International Journal of Pharmaceutical Sciences and Drug Research, 2024;16(6):931-939

Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : https://ijpsdronline.com/index.php/journal

Research Article

Evaluation of Pharmacognostic, Phytochemical, Antioxidant and Anticancer Properties of Leaves of *Cassia tora* and *Mimusops elengi*

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ARTICLE INFO

Article history:

Received: 25 July, 2024

Revised: 10 October, 2024

Accepted: 16 October, 2024

Published: 30 November, 2024

Keywords:

Mimusops elengi, *Cassia tora*, HPTLC, Antioxidant, Anticancer.

DOI:

10.25004/IJPSDR.2024.160602

ABSTRACT

The objective of the present research work was to study the selected medicinal plants regarding their botanical, phytomoieties present, free radical scavenging, and antineoplastic properties of the alcoholic extract of leaves from *Mimusops elengi* (MEAE) and *Cassia tora* (CTAE). An attempt was also made to compare the results obtained with proven anticancer marker compounds like curcumin, quercetin, gallic acid, and ursolic acid. Pharmacognostic studies were conducted using standard methods and protocols. Phytochemical analysis was performed using HPTLC studies. The antioxidant potential of selected plant extracts has been evaluated using a DPPH assay. The selected activity was evaluated using a sensitive and reliable MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay towards MCF-7 (breast), HeLa (Cervical), and HepG2 (liver) cells. Self-programmed cell death or apoptosis assay was carried out on MCF-7 cancer cells to explore the possible mechanism of the anticancer effect of these plant extracts. Pharmacognostic evaluations confirmed the identity, quality, and safety of the botanical specimens used. HPTLC analysis revealed distinct phytochemical profiles rich in gallic acid, quercetin, ursolic acid, and similar compounds in both extracts. The study demonstrated concentration-dependent antioxidant activity, with MEAE exhibiting stronger activity than CTAE. The results thus obtained in the MTT assay exhibited that cancer cell multiplication of MCF-7, HeLa, and HepG2 cancer cells was prevented by the selected extracts of MEAE and CTAE in a reproducible dose-dependent manner over time. Results of the apoptosis assay gave the authors a probable thought and direction that the anticancer activity of CTAE and MEAE towards MCF-7 breast cancer cells may be due to induction of apoptosis.

Introduction

Traditional Indian systems of medicine have been employed for millennia to treat ailments and promote a balanced, healthy lifestyle.[1] It blends our modern lifestyle with the ancient wisdom of using natural resources.[2] Historically, mankind has been dependent on mother nature for its sufferings.[3] Plants have been primary sources of prevention and therapeutics. Off-late it has been observed that the quality of life of humans is continuously deteriorating, leading to a rise in diseases.[4] Several cancer chemotherapeutics, such as vincristine, etoposide, paclitaxel, and topotecan, are derived from plants and are renowned for their effectiveness in treatment.[5] In addition to terrestrial plants, significant discoveries have been made in marine environments, microbes, and even slime molds, yielding promising compounds for combating cancer.[6] Despite the tremendous strides made in cancer research, including the development of targeted therapies and immunotherapies, cancer remains a formidable global health challenge.[7] Natural products have been recognized as an effective approach in fighting against various diseases, including cancer, as they are widely accessible, economical, and have lesser toxic side effects.[8] Cancer is a deadly disease driven by increased oxidative stress, which accelerates its progression by triggering genetic mutations, damaging cell membranes

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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through lipid peroxidation, and causing harmful DNA damage.[9] Plant extracts with promising bioactive phytochemicals, like polyphenols (curcumin and gallic acid), flavonoids (quercetin), and terpenoids (ursolic acid), have been shown to neutralize free radicals and alleviate oxidative-stress-related conditions including cancer.[10] The medicinal plants shortlisted for the research work after an exhaustive literature search were *Cassia tora* and *Mimusops elengi*.[11]

