Journal of Applied and Natural Science
17(3), 1362 - 1372 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Bioactive seed oils of mangrove associates: Phytochemical profiling, antioxidant activity and *in vitro* cytotoxicity studies

James Baben George
Department of Chemistry, St. Berchman's College (Autonomous), Changanasserry, Kottayam – 686101 (Kerala), India
Neethu Cyril*
Department of Chemistry, Assumption College Autonomous, Changanasserry, Kottayam – 686101 (Kerala), India
Sam John
Department of Chemistry, St. Berchman's College (Autonomous), Changanasserry, Kottayam – 686101 (Kerala), India
Sylas V.P
School of Environmental Sciences, Mahatma Gandhi University, Kottayam – 686560 (Kerala), India
Laigi Joseph
Department of Chemistry, T.M.J.M Government College, Manimalakkunu, Koothattukulam, Piravom – 686662 (Kerala), India
Saratchandran A. Divakaran
Department of Botany, Sree Keralavarma College, Thrissur – 680011 (Kerala), India
Pramod Kumar N
Department of Botany, NSS College, Ottapalam, Palakkad – 679103 (Kerala), India

*Corresponding author. E-mail: neethucyril@gmail.com

Article Info
https://doi.org/10.31018/jans.v17i3.6823
Received: May 13, 2025
Revised: August 27, 2025
Accepted: September 07, 2025

How to Cite
George, J. B. *et al.* (2025). Bioactive seed oils of mangrove associates: Phytochemical profiling, antioxidant activity and *in vitro* cytotoxicity studies. *Journal of Applied and Natural Science*, 17(3), 1362 - 1372. https://doi.org/10.31018/jans.v17i3.6823

Abstract
Mangrove-associated plants are rich reservoirs of unique bioactive compounds, owing to their adaptation to harsh coastal environments, yet many remain underexplored for their therapeutic potential. Seed oils from such species offer a promising source of natural antioxidants and anticancer agents. The present study aimed to conduct phytochemical characterization, antioxidant assessment, and anticancer evaluation of seed oils extracted from selected mangrove-associated plants — *Thespesia populnea*, *Canavalia cathartica*, and *Derris trifoliata* — using *n*-hexane via Soxhlet extraction. Preliminary phytochemical screening revealed that *T. populnea* oil possessed the highest total phenolic (65.27 ± 0.25 mg GAE/g) and flavonoid (233.57 ± 1.51 mg QE/g) contents. Gas Chromatography–Mass Spectrometry (GC–MS) and High-Performance Thin-Layer Chromatography (HPTLC) profiling confirmed the presence of diverse bioactive compounds such as tocopherols, flavonoids, sterols, terpenoids, and polyunsaturated fatty acids (PUFAs). Antioxidant activity was assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) and Superoxide Dismutase (SOD) assays showed that oil of *T. populnea* exhibited the strongest radical scavenging ability, correlating with its rich flavonoid content. In contrast, *D. trifoliata* seed oil demonstrated significant cytotoxicity against Dalton's Lymphoma Ascites (DLA) cells (IC_{50} of 62.3 µg/mL) and moderate antiproliferative activity against MCF-7 breast cancer cells (EC_{50} of 80.50 µg/mL), supported by morphological evidence of apoptosis. These bioactivities are likely attributed to the presence of phytochemicals such as the triterpenoid C(14a)-Homo-27-norgammacer-14-ene, sterols like stigmasterol and γ-sitosterol, and flavonoids including rotenone. Overall, the findings suggest that these seed oils, especially those from *T. populnea* and *D. trifoliata*, offer promising prospects as sources of natural antioxidants and potential anticancer agents.

Keywords: Antioxidant, *Canavalia cathartica, Derris trifoliata, Thespesia populnea*, MCF-7

INTRODUCTION

Mangrove ecosystems are among the most productive and biologically diverse habitats on Earth, playing a crucial role in shoreline protection, carbon sequestration, and sustaining coastal biodiversity (Asari *et al*., 2021, Inoue, 2019, Rahman *et al.,* 2024). However, beyond their ecological importance, mangroves are

increasingly recognized for their potential as sources of novel therapeutic agents (Kiran Kumar and Pola 2023, Sadeer, Zengin, and Mahomoodally 2023, Thatoi *et al*. 2013). Despite this, a significant number of mangrove and mangrove-associated plants remain underexplored or underutilized (Bandaranayake 1998), especially concerning their phytochemical composition and pharmacological applications. The harsh and dynamic environment in which mangrove plants thrive, marked by high salinity, tidal fluctuations, strong winds, and intense sunlight, has driven the evolution of unique physiological adaptations (Ashraf *et al*. 2018, Sudhir, *et al.,* 2022). These stress conditions often lead to the production of diverse secondary metabolites such as alkaloids, flavonoids, phenolic compounds, terpenoids, and fatty acids, which are known to exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Ahlawat *et al*. 2024, Prathamanjali *et al.,* 2025). These natural defense compounds, originally evolved to protect the plant, can have significant therapeutic implications.

The rich presence of bioactive phytochemicals in plants has encouraged researchers and industries alike to explore their potential in developing plant-based foods, medicines, and nutritional supplements. Natural antioxidants derived from plants play a crucial role in disease prevention. An imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the body's ability to detoxify these reactive intermediates can lead to oxidative stress, which is associated with a wide range of physiological disorders (Dhawan, 2014, Roberts *et al. (*2010). Environmental factors such as exposure to high-energy radiation, pollutants, pesticides, xenobiotics, smoking, poor nutrition, and mental stress can significantly increase the generation of these reactive species (Aruoma 1998, Rahal *et al*. 2014). Psychological states, including emotional stress and disease conditions, further contribute to free radical production (Phaniendra, Jestadi, and Periyasamy 2015). Antioxidants counteract these harmful effects by neutralizing free radicals, thereby protecting the body from cellular damage (Mandelker 2011, Sindhi *et al*. 2013). Plants, therefore, serve as vital reservoirs of natural antioxidants, largely due to their content of diverse phytochemicals such as fatty acids, alkaloids, steroids, flavonoids, polyphenols, saponins, and terpenoids.

