE-water and Soxhlet-water also showed notable activity, with estimated IC_{50} values of 14.18 and 15.60 µg/mL, respectively. SFE was fractionated into ten fractions. F2 and F3 produced the greatest reduction in MCF-7 cell viability, with viability values of 32.39% and 31.88%, respectively, at the screening concentration. F3 showed the lowest viability among the tested fractions and was prioritised for further phytochemical profiling.

Conclusion: SFE was the most suitable extraction method for enriching constituents that reduced MCF-7 cell viability under the tested conditions. These findings support further LC-MS-guided identification and mechanistic validation of active SFE fractions. However, because untreated cells served as the assay control and no reference anticancer drug or assessment of normal-cell selectivity was included, the results should be interpreted as preliminary cytotoxicity screening evidence rather than as confirmed anticancer efficacy.

KEYWORDS: *Turraea villosa; root extract; supercritical fluid extraction; accelerated solvent extraction; MCF-7; MTT assay; bioassay-guided fractionation; cytotoxicity.*

1. INTRODUCTION

Cancer remains a major cause of morbidity and mortality worldwide, and natural products continue to provide structurally diverse starting points for anticancer drug discovery [1-3]. Plant-derived metabolites have substantially contributed to oncology drug development because they span a broad chemical space and often interact with multiple biological targets [2,3]. The Meliaceae family is particularly relevant because it contains limonoids, triterpenoids, phenolics, and related constituents that have been reported to exhibit cytotoxic and antiproliferative activities [4,5].

Turraea villosa remains comparatively underexplored in terms of extraction-dependent cytotoxicity. For medicinal plants, the extraction method is a critical determinant of extract yield, chemical composition, and bioactivity [6,7]. Conventional Soxhlet extraction, ultrasonication-assisted extraction, accelerated solvent extraction, and supercritical fluid extraction differ in solvent use, temperature, pressure, matrix penetration, and selectivity for polar or non-polar constituents [7-10]. Therefore, a systematic comparison of extraction methods is necessary before downstream phytochemical profiling or formulation development.

The present manuscript focuses solely on the extraction-to-bioassay phase of the work. It reports root collection and authentication, preparation of seven extracts, percentage yield, MTT-based cell viability screening in MCF-7 breast cancer cells, selection of SFE as the prioritised extract, fractionation into ten fractions, and identification of the most active fraction. LC-MS, docking, and molecular dynamics analyses are reserved for a separate companion manuscript.

2. MATERIALS AND METHODS

2.1 Root material collection and authentication

Fresh *Turraea villosa* root material was collected from the Gaganbavada forest, Kolhapur, Maharashtra, India (16.5457904° N latitude and 73.8311296° E longitude). A herbarium sheet was prepared, and the plant material

was authenticated by the Botanical Survey of India. The voucher specimen No. BSI/WRC/Tech./2024/JVD-49 was deposited for reference. The collected root material was dried, pulverised with a laboratory grinder, sieved through sieve No. 40, and stored in a sealed container until extraction.

2.2 Preparation of extracts

Seven root extracts were prepared to compare the effects of extraction technique and solvent polarity: ASE-methanol, ASE-water, ultrasonication-methanol, ultrasonication-water, Soxhlet-methanol, Soxhlet-water, and SFE [7-10]. Extracts were concentrated after extraction and stored under refrigerated/low-temperature conditions until further analysis.

Soxhlet extraction: Dried and finely powdered root material (10 g) was accurately weighed and placed in an extraction thimble. The Soxhlet extractor was assembled with a round-bottom flask containing the selected solvent (methanol or water) and attached to a condenser. The solvent was heated to reflux, and extraction cycles were continued for 6-24 h, until the siphoned solvent became clear. After cooling, the extract-containing solvent was collected and concentrated using a rotary evaporator under reduced pressure to obtain the crude extract.

Ultrasonication-assisted extraction: Powdered root material (10 g) was transferred to a conical flask containing 100 mL methanol and sonicated in a bath-type ultrasonicator for 30 min at ambient temperature. The extract was filtered through Whatman No. 1 filter paper and centrifuged at 7000 rpm for 15 min to remove residual particulates. The same procedure was repeated using 100 mL distilled water under identical conditions.