C. tora (Chakwad), a member of the Fabaceae family, thrives across India and other tropical regions. Historically, this versatile plant has been utilized in traditional medicine, with its roots, leaves, flowers, fruits, and seeds employed to address a diverse range of ailments, including constipation, leprosy, skin disorders, eye conditions, liver ailments, and rheumatic diseases.[12] Previous studies have detailed the chemical compounds in *C. tora* that contribute to its medicinal properties, including alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenols.[13] Numerous studies have shown that the plant *C. tora* possesses potent anti-inflammatory, antimicrobial, antioxidant, and anticancer properties.[11,12,14] The plant is found to be a rich reservoir of promising phytochemicals like quercetin, epicatechin, gallic acid, apigenin, chrysophanol, kaempferol, and leucopelargonidine which may act as potent antioxidants and protect against DNA and cancer.[15] Several promising phytoconstituents, including quercetin, luteolin, formononetin, and chrysophanol, have been isolated from leaves of *C. tora* and shown to exhibit activity against cancer cells.[12,16] *M. elengi* (Bakul or Maulsiri), from the Sapotaceae family, is a valued Indian native plant renowned for its extensive traditional medicinal heritage. Various parts of the plant, like seed, fruit, bark, leaf, and flower, are used to treat dental problems, maintain oral hygiene, and treat conditions like fever, diarrhea, gonorrhea, leucorrhea, and constipation.[17] From this species, several triterpenoids, steroidal glycosides, flavonoids, phenolic compounds, and alkaloids have been identified. Previous studies show that extracts from *M. elengi* aerial parts are rich in antioxidant compounds such as hentriacontane, gallic acid, lupeol, β-sitosterol, β-carotene, hederagenin, taraxerol, ursolic acid, and quercetin which may protect against DNA damage caused by free radicals.[18] Several preclinical studies have demonstrated that the plant possesses potent wound healing,[19] antimicrobial,[20] hepatoprotective,[21] anti-inflammatory,[22] anticancer,[23] and antioxidant activities.[17]

This study aims to analyze *C. tora* and *M. elengi* leaves using pharmacognostic and chromatographic techniques, focusing on compounds with antioxidant and anticancer properties. The study offers a scientifically conducted reference study offering opportunities for future researchers regarding developing natural health supplements and therapeutics, particularly for oxidative stress-related conditions, including cancer. Research highlights bioactive compounds like gallic acid, quercetin, and ursolic acid as promising compounds for targeted cancer therapies with potentially fewer side effects. Pharmacognostic validation ensures safety and regulatory compliance, supporting the use of plant extracts like MEAE and CTAE as cost-effective, eco-friendly alternatives to synthetic drugs.

Materials and Methods

Collection and Authentication of Selected Plant Part

The fresh and matured leaves of *M. elengi* and *C. tora* were personally collected from their native surroundings in the month of November 2020 from the local areas of Lucknow and authenticated at CSIR-NISCAIR, Delhi, by a scientist Dr. Sunita Garg.

Morphological, Microscopical, Physicochemical, and Phytochemical Studies

These studies were conducted as per standard methods and protocols.[24-27]

Extraction

The dried and coarsely powdered leaves of selected plants were extracted with aqueous ethanol using the soxhlet method.[28]

Phytochemical profiling of quercetin, gallic Acid, and ursolic acid using HPTLC

High-performance thin-layer chromatography (HPTLC) analysis was carried out using a CAMAG system equipped with silica gel $60F_{254}$ plates as the stationary phase. The test solutions were prepared from CTAE and MEAE extracts in concentrations of 5 to 10 mg per 5 to 10 mL of methanol. The chromatographic process involved the precise application of these solutions onto the plates, followed by development using specific mobile phases suited to the compounds of interest. After the chromatographic separation, the plates were treated with distinct derivatizing agents to enhance the visibility of the compounds. The plates were then analyzed under white light, which provides a general view of all the compounds, and under UV light at wavelengths of 254 and 366 nm (HPTLC analysis parameters for quercetin, gallic acid, and ursolic acid are summarized Table 1).

Antioxidant activity via DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging assay

Standard ascorbic acid and MEAE/CTAE test extract solutions (1-mL) at concentrations of 250 to 1000 µg/mL were mixed with 3 mL of 0.5 mmol DPPH solution in methanol. The mixture was then incubated at 37°C for 30 minutes. After incubation, absorbance was measured

Table 1: HPTLC analysis parameters for quercetin (QC), gallic acid (GA), and ursolic acid (UA)

Parameters	*Quercetin*	*Gallic acid*	*Ursolic acid*
Mobile Phase	Toluene:Ethyl acetate:Acetic acid (5:5:1)	Toluene:Ethyl acetate:Acetic acid (5:5:1)	Toluene:Ethyl acetate: (8.5:15)
Standard Solutions	0.5/1 mg of quercetin in 1-mL of methanol	0.5/1 mg/mL of gallic acid in 1-mL of methanol	5 mg of ursolic acid in 10 mL of methanol.
Derivatizing Agents	2-Aminoethyl diphenylborinate (NP)	2-Aminoethyl diphenylborinate (NP)	Anisaldehyde sulfuric acid

at 517 nm using a UV-vis spectrophotometer. Three measurements were taken, and their average was calculated, after which the DPPH scavenging activity was determined using the formula:[29]

Percentage (%) Inhibition = $(A_{control} - A_{sample} / A_{control}) \times 100$

In this formula, A represents the absorbance, control refers to the DPPH solution without any sample, and the sample represents the DPPH solution containing the test /standard compound.