This work focuses on evaluating the antioxidant and anticancer properties of seed oils from three underutilized mangrove-associated species—*Thespesia populnea*, *Canavalia cathartica*, and *Derris trifoliata.* Through the integration of preliminary phytochemical screening using Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Thin-Layer Chromatography (HPTLC) analysis and *in vitro* antioxidant and cytotoxicity assays, the study aimed to establish the pharmacological potential of these oils and thereby advocates for the conservation of mangrove ecosystems.

MATERIALS AND METHODS

Materials

The seeds of three wetland species (Fig.1) – *T. populnea, C. cathartica*, and *D. trifoliata* were collected from wetlands of Southern Kerala, India. *n*-hexane, methanol, ferric chloride, potassium acetate, aluminum chloride, sodium carbonate, sodium dihydrogen phosphate, Ethylenediaminetetraacetic acid (EDTA), sodium hydrogen phosphate and hydrochloric acid were supplied by Merck Millipore. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS$^{\bullet+}$), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), riboflavin, nitro blue tetrazolium (NBT), Folin-Ciocalteu reagent, penicillin-streptomycin solution, amphotericin B solution, thiazolyl blue tetrazolium Blue (MTT) were purchased from Sigma-Aldrich. DLA and MCF-7 (Human breast cancer) cell lines were initially procured from the National Centre for Cell Sciences (NCCS), Pune, India.

Extraction of seed oils

Freshly collected seeds were shade-dried under indirect sunlight for one week. It was then ground in a blender. The powder was extracted with n-hexane for 8 hours using a Soxhlet extractor – SoxTRON (SOX-3) apparatus at 68°C. The solvent was removed from the sample at 45°C under reduced pressure using a rotary evaporator to obtain crude oil samples, which were then stored in a freezer at -20°C.

Preliminary phytochemical evaluation of seed oils

Determination of total phenolics

Total phenolic content was estimated colorimetrically using the Folin-Ciocalteu method, following the procedure described by Singleton *et al.*. For the assay, 500 µL of the seed oil was mixed with 2 mL of 10% Folin-Ciocalteu reagent and subsequently neutralized with 4 mL of 7.5% sodium carbonate solution. The reaction mixture was incubated at room temperature for 30 minutes with intermittent shaking. Absorbance was recorded at 765 nm using a spectrophotometer. A calibration curve was constructed using gallic acid, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract (mg/g GAE).

Determination of total flavonoids

Total flavonoid content of the samples was determined using the Aluminum chloride colorimetric method, with quercetin as the reference standard (Pękal and Pyrzynska 2014). An aliquot of 500 µL of the sample was mixed with 1.5 mL of 95% ethanol, followed by 100 µL of 10% aluminum chloride and 100 µL of 1 M potassium acetate. The final volume was adjusted to 5 mL using distilled water. The mixture was incubated at room temperature for 30 minutes, and the absorbance of the

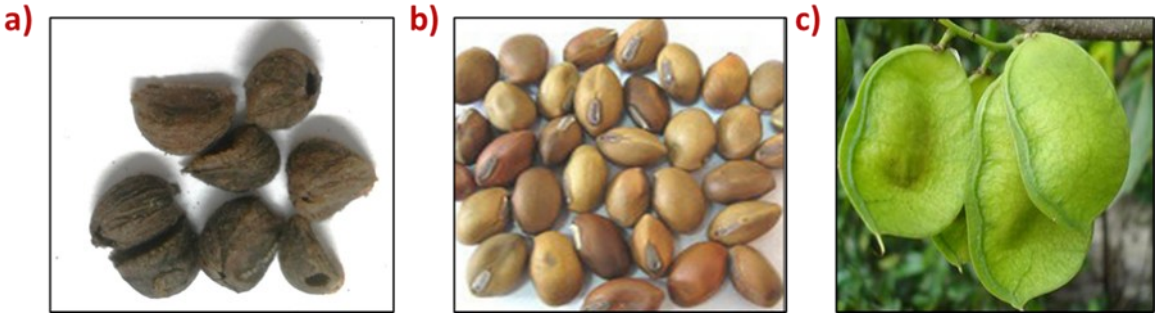

Fig. 1. *Seeds of mangrove associates (a) Thespesia populnea (b) Canavalia cathartica and (c) Derris trifoliata*

resulting yellow complex was measured at 415 nm using a UV-Vis spectrophotometer. A blank was prepared by substituting the aluminum chloride with distilled water. A standard curve was plotted using quercetin and the flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of dry extract.

Identification of compounds by Gas Chromatography-Mass Spectroscopy (GC-MS)

For general profiling, samples were separated on DB-5 fused-silica capillary column in a Gas Chromatography (Model: GC-MS-QP 2010, Shimadzu, Japan, ionization energy of 70 eV) using a DB Wax column (30 m×0.25 mm×0.25 µm). The compounds were identified by comparison with the NIST mass spectral database (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). The fatty acid composition of the seed oil was determined by converting it into fatty acid methyl esters (FAMEs), followed by GC-MS. A 0.25 g sample was refluxed with 20 mL of 0.5 M methanolic KOH for 20 min at 55°C, then cooled and esterified with 1.5 mL of H_2SO_4 and 15 mL of methanol. The mixture was refluxed for 30 min, cooled to room temperature, and then 10 mL of n-heptane was added, it was refluxed again for 10 min. The top layer was collected through anhydrous sodium sulphate and transferred to 2 mL autosampler vials for GC-MS analysis. The methyl esters of fatty acid were separated by GC-MS and compared with NIST spectral database.