Accelerated solvent extraction: Powdered root material (10 g) was thoroughly mixed with 10 g diatomaceous earth to ensure uniform dispersion and prevent clogging. The mixture was packed into an ASE extraction cell, and extraction was performed at 45°C under nitrogen pressure of 1400 psi for 45 min. Methanol and distilled water were used separately as extraction solvents. Extracts were collected and refrigerated until analysis.

Supercritical fluid extraction: Powdered root material (100 g) was loaded into an extraction bag and placed in the extraction vessel of the SFE apparatus. Extraction was performed using supercritical CO2 at 350 bar and 55°C for 90 min with a CO2 flow rate of 30 g/min. Ethanol was used as a co-solvent. The extract was collected and stored at low temperature until analysis.

Table 1. Extraction conditions used for preparation of *Turraea villosa* root extracts.

Extraction method	Sample amount	Solvent/medium	Key operating parameters
Soxhlet extraction	10 g root powder	Methanol or water	Reflux extraction in Soxhlet apparatus for 6-24 h, until the siphoned solvent became clear; extract concentrated under reduced pressure using rotary evaporation.
Ultrasonication-assisted extraction	10 g root powder	100 mL methanol or 100 mL distilled water	Bath-type ultrasonication for 30 min at ambient temperature; filtration through Whatman No. 1 paper; centrifugation at 7000 rpm for 15 min.
Accelerated solvent extraction	10 g root powder + 10 g diatomaceous earth	Methanol or distilled water	Extraction at 45°C under nitrogen pressure of 1400 psi for 45 min; extracts collected and refrigerated.
Supercritical fluid extraction	100 g root powder	Supercritical CO2 with ethanol co-solvent	SFE at 350 bar and 55°C for 90 min with CO2 flow rate of 30 g/min and ethanol as co-solvent; extract collected and stored at low temperature.

2.3 Percentage yield

The dried weight of each extract was recorded. Percentage yield was calculated using the following equation:
Percentage yield (% w/w) = (weight of dried extract/weight of dry root powder) × 100

2.4 MTT assay for extract and fraction cytotoxicity screening

The MTT assay was used to assess the ability of extracts and fractions to reduce MCF-7 cell viability by measuring mitochondrial metabolic activity [11,12]. MCF-7 breast cancer cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, and maintained at 37°C in a humidified 5% CO2 incubator.

Cells were seeded into 96-well microtiter plates at approximately 1 × 10^4 cells/well and incubated overnight to allow attachment. For crude-extract screening, cells were treated with different extract concentrations and incubated for 48 h under standard culture conditions. Untreated cells served as the assay control. No reference anticancer drug was included in the final analysed dataset.
After treatment, freshly prepared MTT reagent (5 mg/mL in phosphate-buffered saline) was added to each well at 10 µL per 100 µL culture medium, yielding a final MTT concentration of approximately 0.5 mg/mL. Plates were incubated at 37°C for 3-4 h to allow formazan crystal formation. The medium was then carefully removed, and 100 µL DMSO was added to dissolve the formazan crystals. Plates were gently shaken for 10-15 min to ensure complete dissolution. Absorbance was measured at 570 nm using a microplate reader, with background/reference correction at 630-690 nm where applicable.
For SFE fraction screening, cells were seeded at 10,000 cells/well, treated with SFE fractions at the selected screening concentration of 10 µg/mL, and incubated for 48 h. Fraction treatments were performed in triplicate. Cell viability was calculated using the following equation:
Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100
The IC_{50} was defined as the estimated concentration required to reduce MCF-7 cell viability by 50% under the assay conditions. IC50 values were derived from concentration-response viability data using nonlinear regression in GraphPad Prism and were used for comparative ranking of extract activity rather than definitive potency benchmarking, because no reference anticancer drug was included in the final analysed dataset.