In-vitro screening of anticancer activity

For the screening process, MCF-7, HeLa, and HepG2 cancer cell lines were obtained from NCCS Pune, India. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), enriched with 10% fetal bovine serum (FBS) and 1% antibiotics (Penicillin and Streptomycin) and maintained at 37°C with 5% CO_2 in a humidified incubator.

MTT assay for evaluation of cytotoxicity on MCF-7, HeLa, and HepG2 cancer cells

In this experiment, 5000 cells per well were seeded in 96-well plates and incubated at 37°C with 5% CO_2 for a few hours. The cells were then exposed to varying concentrations (10–500 µg/mL) of plant extracts (CTAE and MEAE) prepared in DMSO for 24 hours. As standards, quercetin, gallic acid, curcumin, and methotrexate were tested at concentrations of 10 to 200 µg/mL. After a 24-hour treatment, the plates were incubated at 37°C in 5% CO_2 and cell toxicity and IC_{50} values were determined. Following incubation 20 µL of 5 mg/mL MTT was added to each well, and the plates were further incubated for another 4 to 5 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a MultiSkan UV-vis spectrometer to determine cell viability relative to controls.[30] Three readings were noted down and the average of their readings was then taken.

Apoptotic (AO/EtBr) double staining assay

In this experiment, 50,000 cells per well were seeded in a 24-well plate and incubated at 37°C with 5% CO_2 in DMEM with FBS and antibiotics. After cells attained morphology, they were treated with extracts at concentrations of 100, 200, and 500 µg/mL and incubated for 24 hours. The cells were then washed, trypsinized, and suspended in PBS. A staining solution (100 µg/mL AO and EtBr) was added to the cell suspension, and the mixture was examined under a fluorescent microscope. AO stained both live and dead cells, while EtBr stained only dead cells with compromised membranes.[31]

Results

Morphological Studies

The leaves of *C. tora* are a compound paripinnate, up to 8 cm long, with three pairs of opposite pale yellowish-green leaflets. They have a characteristic odor, bitter taste, and papery texture oblong shape with a rounded apex, entire margin, and reticulate venation. The leaflets with pubescent surfaces are up to 2 to 5 cm long and 1 to 2 cm wide (as depicted in Fig. 1).

The fresh leaves of *M. elengi* are glossy, light to dark green, with a distinctive odor, and astringent taste. The leaves are up to 7 to 11 cm long and 3 to 5 cm wide. Leaves are variable, petiolate, elliptic, ovate to lanceolate in shape, with an acuminate apex, undulate margin, and reticulate venation (as depicted in Fig. 2).

Microscopical, Physicochemical, and Phytochemical Studies

The observations of microscopical, physicochemical, and phytochemical analysis are placed in Figs 3-6 and Tables 2, 3, and 4.

Fig. 1: Morphology of *C. tora* leaves

Fig. 2: Morphology of *M. elengi* leaves

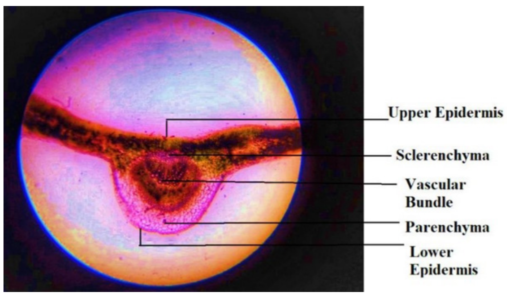

Fig. 3: T. S. of *C. tora* leaf

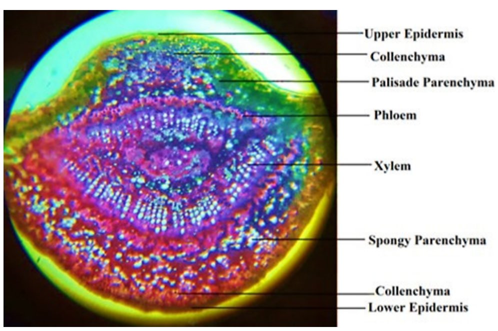

Fig. 4: T. S. of *M. elengi* leaf

Phytochemical Profiling of Gallic Acid, Quercetin, and Ursolic Acid using HPTLC

Standards gallic acid (GA) and quercetin (QC) appeared as a prominent blue and yellow colored band at R_f values of 0.4 and 0.5 mm, while ursolic acid (UA) appeared as a yellow-colored band at an R_f value of 0.2 mm. The HPTLC analysis of CTAE showed the presence of 7 major components with