High Performance Thin Layer Chromatography (HPYLC) analysis

Seed oils of *T. populnea, C. cathartica*, and *D. trifoliata* were spotted on a TLC plate and developed using the solvent system ethyl acetate: hexane: methanol (1:1:0.125) in an HPTLC chamber. The developed HPTLC plate was sprayed with the freshly prepared p-anisaldehyde-sulfuric acid reagent. The plate was then heated at 105°C for 3-5 minutes for the development of color bands. The HPTLC plate was visualized under white light and at 366 nm.

Antioxidant activities of Seed oils

Antioxidant activities of seed oils are commonly evaluated using the assays: DPPH, which measures free radical scavenging capacity, and $ABTS^{\cdot+}$, which assesses the ability to neutralize $ABTS^{\cdot+}$ radicals, and SOD assay, which quantifies the oil's potential to mimic superoxide dismutase in disarming superoxide radicals (Ochida *et al*., 2024, Okoh *et al*., 2014).

1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The DPPH radical scavenging activity of the samples was assessed following the method described by Aquino *et al* (Aquino *et al*. 2001). DPPH, in its radical form, exhibits a characteristic absorbance at 515 nm, which diminishes upon reaction with an antioxidant. Various concentrations of the sample aliquots were added to a freshly prepared DPPH solution (0.25 g/L in methanol), and the total volume was adjusted to 2 mL with methanol. The mixtures were incubated in the dark at room temperature for 20 minutes, after which the absorbance was recorded at 515 nm using a UV-Vis spectrophotometer. All measurements were performed in duplicate. The radical scavenging activity was calculated as a percentage of inhibition relative to a control.

% Inhibition = (Absorbance of control- Absorbance of sample) x 100 / Absorbance of control …..Eq. 1

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) scavenging activity

$ABTS^{\cdot+}$ radical scavenging activity was evaluated following the method described by Barton *et al* (Barton 2010). The $ABTS^{\cdot+}$ radical cation was generated through the reaction between $ABTS^{\cdot+}$ and sodium persulfate ($Na_2S_2O_8$). To assess antioxidant activity, 2 mL of the $ABTS^{\cdot+}$ solution was mixed with varying concentrations of the seed oil. After an incubation period of 6 minutes at room temperature, the absorbance was measured at 734 nm using a UV-Vis spectrophotometer. All measurements were performed in duplicate.

% Inhibition = (Absorbance of control- Absorbance of sample) x 100 / Absorbance of control …..Eq. 2

Superoxide dismutase (SOD) scavenging activity

Superoxide radical scavenging activity was assessed using the nitro blue tetrazolium (NBT) reduction method (McCord and Edeas 2005), which is based on the light-induced generation of superoxide radicals by riboflavin. These radicals reduce NBT to a blue-colored, water-soluble formazan complex. The reaction mixture consisted of 6 µM EDTA, 3 µM sodium cyanide

(NaCN), 2 µM riboflavin, 50 µM NBT, varying concentrations of the seed oil, and 67 mM phosphate buffer (pH 7.8), in a total volume of 3 mL. The test tubes were uniformly illuminated using an incandescent lamp for 15 minutes. Absorbance was recorded at 560 nm before and after illumination. The percentage of superoxide scavenging was calculated by comparing the absorbance of the seed oils-treated samples to that of the control.

Antiproliferative activities of seed oils

The antiproliferative potential of seed oils was assessed through *in vitro* short-term cytotoxicity studies using Dalton's Lymphoma Ascites (DLA) cells (Larramendy and Soloneski 2018), with cell viability quantitatively evaluated by the MTT assay (Kumar *et al.* 2018).

Short term cytotoxicity

In vitro short-term cytotoxicity of the three selected seed oils was evaluated using Dalton's Lymphoma Ascites (DLA) cells. Tumor cells were collected from the peritoneal cavity of tumor-bearing mice and washed three times with normal saline to remove impurities. Cell viability was assessed using the trypan blue exclusion method. A viable cell suspension (1×10^6 cells in 0.1 mL) was added to test tubes containing various concentrations of the oil samples. The volume was adjusted to 1 mL using phosphate-buffered saline (PBS). A control group containing only the cell suspension was included for comparison. All assay mixtures were incubated at 37°C for 3 hours. Following incubation, 0.1 mL of 1% trypan blue was added to each tube and allowed to react for 2–3 minutes. The stained (non-viable) and unstained (viable) cells were then counted separately using a hemocytometer. Cells that absorbed the dye were considered dead, while viable cells remained unstained.

% cytotoxicity = No. of dead cells x 100 / (No. of live cell +No. of dead cell) …….Eq. 3

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT assay was employed for the evaluation of the antiproliferative activity of seed oils on human breast carcinoma cell line, MCF-7. The percentage cell viability was determined by measuring the optical density (OD) of formazan formed in viable cells at 570 nm. The cell line was cultured in 25 cm² tissue culture flasks using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), L-glutamine, sodium bicarbonate, and an antibiotic-antimycotic solution comprising penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL). Cultures were maintained at 37°C in a humidified incubator with 5% CO_2 (NBS Eppendorf, Germany). Confluent monolayers, approximately two days old, were trypsinized and suspended in 10% growth medium. A 100 µL aliquot containing 5×10^4 cells was seeded into each well of a 96-well tissue culture plate and incubated under standard culture conditions.

The test samples (1 mg/mL) were dissolved in DMEM using a cyclomixer and filtered through a 0.22 µm Millipore syringe filter to ensure sterility. After the cells reached optimal confluency, the growth medium was aspirated and replaced with serial two-fold dilutions of the sample prepared in 5% DMEM at final concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL. Each concentration (100 µL) was added in triplicate and incubated at 37°C in a 5% CO_2 atmosphere for 24 hours. Following incubation, the medium was carefully removed, and 30 µL of reconstituted MTT solution (15 mg in 3 mL PBS, sterile filtered) was added to each well. The plate was gently shaken and incubated for 4 hours to allow for formazan crystal formation. Subsequently, the supernatant was removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan. The absorbance was measured at 570 nm using a microplate reader. Post-incubation, morphological changes such as cell rounding, shrinkage, cytoplasmic granulation, and vacuolization were assessed under an inverted phase-contrast tissue culture microscope (Olympus CKX41, Optika Pro5 CCD camera). Images were captured to document cytotoxic effects.