2.5 Statistical analysis

Percentage inhibition data from extract screening were expressed as mean ± SD. Because the three replicate measurements were entered as matched replicate rows across extract groups in GraphPad Prism (version 8.0.1), percentage inhibition values were analysed using repeated-measures one-way ANOVA with the Geisser-Greenhouse correction. A p-value < 0.05 was considered statistically significant. Dose-response curve fitting was performed using nonlinear regression, and goodness of fit was assessed using R^2 values. The statistical analysis was used to support extract prioritisation within this screening dataset and was not used as evidence of therapeutic efficacy.

2.6 Bioassay-guided fractionation of the superior extract

The SFE extract was selected for fractionation because it produced the highest extract yield and the lowest estimated IC_{50} among all crude extracts. Approximately 5 g of SFE extract was dissolved in a minimal volume of analytical-grade methanol, briefly sonicated to ensure complete dissolution, and filtered through Whatman No. 1 filter paper to remove particulate matter. The extract solution was adsorbed onto a small quantity of silica gel and dried to obtain a free-flowing powder for chromatographic loading [13].
Flash column chromatography was performed using silica gel (60-120 mesh) as the stationary phase. The column was packed by the wet slurry method using n-hexane as the initial solvent. The adsorbed sample was carefully loaded onto the top of the packed column without disturbing the stationary phase. Elution was carried out using a stepwise polarity gradient from non-polar to more polar solvent systems. Fractions were collected, evaporated to dryness, weighed individually, and grouped according to similar chromatographic behaviour. Ten major fractions, F1-F10, were obtained and screened in MCF-7 cells using the MTT assay at 10 µg/mL. The fraction showing the lowest cell viability was prioritised for further phytochemical characterisation [13].

3. RESULTS

3.1 Percentage yield of extracts

The extraction yield varied across methods and solvents, indicating that extraction technology and solvent polarity influenced phytoconstituent recovery. SFE produced the highest yield (12.15% w/w), followed by ASE-methanol (11.60% w/w), Soxhlet-water (10.65% w/w), ultrasonication-methanol (10.20% w/w), Soxhlet-methanol (9.80% w/w), ASE-water (9.30% w/w), and ultrasonication-water (8.50% w/w).

Table 2. Percentage yield of *Turraea villosa* root extracts prepared by different extraction methods.

Extraction method	Solvent/medium	Yield (% w/w)
ASE	Methanol	11.60
ASE	Water	9.30
Ultrasonication	Methanol	10.20
SFE	Supercritical CO2 with ethanol co-solvent	12.15
Soxhlet	Methanol	9.80
Soxhlet	Water	10.65
Ultrasonication	Water	8.50