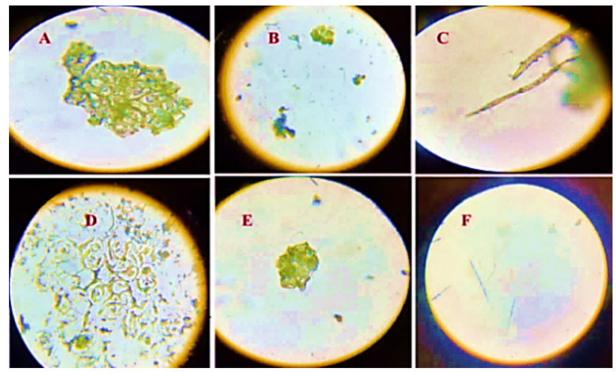

Fig. 5: Powder microscopy of *C. tora* leaf (A) Stone cells, (B) Calcium oxalate crystals, (C) Fibers, (D) Stomata, (E) Epidermal cells, and (F) Acicular calcium oxalate crystals

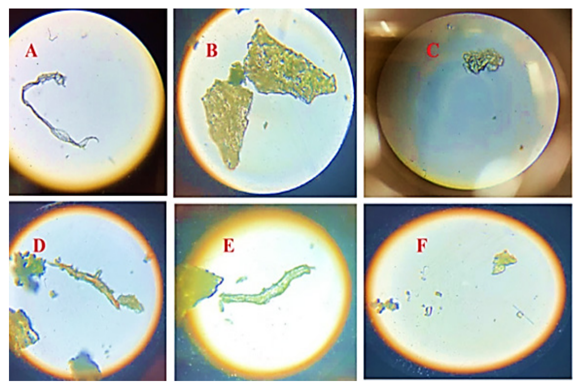

Fig. 6: Powder microscopy of *M. elengi* leaf (A) Thin non-lignified fibers, (B) Epidermal cells, (C) Stone cells, (D) Lignified fibers (E) Non-lignified fibers, and (F) Acicular and prismatic calcium oxalate

Table 2: Quantitative microscopical parameters of *C. tora* and *M. elengi* leaves

Quantitative microscopical parameters					
Test drug extract	*Stomatal No.*	*Stomatal index*	*Vein islet number*	*Vein termination number*	*Palisade ratio*
C. tora	15.7 ± 0.36	17.18 ± 0.4	16.3 ± 0.5	13.2 ± 0.5	15.1 ± 0.7
M. elengi	11.5 ± 0.4	20.4 ± 0.5	12.58 ± 0.7	6.54 ± 0.53	6.4 ± 0.1

Table 3: Physicochemical parameters of leaf powder of *C. tora* and *M. elengi*

Physicochemical parameters						
Test drug extract	Total ash	Acid insoluble ash	Water soluble ash	Moisture content	Water soluble extractive	Alcohol soluble extractive
C. tora	13.24 ± 0.5	4.61 ± 0.1	6.94 ± 0.79	6.43 ± 0.24	24.64 ± 0.6	10.24 ± 0.25
M. elengi	4.85 ± 0.53	1.89 ± 0.18	2.58 ± 0.34	5.35 ± 1.07	21.99 ± 0.38	14.94 ± 0.70

Table 4: Phytochemical analysis of *C. tora* and *M. elengi*

Phytoconstituents	*M. elengi*	*C. tora*
Alkaloids	+	+
Saponins	+	+
Glycosides	+	+
Phenols	+	+
Tannins	+	+
Steroids	+	+
Triterpenoids	+	+
Flavonoids	+	+

Where the + sign means presence and the - sign means the absence of phytoconstituent.

R_f values ranging from 0.049 to 0.850. In the extract of CTAE, a peak of GA (fifth) and QC (sixth) appeared at an R_f value of 0.369 and 0.494. In the CTAE, no distinct peak of UA was detected. The analysis of the MEAE identified five key components, with R_f values ranging between 0.406 and 0.883. In the MEAE extract, peaks corresponding to GA (R_f 0.406), QC (R_f 0.535), and UA (R_f 0.19) were observed, with 0.17% of ursolic acid (UA) being quantified (HPTLC analytical data is shown in Figs 7-10 and Tables 5 and 6).

DPPH-based in-vitro antioxidant evaluation

In this study, both CTAE and MEAE showed concentration-dependent antioxidant activity. Although their activity was lower than the standard compound ascorbic acid, which achieved 98% inhibition, CTAE and MEAE still showed significant antioxidant activity with 80.72 and 85.24% inhibition, respectively (as shown in Fig. 11).