The percentage cell viability was calculated using the formula:

% cell viability= Mean of OD samplesx100 / Mean of OD control group ……..Eq. 4

Statistical analysis

For each plant species, the data were presented as mean ± standard deviation (SD) for the three determinations.

RESULTS AND DISCUSSION

Preliminary phytochemical studies of seed oils

As reported in the literature, the oil extraction using n-hexane results in the highest oil yield (Keneni, Bahiru, and Marchetti 2021). Hence, the seeds of *T. populnea, C. cathartica*, and *D. trifoliata* were extracted with n-hexane to obtain the oil using a Soxhlet extractor. The total polyphenolic content in the seed oils of *T. populnea, C. cathartica*, and *D. trifoliata* was found to be 65.27 ± 0.25 mg GAE/g dry weight, 29.90 ± 0.192 mg GAE/g dry weight and 28.28 mg GAE/g dry weight, respectively. The total flavonoid content for *T. populnea, C. cathartica*, and *D. trifoliata* was found to be 233.57 ± 1.51 mg QE/g dry weight, 19.9 ± 0.17 mg QE/g dry weight and 30 ± 1.76 mg QE/g dry weight. Numerous studies have investigated the bioactive properties of plant secondary metabolites, with a particular focus on phenolic compounds and flavonoids (Aquino *et al*. 2001, de Lima *et al*. 2024, Mutha *et al*. 2021, Taghiza-

deh and Jalili 2024). Among the three samples analyzed, oil of *T. populnea* exhibited the highest total phenolic and flavonoid contents. Plants might synthesize these secondary polyphenolic compounds as an adaptive response to cope with environmental stressors. Plant secondary metabolites produced in response to abiotic stresses have potential applications in pharmaceutical product development.

GC- MS analysis of unsaponifiable matter in the seed oil of *T. populnea* showed the presence of polyphenolic compounds like tocopherol and γ-sitosterol, as well as terpenes like aromadedrone and cadenine. Results are shown in Fig. 2. Previous studies about *T. populnea* have reported the presence of hepatoprotective phenolic acids (Yuvaraj and Subramoniam, 2009) as well as anticancer sesquiterpene quinones such as thespone, thespesone, mansonone-D, and mansonone-H (Johnson *et al.* 1999). Additionally, Sompong Boonsri *et al*. confirmed the presence of cytotoxic and antibacterial sesquiterpene quinones, including mansonone E and (+)-gossypol (Boonsri *et al*. 2008) in *T. populnea*. Presence of compounds including sterols and triterpenic alcohols which may contribute to the total polyphenolic content of *C. cathartica* (Gaydou, Viano, and Bourreil 1992). GC- MS analysis of unsaponifiable matter in the seed oil of *C. cathartica* showed the high amounts of polyphenolic compounds like lupeol (73%), stigmasterol (3.4%), sitosterol (3.5%), beta-amyrin (1.%), lupen-3-one (2.03 %). Data from GC- MS general profiling of *D. trifoliata* showed the presence of C (14a)-Homo-27-norgammacer-14-ene (13.81%), a triterpenoid, stigmasterol (10.12%), γ- sitosterol (1.55%) and rotenone (11.40%). The seed oil of *D. trifoliata* has been reported to contain flavonoids such as rotenoids and their derivatives (Anwer and El-Sayed 2025, Qian *et al*. 2024, Tewtrakul, Cheenpracha, and Karalai 2009). In addition, various polyphenolic compounds—including isoflavonoids such as tephrosin and toxicarol, as well as pterocarpans, triterpenes, and coumarins—have been identified in the seeds of *D. trifoliata*. These compounds exhibit a broad spectrum of biological activities, including insecticidal, antioxidant, antimicrobial, antifungal, antihyperglycemic, and cytotoxic effects (Nha Trang *et al*. 2024).