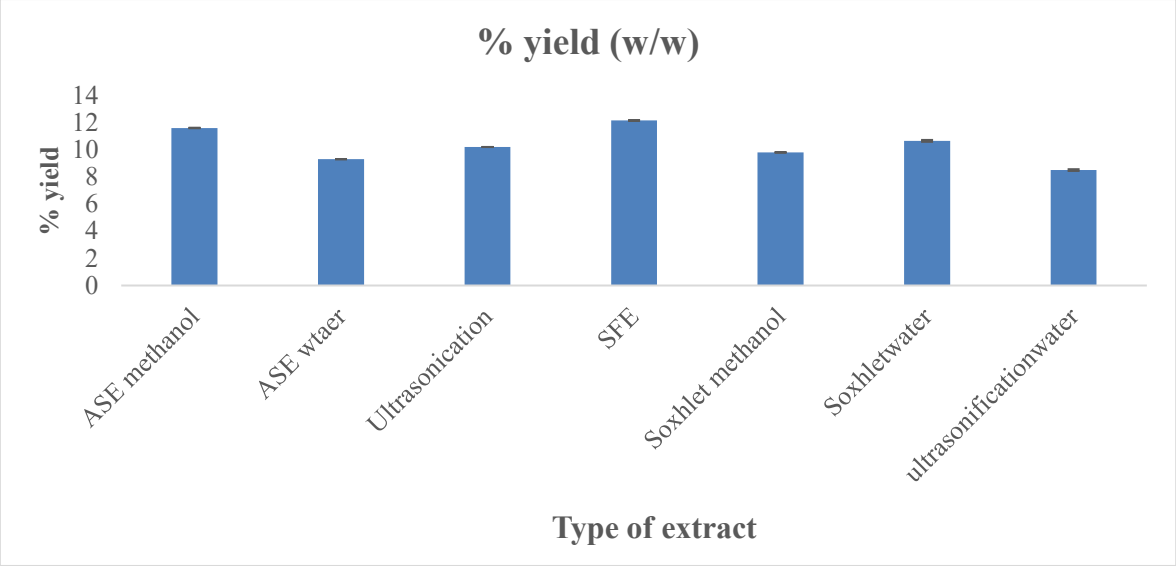

Fig. 1. Percentage yield of *Turraea villosa* root extracts prepared by different extraction methods.

3.2 MTT cytotoxicity screening of crude extracts in MCF-7 cells

All extract preparations produced varying degrees of reduction in MCF-7 cell viability. SFE produced the greatest reduction in cell viability among the crude extracts, with 45.94 ± 0.29% inhibition, 54.04% viability at 10 µg/mL, and an estimated IC_{50} of 11.02 µg/mL. ASE-water and Soxhlet-water also showed notable activity, with estimated IC_{50} values of 14.18 and 15.60 µg/mL, respectively. Ultrasonication-water was the least active extract, with 86.96% viability at 10 µg/mL and an estimated IC_{50} of 59.56 µg/mL.

Table 3. Cytotoxic activity of *Turraea villosa* root extracts against MCF-7 cells determined by MTT assay.

Extract	Solvent/medium	Extraction method	Average absorbance	Viability at 10 µg/mL (%)	Inhibition (%)	Estimated IC50 (µg/mL)	R^2
Extract 1	Methanol	ASE	0.587	72.92	27.01 ± 0.20	20.48	0.9996
Extract 2	Water	ASE	0.497	61.74	38.28 ± 0.62	14.18	0.9949
Extract 3	Methanol	Ultrasonication	0.617	76.65	23.28 ± 1.05	25.33	0.9975
Extract 4	Supercritical CO2	SFE	0.435	54.04	45.94 ± 0.29	11.02	0.9873
Extract 5	Methanol	Soxhlet	0.669	83.11	16.90 ± 0.89	32.85	0.9889
Extract 6	Water	Soxhlet	0.519	64.47	35.46 ± 0.98	15.60	0.9965
Extract 7	Water	Ultrasonication	0.712	86.96	13.00 ± 1.28	59.56	0.9993
Control	-	-	0.805	100.00	-	-	-

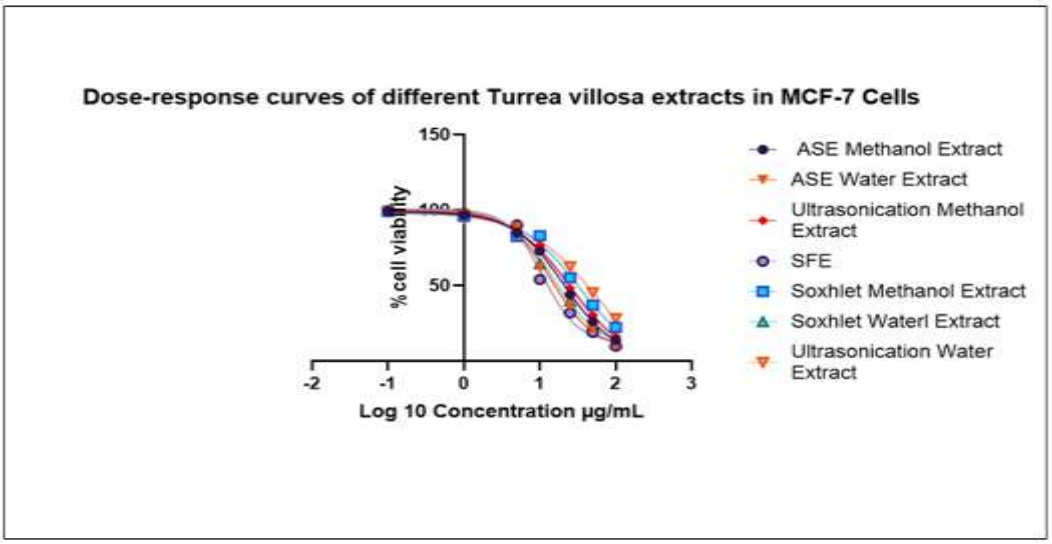

Fig. 2. Dose-response curves of *Turraea villosa* root extracts in MCF-7 breast cancer cells.