MTT assay for cytotoxicity evaluation in cancer cells

The MTT assay results demonstrated that both MEAE and CTAE extracts effectively inhibited the growth of MCF-7, HeLa, and HepG2 cancer cell lines in a dose-dependent manner, with the extent of inhibition increasing over time, as shown in Figs 12-14. Among the three cell lines, MCF-7 cells exhibited the highest sensitivity to the extracts, displaying the strongest cytotoxic effects as tabulated in Table 7.

Apoptotic (AO/EtBr) double staining assay

The apoptotic assay was utilized to monitor the effects of treatments on the nuclei of cancer cells. This method allows for the precise differentiation between normal cells and those undergoing apoptosis, providing clear insights into the mechanism of action of these extracts. The fluorescent microscopy analysis highlights the dose-dependent anticancer effects of MEAE and CTAE, which are likely due to apoptosis induction in MCF-7 cancer cells (as depicted in Fig. 15).

Table 5: HPTLC peak table of *C. tora* (CTAE)

Parameter	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5 GA	Peak 6 QC	Peak 7
Start R_f	0.036	0.053	0.092	0.260	0.307	0.422	0.715
Max R_f	0.040	0.068	0.110	0.281	0.349	0.465	0.806
End R_f	0.049	0.089	0.128	0.292	0.369	0.494	0.850
Height (Start)	0.0000	0.0000	0.0000	0.0029	0.0042	0.0159	0.0213
Height (Max)	0.0142	0.0626	0.0171	0.0147	0.0244	0.1210	0.0931
Height (End)	0.0000	0.0000	0.0000	0.0046	0.0128	0.0304	0.0116
%Area	0.65	7.29	2.03	1.76	6.63	30.47	51.17

Table 6: HPTLC peak table of *M. elengi* (MEAE)

Parameter	*Peak 1 GA*	*Peak 2*	*Peak 3 QC*	*Peak 4*	*Peak 5*
Start R_f	0.347	0.414	0.483	0.561	0.704
Max R_f	0.383	0.460	0.504	0.654	0.786
End Rf	0.406	0.482	0.535	0.704	0.883
Height (Start)	0.0086	0.0116	0.0406	0.0262	0.0685
Height (Max)	0.0333	0.0671	0.0799	0.1636	0.1792
Height (End)	0.0137	0.0409	0.0266	0.0685	0.0077
Area%	3.54	7.69	7.75	34.83	46.19

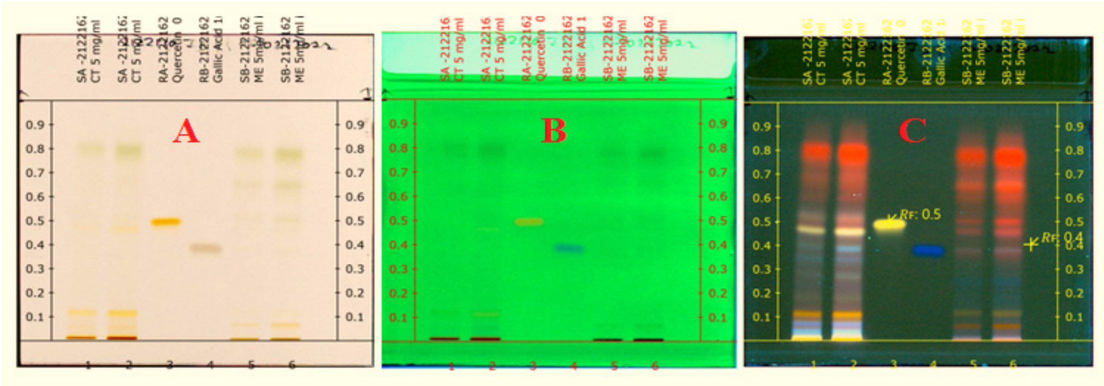

Fig. 7: HPTLC plate images for the identification of gallic acid and quercetin (A) under white light, (B) under 254 nm, and (C) under 366 nm

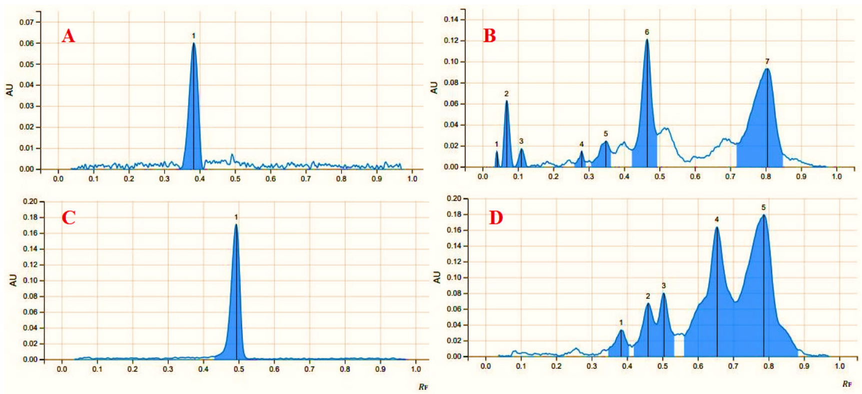

Fig. 8: HPTLC chromatogram (A) Gallic acid, (B) *C. tora,* (C) Quercetin, and (D) *M. elengi*