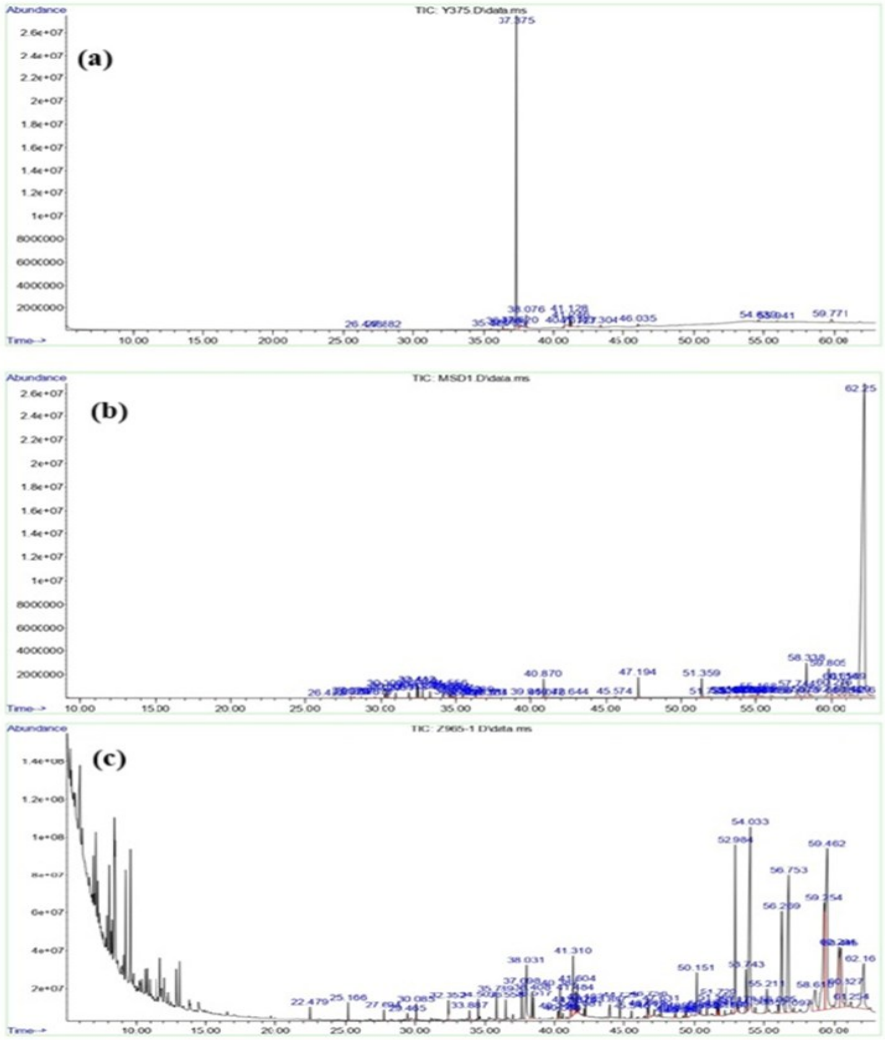

Fig. 2. *Gas Chromatography–Mass Spectrometry profile of seed oil of (a) Thespesia populnea (b) Canavalia cathartica, and (c) Derris trifoliata*

The HPTLC profiling of seed oil, as illustrated in Fig. 3, revealed a distinct banding pattern indicative of phytochemical diversity among the samples. The chromatograms (Fig. 3a and 3c) displayed 9, 15, and 18 bands corresponding to *T. populnea, C. cathartica*, and *D. trifoliata* respectively, reflecting the presence of bioactive phytochemicals. Derivatization with *p*-anisaldehyde/sulfuric acid reagent (Fig. 3b) enabled clear visualization of compound classes through color differentiation (viewed at 366 nm wavelength), ranging from grey-blue to violet and green. According to literature, blue bands are typically associated with monoterpenes and their alcohols, dark purple with triterpenes and phytosterols, grey with steroids and terpene esters, and brown with diterpenes, thereby facilitating preliminary identification of the compound classes present in the sample (Plainfossé *et al*., 2023). The fatty acid composition of the seed oils from *T. populnea, C. cathartica*, and *D. trifoliata* was determined using GC-MS. Results are given in Table 1. The fatty acid profiles of the three seed oils revealed a predominance of long-chain polyunsaturated fatty acids—particularly linoleic acid, oleic acid and α-linolenic acid. *T. populnea* seed oil contained roughly 52% unsaturated fatty acids, with linoleic acid (9Z,12Z-octadecadienoic acid) as the predominant lipid, followed by oleic acid. In *C. cathartica*, α-linolenic acid accounted for 13.41% of total fatty acids. The oil of *D. trifoliata* was distinguished by an unusually high content (42.3%) of cis-11-octadecenoic acid. These long-chain polyunsaturated fatty acids had been reported to confer significant cardiometabolic and anti-inflammatory benefits (Anand and Kaithwas 2014). Linoleic acid supplementation was shown to reduce low-density lipoprotein cholesterol and attenuate atherogenesis (McLeod *et al*., 2004), whereas α-linolenic acid served as a precursor for bioactive omega-3 fatty acids and exerted modulatory effects on inflammatory pathways (Zhu *et al*., 2024).

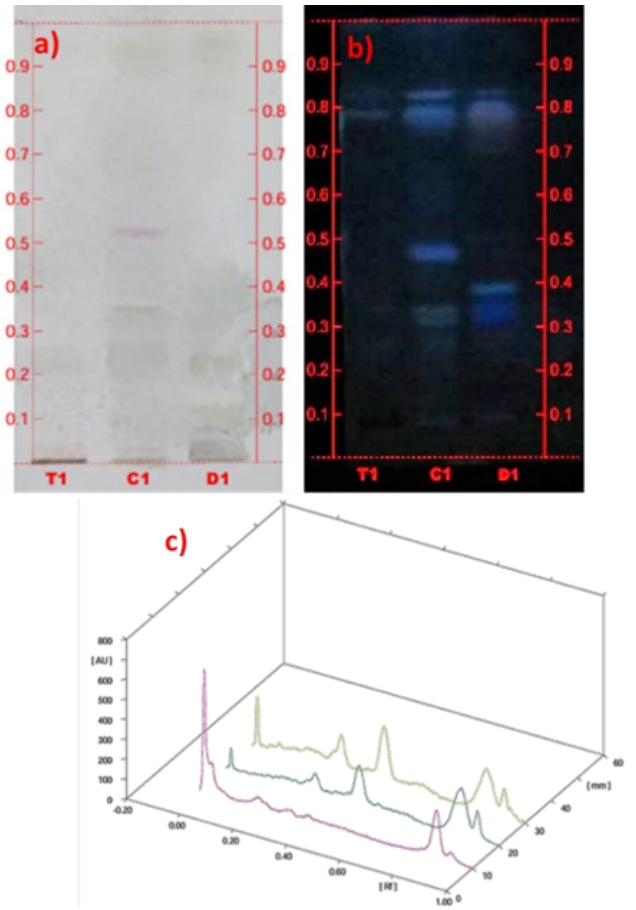

Fig. 3. *High–Performance Thin-Layer Chromatography (HPTLC) profile of seed oils of (a) Thespesia populnea (T1) (b) Canavalia cathartica (C1) and (c) Derris trifoliata (D1) after derivatization with p-anisaldehyde/sulfuric acid reagent under (a) white light and (b) 366 nm, (c) High–Performance Thin-Layer chromatogram of seed oils*