Repeated-measures one-way ANOVA with Geisser-Greenhouse correction indicated significant extract-dependent variation in percentage inhibition among the tested extracts ($p = 0.0002$; corrected model $R^2 = 0.9981$). These results support selecting SFE for further fractionation, given its comparatively higher inhibition and lower estimated IC_{50} under the present assay conditions.

3.3 Concentration-dependent cell viability pattern

A concentration-dependent reduction in MCF-7 cell viability was observed across extract treatments. The concentration-response pattern supported dose-dependent cytotoxicity screening and was used to fit nonlinear curves and estimate IC_{50} values. The values in Table 4 are presented as representative viability values from the dose-response dataset and support the comparative activity ranking obtained in the extract-screening assay.

Table 4. Representative concentration-response viability pattern of *Turraea villosa* root extracts in MCF-7 cells.

Concentration (µg/mL)	ASE Methanol	ASE Water	Ultrasonication Methanol	SFE	Soxhlet Methanol	Soxhlet Water	Ultrasonication Water
0	100	100	100	100	100	100	100
10	72.92	61.74	76.65	54.04	83.11	64.47	86.96
20	50.89	41.25	57.12	35.22	67.48	44.72	75.14
40	31.22	24.86	37.64	21.08	48.92	27.42	62.18
60	22.84	18.11	28.56	15.46	37.81	20.35	51.86
80	18.12	14.39	23.12	12.34	30.96	16.25	43.82
100	15.06	12.02	19.49	10.29	26.21	13.67	37.41
Estimated IC_{50} (µg/mL)	20.48	14.18	25.33	11.02	32.85	15.60	59.56

3.4 Screening of SFE fractions

The superior SFE extract was fractionated into 10 major fractions and screened using the MTT assay at 10 µg/mL. The control absorbance was 0.4762, corresponding to 100% cell viability. F2 and F3 produced the greatest reduction in MCF-7 cell viability, with viability values of approximately 32.39% and 31.88%, respectively. F3 showed the lowest cell viability among all fractions and was therefore prioritised for further chemical and computational evaluation. F4, F5, and F6 also reduced cell viability, indicating that bioactive constituents may be distributed across mid-polar fractions.

Table 5. MCF-7 cell viability after treatment with SFE fractions of *Turraea villosa* root extract at 10 µg/mL.

Fraction	Average absorbance	Cell viability (%)	Activity interpretation
F1	0.3505	73.62	Weak activity
F2	0.1542	32.39	Marked viability reduction
F3	0.1518	31.88	Lowest viability; prioritized
F4	0.2199	46.19	Moderate viability reduction

F5	0.1751	36.78	Marked viability reduction
F6	0.1754	36.86	Marked viability reduction
F7	0.3413	71.69	Weak activity
F8	0.3248	68.23	Weak to moderate activity
F9	0.3473	72.96	Weak activity
F10	0.3660	76.88	Weak activity
Control	0.4762	100.00	Untreated control