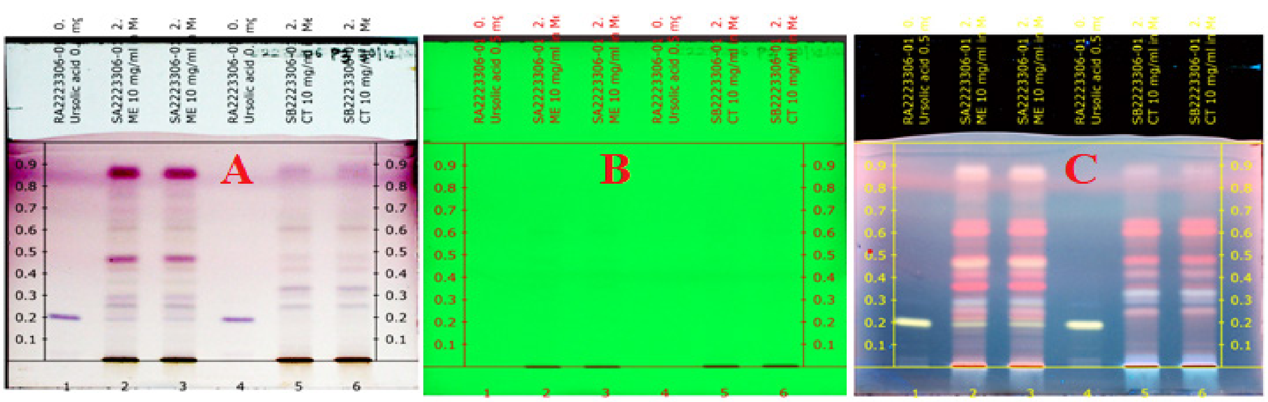

Fig. 9: HPTLC plate images for the identification of ursolic acid (A) under white light, (B) under 254 nm, and (C) under 366 nm

Discussion

Cancer is one of the silent killers in the population because it remains undetected and asymptomatic for many years. By the time it is detected, it is too late for a patient to recover as cancer enters a progressive stage, making it quite difficult to manage.[32] As per W.H.O. data, 80% of the population depends on natural resources for the alleviation of their sufferings. Medicinal plant-based therapeutic agents offer many advantages over conventional means of cancer management like being economical and readily available.[33] Hence, it was worthwhile to explore the effects of traditional Ayurvedic drugs *C. tora* (H: Chakwad, Sanai) and *M. elengi* (H: Maulsiri, Bakul) on cancer cells in a scientifically acceptable methodology with the help of modern techniques and instrumentation. The results thus obtained in this research work open a treatise of

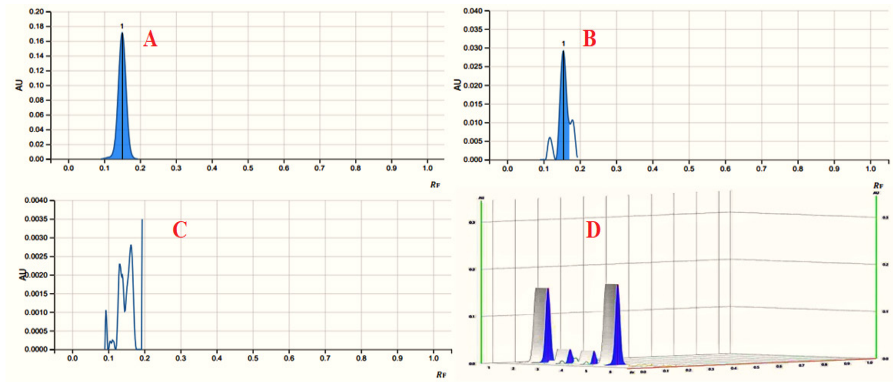

Fig. 10: HPTLC chromatogram of (A) Ursolic acid, (B) MEAE showing a peak of ursolic acid, (C) CTAE, and (D) 3D Profile of ursolic acid concerning a test sample of MEAE

Table 7: IC_{50} values of MEAE and CTAE

Drug samples	Cancer cell lines		
	MCF-7	HeLa	HepG2
MEAE	329	895	582
CTAE	347	903	676
Quercetin	226	210	235
Curcumin	210	154	176
Gallic acid	193	172	196
Methotrexate	191	82	147

medicinal plants plethora in our country, which is highly desirable due to its diverse geographical diversity. The promising results thus obtained might generate interest in international natural product-based researchers to venture deep into its anticancer-responsible phytomoieties along with exploration of the mechanism of action of such natural-based phytocompounds.