Antioxidant activities of seed oils

In vitro antioxidant properties of seed oils were evaluated by four different assays based on electron transfer and hydrogen atom transfer methods. DPPH, $ABTS^{\cdot+}$ and SOD assays were employed for the determination of antioxidant activity. The variation in percentage of inhibition of these free radicals with concentration of seed oil were plotted and shown in Fig. 4. The DPPH assay is based on the capability of stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors, especially phenolics, and get reduced to the corresponding pale yellow hydrazine (Pyrzynska and Pękal 2013). The scavenging effect of the seed oils on the DPPH radical decreased in the order: Ascorbic acid > *T. populnea* > *D. trifoliata* > *C. cathartica*. The quality of the antioxidants in the sample was determined by their IC_{50} values (Table 2). A low IC_{50} value indicates strong antioxidant activity in the sample (Lin *et al*. 2003). From the graph, it was observed that the DPPH scavenging ability of seed oil of *T. populnea* showed an IC_{50} value of 32.93 µg/mL lower than the other two seed oils. The reference compound, ascorbic acid exhibited an IC_{50} value of 2.48 µg/mL. Therefore, seed oil of *T. populnea* showed strong antioxidant activity compared to other two oils. This can be correlated with higher abundance in phenolic compounds, especially flavonoids. Based on present findings, the predominant phenolic compounds in *Thespesia* appear to belong to the flavonoid class, with a content of 233.57 ± 1.51 mg QE/g dry weight. In contrast, HPTLC analysis confirmed the presence of triterpene alcohols and sterols as the principal phenolic constituents in the oils of *C. Cathartica* and *D. trifoliata*. These compounds are likely to exhibit lower hydrogen-donating capacity compared to flavonoids, which may account for the comparatively lower DPPH radical scavenging activity observed in these oils.

DPPH radical scavenging capacity of *T. populnea* seed oil was closely linked to the structural characteristics of its flavonoid constituents. Flavonoids were recognized for their radical scavenging potential, primarily attribut-

Table 1. Fatty acid composition of the oils from *(a) Thespesia populnea (b) Canavalia cathartica and (c) Derris trifoliata*

Name of fatty Acid	Formula	*T. populnea* (%)	*C. cathartica* (%)	*D. trifoliata* (%)
2-methyl decanoic acid	$C_{11}H_{22}O_2$		2.423	
Dodecanoic (Lauric acid)	$C_{12}H_{24}O_2$	1.698	3.386	3.708
Methyl tetradecanoic	$C_{15}H_{30}O_2$	1.536		2.855
Hexadecanoic (Palmitic acid)	$C_{16}H_{32}O_2$	33.32	22.049	26.27
Octadecanoic (Stearic acid)	$C_{18}H_{36}O_2$	3.13		7.707
9- octadecenoic (Oleic acid)	$C_{18}H_{34}O_2$	18.46	46.480	
11-octadecenoic	$C_{18}H_{34}O_2$			42.272
9, 12-octadecadienoic (Linoleic acid)	$C_{18}H_{32}O_2$	34.00	12.420	10.870
9,12,15 octadecatrienoic acid (α linolenic acid)	$C_{20}H_{40}O_2$	…..	13.241	……
9(Z), 15(Z)-octadecadienoic acid	$C_{18}H_{32}O_2$	0.251	….	….

Other fatty acids are present in minor amounts

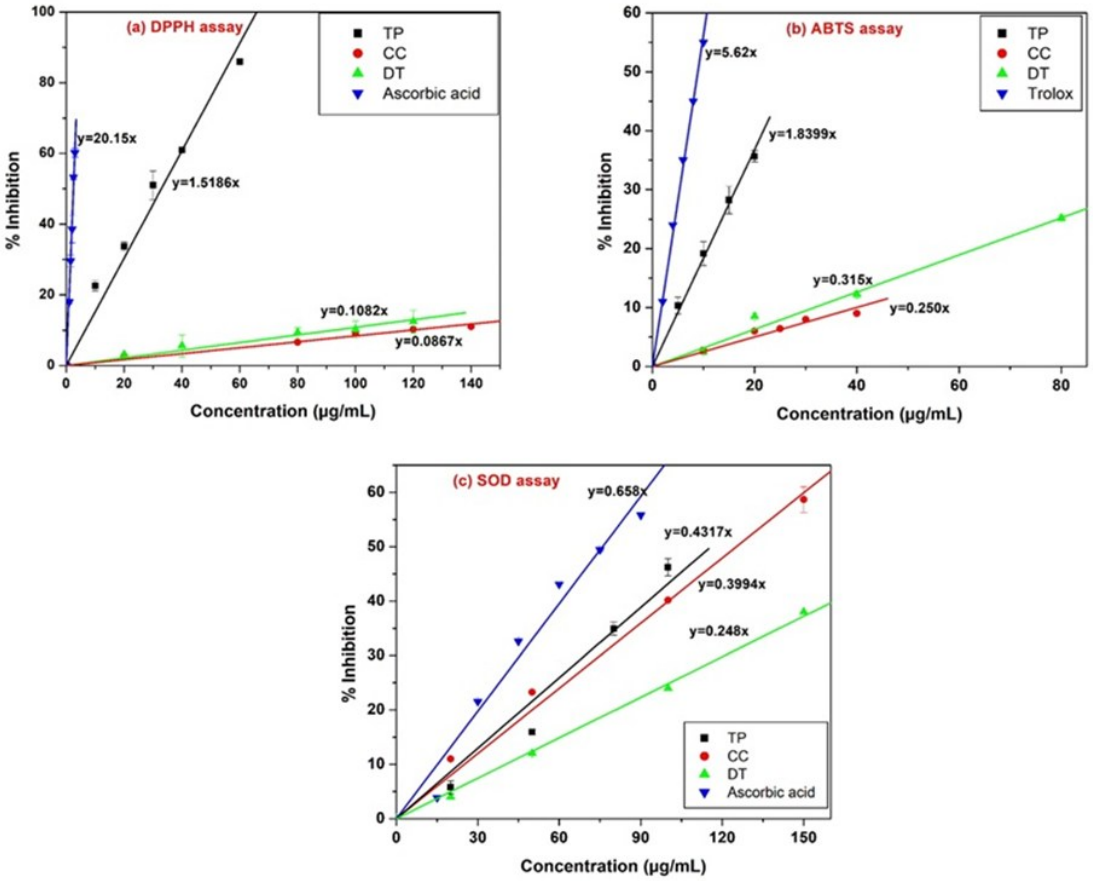

Fig. 4. *(a) 1,1-diphenyl-2-picrylhydrazyl (DPPH) (b) 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) and (c) Superoxide dismutase (SOD) radical scavenging potential of seed oils of (a) Thespesia populnea (TP) (b) Canavalia cathartica (CC) and (c) Derris trifoliata (DT)*