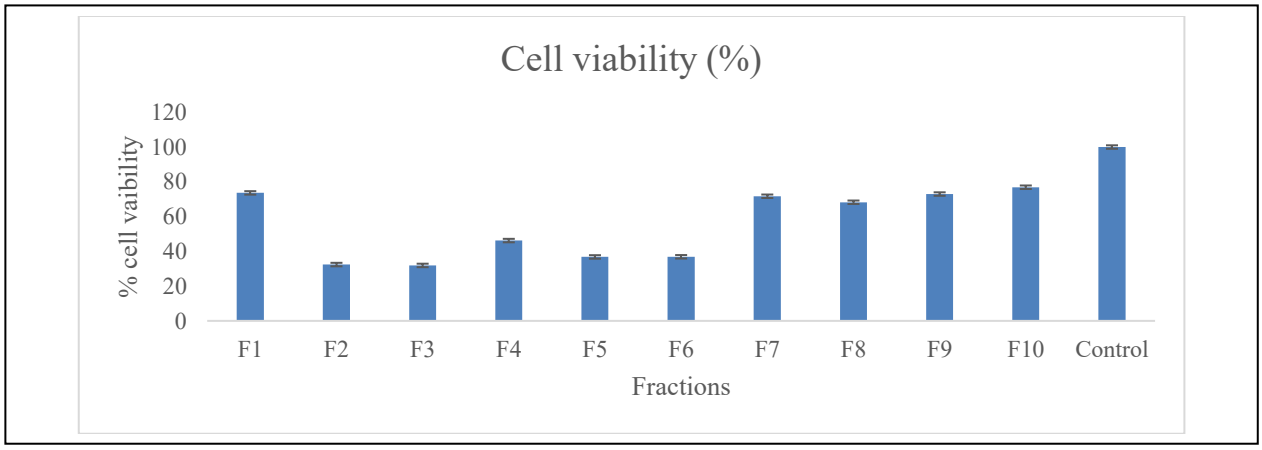

Fig. 3. Percentage cell viability of MCF-7 cells after treatment with SFE fractions.

3.5 Dose-response behaviour of the selected F3 fraction

The selected F3 fraction showed a concentration-dependent decrease in MCF-7 cell viability. Viability declined from 82.70% at 0.1 µg/mL to 47.15% at 10 µg/mL and to 20.40% at 100 µg/mL. Based on the dose–response profile, the estimated IC_{50} of the F3 fraction was approximately 8 µg/mL under the present assay conditions. This finding indicates enrichment of constituents that reduce MCF-7 cell viability in F3, thereby justifying its selection for LC-MS profiling in the companion study.

Table 6. Dose-response results for the selected F3 fraction.

F3 concentration(µg/mL)	Average absorbance	Cell viability (%)
0.1	0.3820	82.70
1	0.3183	68.90
5	0.2554	55.30
10	0.2177	47.15
25	0.1667	36.10
50	0.1296	28.05
75	0.1079	23.35
100	0.0942	20.40

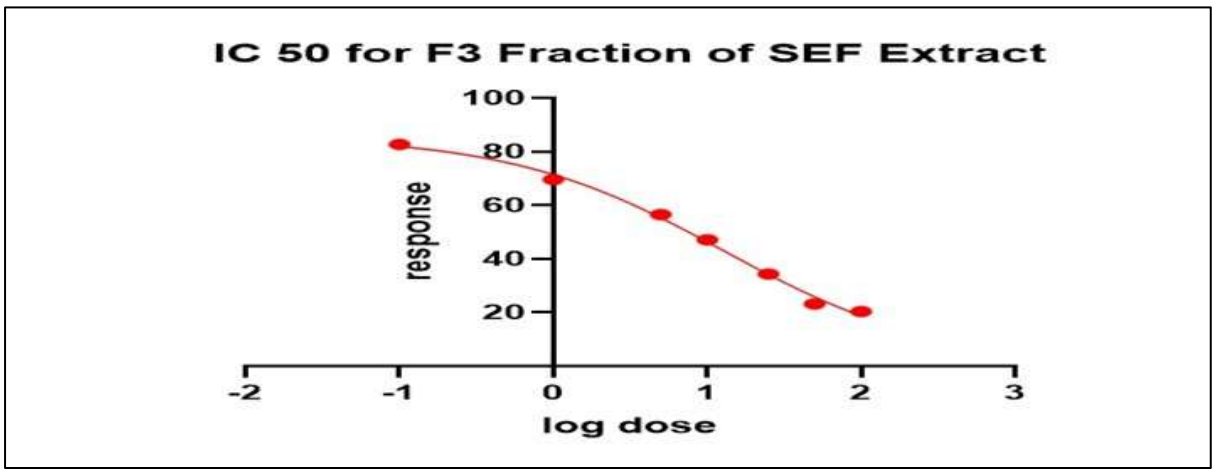

Fig. 4. Dose-response curve for IC_{50} determination of F3 fraction from SFE extract