The morphological and anatomical traits help in differentiating *C. tora* and *M. elengi* from similar species. The characteristic odor and bitter taste of *C. tora* support its traditional use for rheumatic ailments and constipation, while the glossy leaves and undulated margin of *M. elengi* align with its applications in dental care and fever, reflecting their potential as a source of bioactive compounds (Figs 1 and 2). Transverse sections reveal distinctive vascular structures in both species, aiding accurate identification (Figs 3 and 4). Powder microscopy confirms their identity through features like stomata and calcium oxalate crystals (Figs 5 and 6). Phytochemical analysis shows both plants contain a rich variety of secondary metabolites essential for their medicinal potential (Table 4). These parameters can serve as distinctive markers for identifying and authenticating selected plant specimens and providing valuable insights for the compilation of a detailed monograph.[34,35] HPTLC analysis of *C. tora* and *M. elengi* highlights their phytochemical profiles, revealing key constituents like

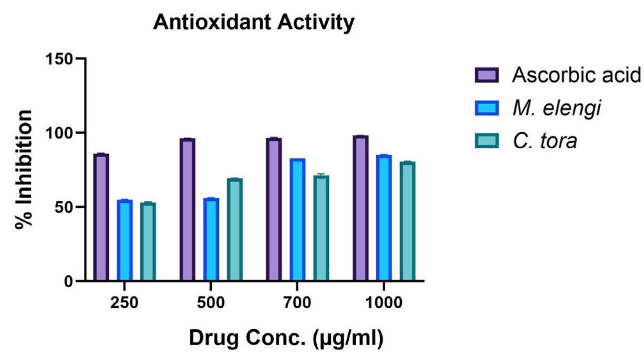

Fig. 11: DPPH assay of *M. elengi* and *C. tora,* one-way ANOVA test, $p = 0.0241$