ed to the presence of phenolic hydroxyl groups that functioned through a hydrogen atom transfer mechanism. Previous investigations had highlighted that specific hydroxylation patterns, particularly on the B-ring and/or the presence of –OH groups, significantly enhanced antioxidant capacity (Cai *et al*. 2006, Wang, Li, and Bi 2018). While phenolic O–H groups were generally considered the primary contributors to antioxidant activity, emerging evidence suggested that C–H bonds also played a role. Notably, in flavonoids bearing a 4-carbonyl and/or 3-hydroxyl group, C–H bonds exhibited lower bond dissociation energies than O–H bonds,

Table 2. Antioxidant activities of seed oils of *Thespesia populnea, Canavalia cathartica and Derris trifoliata*

Seed oil	DPPH (IC_{50} in µg/mL)	ABTS·+ (IC_{50} in µg/mL)	SOD (IC_{50} in µg/mL)
T. populnea	32.93 ± 0.07	27.18 ± 0.02	115.52 ± 0.19
C. cathartica	576.70 ± 3.07	200 ± 2.08	125.15 ± 1.07
D. trifoliata	462.10 ± 2.07	158.73 ± 4.07	201.61 ± 3.07
Ascorbic acid	2.48 ± 0.03		75.98 ± 0.23
Vitamin E			
Trolox		8.86 ± 0.02	

Means are presented ± standard error of triplicate measurements

Table 3. Cytotoxicity screening of seed oils of *(a) Thespesia populnea (b) Canavalia cathartica and (c) Derris trifoliata* against Dalton's Lymphoma Ascites (DLA) cell lines

Seed oil	IC_{50} (µg/mL)
T. populnea	100.6 ± 2.3
C. cathartica	>150
D. trifoliata	62.3 ± 5.3

Means are presented ± standard error of triplicate measurements

thereby contributing to hydrogen donation and radical stabilization (Vo *et al*. 2019). This property may explain the unexpectedly high DPPH radical scavenging activity observed in T. populnea seed oil, likely due to the presence of structurally favourable flavonoids.

Antioxidant capacity of the seed oils was also evaluated using ABTS·$^{+}$ method and SOD assay. In both ABTS·□ and SOD assays, greater antiradical activities were shown for *T. populnea* oil with an IC_{50} value of 27.18 µg/mL and 115.52 µg/mL, respectively. Although the results with ABTS·$^{+}$ showed same trend as that of DPPH, ABTS·$^{+}$ is less selective and hence more sensitive than DPPH in the reaction with hydrogen donors. ABTS·$^{+}$ reacts with any hydroxylated aromatics irrespective of their real antioxidative potential (Gaber, El-Dahy, and Shalaby 2023, Nikolaos Nenadis *et al*. 2004).

The SOD assay utilizes light-activated riboflavin to generate superoxide radicals, which subsequently reduce NBT to form a blue-colored formazan. The presence of SOD or antioxidant compounds in the seed oil inhibits this reaction by scavenging the superoxide radicals, resulting in reduced formazan formation. The extent of this inhibition was assessed by monitoring the decrease in absorbance at 560 nm, providing a measure of antioxidant activity (Haida and Hakiman 2019, Lang *et al*. 2024). Oil of *T. populnea* exhibited the highest superoxide scavenging activity, which was directly related to its higher content in phenolics, mainly flavonoids, compared to the other two oils (Zeng *et al*. 2020). Mostofa *et al*. reported the superoxide scavenging abilities of polyunsaturated fatty acids, which might indirectly act as antioxidants in vascular endothelial cells, hence diminishing inflammation and, in turn, the risk of atherosclerosis and cardiovascular disease (Mostofa *et al*. 2024, Shramko *et al*. 2021). Therefore, the SOD scavenging ability of oil of *T. populnea* could also be correlated to the presence of 34% of 9, 12-octadecadienoic acid.

In these assays, *T. populnea* seed oil demonstrated significantly higher antioxidant activity compared to the other two oils. This suggests that the antioxidant potential of *T. populnea* oil may not solely arise from common polyphenolic compounds, but could also be attributed to the presence of distinctive flavonoid constituents and polyunsaturated fatty acids (Gonçalves *et al*., 2024).

In vitro antiproliferative activities of seed oils

The cytotoxic activity and antiproliferative effects of seed oils were investigated using the trypan blue dye exclusion method on DLA cells and the MTT assay on the breast cancer cell line MCF- 7. The results of cytotoxic activity on DLA cell lines are given in Table 3.

Oil of *D. trifoliata* exhibited significant cytotoxicity against DLA cell lines compared to the other two oils. It was observed that the cytotoxic effect on DLA cell lines increased with the increase in the concentration of oil. Hence, the seed oil was further studied for its antiproliferative activity on MCF-7 breast cancer cell lines. The median effective concentration, EC_{50} value, for cell mortality in MCF-7 cell lines after exposure to oil of *D. trifoliata* for 24 hours was found to be 80.50 µg mL^{-1} (Fig. 5).