4. DISCUSSION

The present study demonstrates that the extraction strategy strongly affected both the recovery yield and the MCF-7 cell viability response to*Turraeavillosa* root extracts. SFE produced the highest percentage yield and the greatest reduction in MCF-7 cell viability among the seven extract preparations. This may be attributable to enhanced penetration of the root matrix by supercritical CO2 under high pressure and controlled temperature, combined with ethanol-assisted enrichment of non-polar to moderately polar phytoconstituents [7,10]. The CO2-based extraction environment may also reduce thermal degradation and residual organic solvent burden, which is useful for downstream pharmaceutical development [10].

ASE-methanol showed a high extraction yield, but SFE provided the most favourable combination of yield, reduced cell viability, cleaner extraction conditions, and suitability for subsequent formulation or mechanistic work. ASE-water and Soxhlet-water also showed meaningful cytotoxicity screening responses, suggesting that polar constituents may contribute to activity; however, the comparatively lower estimated $IC_{50}$of SFE supported its selection as the prioritised extract under the tested conditions [7-10].

The fractionation results further support the value of bioassay-guided separation. The activity of the crude SFE extract was not evenly distributed across all fractions. Instead, F2 and F3 produced markedly greater reductions in MCF-7 cell viability, whereas F1, F7, F8, F9, and F10 showed comparatively weak activity. This pattern suggests enrichment of active constituents within a limited fraction range and provides a rational basis for LC-MS-guided compound prioritisation [13].

F3 showed the lowest viability in the fraction-screening assay and exhibited concentration-dependent reduction in cell viability; therefore, it was prioritised for further phytochemical and computational evaluation. Nevertheless, these data should be interpreted cautiously. MTT reduction reflects metabolic activity and does not, by itself, distinguish cytostatic effects from cell death [11,12]. Furthermore, the study did not include normal-cell cytotoxicity or a standard anticancer drug comparator in the final analysed dataset. Therefore, the findings support cytotoxic potential and prioritisation for further study, but they do not establish cancer selectivity or therapeutic efficacy [14,15].

5. CONCLUSION

This study compared seven *Turraea villosa* root extract preparations and identified SFE as the most promising extraction method based on yield percentage and MCF-7 cell viability reduction. SFE achieved the highest yield (12.15% w/w), the lowest estimated crude-extract IC_{50} (11.02 µg/mL), and the greatest inhibition profile among the tested extracts. Bioassay-guided fractionation of the SFE extract identified F2 and F3 as the fractions producing the greatest reduction in MCF-7 cell viability, with F3 showing the lowest cell viability among the tested fractions. IC_{50} of the F3 fraction was approximately 8 µg/mL The findings establish a rational extraction and fractionation workflow for Turraea villosa root-derived cytotoxic constituents and support subsequent LC-MS-guided compound identification and target-based computational prioritisation.

LIMITATIONS

The study was limited to MCF-7 breast cancer cells, and selectivity for normal cells was not evaluated. A reference anticancer drug was not included in the final comparative dataset, limiting direct potency benchmarking. Fraction-level screening identified active fractions, but individual compounds were not isolated in this manuscript. Mechanistic interpretation requires additional assays because MTT results alone cannot confirm apoptosis, cell-cycle arrest, necrosis, or the presence of a defined molecular target [11,12,14,15].

Declarations

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Author contributions / CRediT

Neha Patil: Conceptualisation, methodology, investigation, formal analysis, data curation, visualisation, software, original draft, review and editing, project administration. John Disouza: Conceptualisation, supervision, methodology, validation, review and editing. Mansingraj Nimbalkar: Resources, validation, plant authentication support, review and editing. Popat Kumbhar: Supervision, methodology, validation, resources, review and editing. All authors approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement
This study did not involve human participants or experimental animals. Root material was authenticated by the Botanical Survey of India, and in vitro assays were performed using authenticated cell-culture resources.

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