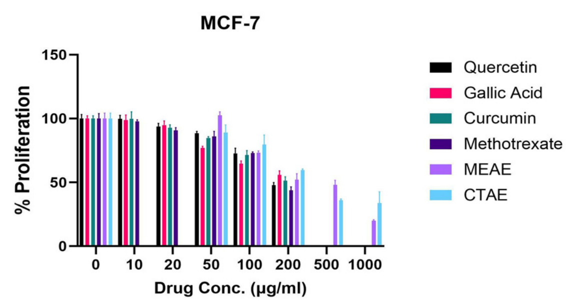

Fig. 12: MTT assay for MCF-7 cancer cell line, one-way ANOVA test, $p = 0.5716$

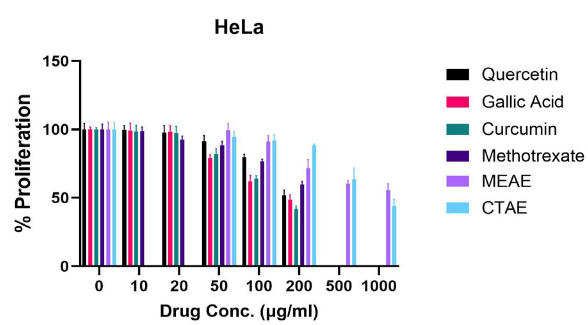

Fig. 13: MTT assay for HeLa cancer cell line, one-way ANOVA test, $p = 0.9817$

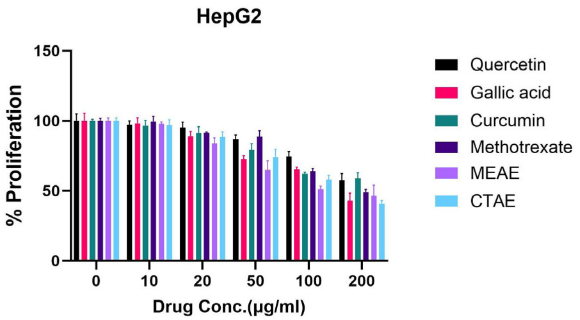

Fig. 14: MTT assay for HepG2 cancer cell line, one-way ANOVA test, $p = 0.9443$

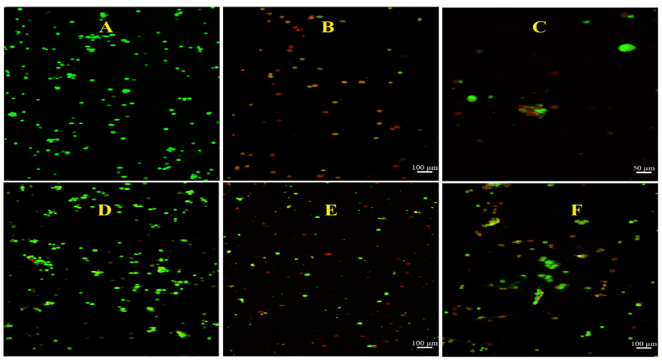

Fig. 15: Apoptosis analysis on MCF-7 cancer cells (A) CTAE at 100 µg/mL treatment, (B) CTAE at 200 µg/mL, (C) CTAE at 500 µg/mL, (D) MEAE at 100 µg/mL treatment, (E) MEAE at 200 µg/mL, (F) MEAE at 500 µg/mL. Nuclei showed green fluorescence to represent live cells, nuclei showed yellow-green fluorescence to represent early apoptotic cells and nuclei showed orange fluorescence to represent late apoptotic cells

gallic acid and quercetin, both known for their antioxidant properties (Figs 7 and 8 and Tables 5 and 6). Both MEAE and CTAE exhibited antioxidant activity (Fig. 11) that might be due to the presence of phytoconstituents such as gallic acid, and quercetin. However, MEAE demonstrated a detectable amount of ursolic acid, a compound known for its potent antioxidant properties, which was absent in CTAE and may refer to the fact that *M. elengi* possessed greater activity than *C. tora* (Figs 9 and 10). Further, the higher activity of the selected extracts observed towards MCF-7 cells highlights their potential as effective agents for targeting breast cancer (Table 7). The results of the apoptotic assay further reinforce these findings, suggesting that the potential anticancer effect of the selected plant extracts may be attributed to apoptosis induction in MCF-7 cancer cells (Fig. 15). After compiling the data thus generated in this research work and critically analyzing it, it is pertinent to state that its high time that an economically developing country like India, should focus more and more on newer medicinal plants to become economically self-reliant in natural product based research, thus giving a new lease of life to our age-old Indian traditional system of medicine, i.e., Ayurveda, making it more acceptable by western countries.

Conclusion

Pharmacognostic studies confirmed the genuine identity and ensured the quality, purity, and safety of the botanical specimens *M. elengi* and *C. tora* used in the study. HPTLC analyses revealed a rich presence of bioactive compounds, including quercetin, ursolic acid, and gallic acid, whose further exploration could provide valuable insights into their contributions to the observed pharmacological activities. In the DPPH study, both CTAE and MEAE showed concentration-dependent antioxidant activity, demonstrating their potential applications in therapies aimed at combating various oxidative stress-related conditions including cancer. The findings from the MTT assay affirm the anticancer potential of selected plant extracts, with MEAE showing a more pronounced effect than CTAE and MCF-7 cells exhibiting the highest sensitivity to the extracts. The observed cytotoxic effects against cancer cell lines highlight the clinical relevance of selected plant extracts as potential candidates for further development into novel anticancer therapies. The apoptotic assay has revealed the promising anticancer properties of selected plant extracts and supports their potential use in breast cancer therapy. Considering their multifaceted phytochemical profiles and demonstrated biological activities, *C. tora* and *M. elengi* leaves hold promise not only as anticancer agents but also as sources of natural antioxidants for holistic health applications.

Acknowledgment

We are deeply thankful to Dr. Sunita Garg at CSIR-NISCAIR, Delhi, for identifying the plant material, and to Mr. Tukaram Thite and Mr. Ramakant R. Yadav of Anchrom Labs for their HPTLC analysis. Sincere thanks to Dr. Meenakshi Gupta of CSJM University, Kanpur for the assistance provided during the cancer cell line studies and to the Department of Chemical Engineering, IIT Kanpur, for conducting the *in-vitro* anticancer testing. We are thankful to ACME Life Tech-LLP, Baddi (H.P.), for providing the gift sample of methotrexate.

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HOW TO CITE THIS ARTICLE: Dixit P, Singh D, Singh NK, Gupta R. Evaluation of Pharmacognostic, Phytochemical, Antioxidant and Anticancer Properties of Leaves of *Cassia tora* and *Mimusops elengi*. Int. J. Pharm. Sci. Drug Res. 2024;16(6):931-939. **DOI:** 10.25004/IJPSDR.2024.160602