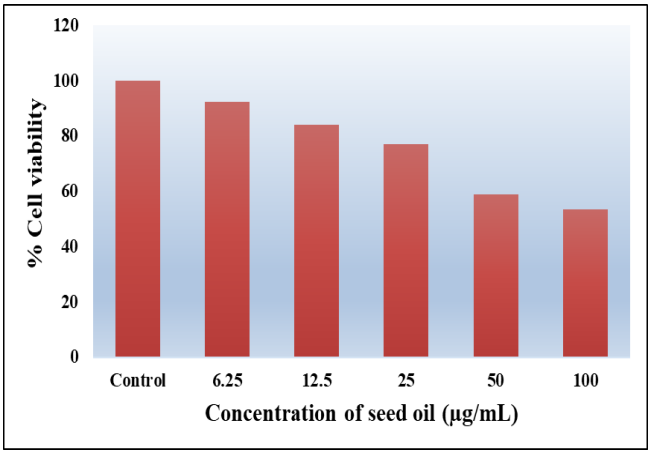

Fig. 5. *Variation of % cell viability with concentration of seed oil of Derris trifoliata*

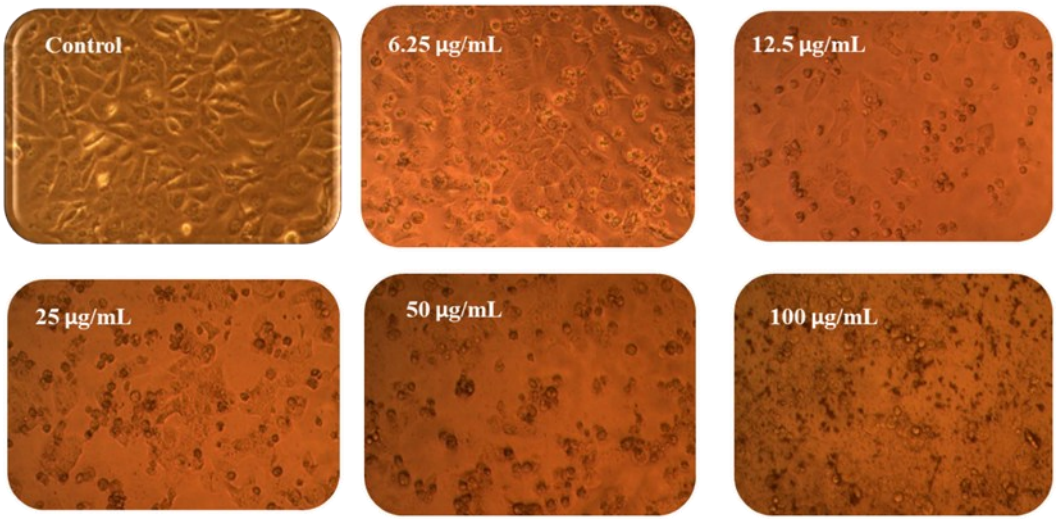

Fig. 6. *An inverted phase contrast tissue culture microscopy image of MCF 7 cell lines on treatment with the seed oil of Derris trifoliata*

The *D. trifoliata* oil showed moderate anticancer activity, which could be due to the presence of phenolic compounds, flavonoids, sterols and terpenoids. Data from GC- MS general profiling of *D. trifoliata* showed the presence of C(14a)-Homo-27-norgammacer-14-ene (13.81%), a triterpenoid, stigmasterol (10.12 %), γ-sitosterol (1.55 %) and rotenone (11.40 %), an isoflavanone. The anticancer activity of the oil may be attributed to the presence of bioactive phytochemicals. Furthermore, images of the cell cultures shown in Fig. 6, captured using an inverted phase contrast tissue culture microscope, revealed apoptotic features and nuclear condensation in MCF-7 cells treated with *D. trifoliata* seed oil. The morphological alterations observed included changes in the refractive index of cells, along with cytoplasmic membrane shrinkage, loss of adhesion to neighboring cells, and membrane blebbing, as illustrated in Fig. 6. These findings indicate that the seed oil of *D. trifoliata* may exhibit anticancer properties and could hold potential for development in pharmaceutical applications.

Conclusion

This study highlights the therapeutic potential of seed oils from selected mangrove-associated plants, *T. populnea, C. cathartica,* and *D. trifoliata,* particularly due to their significant antioxidant and anticancer activities. The present study comprehensively evaluated the phytochemical composition, antioxidant potential, and anticancer activity of seed oils extracted from these plants. GC-MS and HPTLC analyses revealed a rich diversity of bioactive compounds, including flavonoids, polyphenols, sterols, terpenoids, and long-chain polyunsaturated fatty acids. Among the oils studied, *T. populnea* exhibited the highest total phenolic and flavonoid content, which correlated strongly with its superior antioxidant activity in DPPH, ABTS·+ and SOD assays. The potent free radical scavenging capacity of this oil may be attributed to the abundance of structurally favorable flavonoids and essential fatty acids like linoleic acid. Conversely, the seed oil of *D. trifoliata* demonstrated the most pronounced cytotoxic effect against DLA cells and a moderate antiproliferative activity (EC_{50} value of 80.50 µg mL^{-1}) against MCF-7 breast cancer cells, likely due to the presence of bioactive compounds such as rotenone, stigmasterol, and triterpenoids. Morphological alterations observed in treated cancer cells further support the apoptotic potential of *D. trifoliata* seed oil. These findings suggest that the seed oils, particularly those of *T. populnea* and *D. trifoliata*, hold promise as sources of natural antioxidants and anticancer agents, deserving further investigation into their mechanisms of action and potential pharmaceutical applications.

ACKNOWLEDGEMENTS

The authors acknowledge the Inter-University Instrumentation Centre (DST-SAIF, Government of India) and the School of Environmental Sciences, MGU (KSCSTE-SARD, Government of Kerala) for providing the instrumentation facility.

Conflict of interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Dr. Neethu Cyril, upon reasonable request.

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