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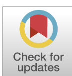
Research paper

Differential cytotoxic effects of *Garcinia mangostana* pericarp extract on leukaemic versus normal human cell lines: insights into selective anticancer activity



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ABSTRACT

Introduction: Garcinia mangostana L. is widely recognised for its traditional medicinal uses and growing relevance in the nutraceutical sector. This research endeavour is designed to scrutinise the phytochemical composition and cytotoxic effects of an alcoholic extract derived from the pericarps of *G. mangostana* (ME) on leukaemic cell lines.

Methods: G. mangostana L. pericarps were subjected to ethanol extraction, and subsequent compound identification was conducted via Capillary Liquid Chromatography-Electrospray Ionisation-Quadrupole Time-of-Flight Tandem Mass Spectrometry (CapLC-ESI-QTOF-MS/MS). In parallel, the extract was fractionated through High-Performance Liquid Chromatography (HPLC). Furthermore, U937, Jurkat and normal human umbilical vein endothelial cell (HUVEC) lines were exposed to varying concentrations of the extract to assess cell proliferation, viability, cytotoxicity, and apoptotic DNA damage.

Results: Investigations confirmed the presence of various xanthones, including β-mangostin, α-mangostin, and garcinone E. Significantly, ME displayed pronounced cytotoxic effects specifically on leukaemic cells, distinguishing its impact on malignant cells as opposed to non-malignant ones, and facilitated apoptotic DNA fragmentation. Moreover, the whole extract consistently displayed greater cytotoxicity compared to individual fractions.

Conclusion: According to the results obtained ME selective cytotoxicity against cancer cell lines and greater biological activity than the single compounds in the extract. These findings underscore ME's potential in cancer prevention, complementing dietary strategies ahead of pharmaceutical interventions.

Introduction

Recent epidemiological research suggest that diets rich in plant-derived foods may confer protection against multiple cancer types, attributed largely to their rich polyphenolic content, including flavonoids, phenolic acids, and stilbenes (Contreras García and Zaragoza Marti, 2019; Papadimitriou et al., 2021).

The exploration and isolation of phytocomplexes and phytochemicals from plants, encompassing herbs, fruits, and vegetables, have revealed biologically active compounds that hold promise as both cancer chemopreventive and chemotherapeutic agents (Chiaino et al., 2020, 2021).

Xanthones were investigated for their anticancer effects in several studies (Kurniawan et al., 2021).

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Compounds such as alpha-mangostin have demonstrated significant anticancer activities, including apoptosis induction and cell cycle arrest, specifically in breast cancer cells. These effects are mediated through the inhibition of NF-kB signalling and induction of reactive oxygen species (Cruz-Gregorio et al., 2023). Other xanthones isolated from Calophyllum inophyllum, such as 1,3,6,7-tetrahydroxy-5-methoxy-4-(1′,1′-dimethyl-2′-propenyl)-8-(3″,3″-dimethyl-2″-propenyl)-xanthone and (2′S)-7-hydroxy caloxanthone B, exhibit in vitro anticancer effects, depending on their structure, showing potent and selective cytotoxicity against HCT-116 colon cancers with IC50 values as low as 3.04 μM (Mah et al., 2015). Further research has explored the anticancer mechanisms of morusignin I, 8-hydroxycudraxanthone G, and cudraxanthone I, revealing that these compounds induce apoptosis and disrupt the MMP through caspase activation, highlighting the critical role of caspase pathways in the anticancer effects of these phytochemicals (Kuete et al., 2014). Prenylated compounds belonging to this phytochemical class endowed with anticancer properties against HepG2, T98, MCF-7 were identified in *Garcinia bracteate* (Xue et al., 2020). Similar observations regarding the potential health benefits of phenolic acids and stilbenes, such as resveratrol (Avcı et al., 2011), can be extended to the compounds found in the tropical fruit *Garcinia mangostana*. The mangosteen tree (*G. mangostana*), an evergreen species, originates from the tropical regions of Southeast Asia including Thailand, Myanmar, Malaysia, Indonesia, and Singapore (Yang et al., 2017).

The fruit, typically 3.5–7.5 cm in diameter, exhibits a round and smooth exterior, with colours ranging from dark purple to red purple. Its 6–10 mm thick rind reveals a red cross-section and purplish-white interior, containing bitter yellow latex and a staining purple juice. The fruit may possess 1–5 fully developed seeds with an ovoid oblong shape, often flattened, measuring 2.5 cm in length and 1.6 cm in width. The seeds are accompanied by 4–8 triangular segments of white, juicy, and soft arils. The flavour of the flesh ranges from slightly to distinctly acidic, making it highly regarded for its luscious and delicious taste (Obolskiy et al., 2009).

Historically, various parts of the mangosteen tree, particularly the pericarp, leaves, and bark, have been traditionally employed to address various medical conditions, including arthritis, diarrhoea, dysentery, inflammation, skin disorders, and wound-healing properties.

Extracts from mangosteen pericarp are rich in bioactive compounds, primarily isoprenylated xanthones, known for their diverse *in vitro* biological effects, including antioxidant, cytotoxic, anti-inflammatory, antibacterial, antifungal, antiviral, and cancer chemopreventive properties (Asfour, 2016; Nawawi et al., 2023; Rashidi et al., 2022). Alpha-Mangostin, the most abundant xanthone in the pericarp, is known for its pharmacological activities, encompassing antioxidant, anti-infective, anticarcinogenic, antidiabetic, neuroprotective, hepatoprotective, and cardioprotective properties (Alam et al., 2023).

This study focuses on the *G. mangostana* ethanolic extract (ME) characterisation and impact on cultured human normal and leukaemic cancer cell lines, building upon the extensive body of research on the anticancer potential of xanthones using various human cancer cell lines (Nauman and Johnson, 2022).

Material and methods

Reagents and chemicals

Chemical reagents, including 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and trypan blue, were acquired from Sigma Chemical Co., St. Louis, MO, USA, and used in their original form. MS grade acetonitrile, water, and formic acid were purchased from Sigma-Aldrich (St Louis, MO).

Preparation of ME

The fruits of *G. mangostana*, originating from Thailand, were procured at a market in Milan. Voucher specimens have been archived at the Department of Biomolecular Science, University of Urbino Carlo Bo, Italy (GM 1, GM2, and GM3). A total of 180 g of *G. mangostana* pericarps were subjected to air-drying and finely ground using a blender to ensure homogeneity. The air-dried and powdered fruits (180 g) were subjected to extraction under continuous magnetic stirring at 300 rpm, using a hydroethanolic solution containing 90% ethanol, for a period of 10 days, all while being shielded from any light source, at 25 °C. Subsequently, the solid components were separated from the liquid through centrifugation at 10 000 x g for 15 minutes, and the obtained liquid was filtered using 0.2 μm pore size Millipore Stericup Filters. The purified extract was then aliquoted into 1 ml vials and subjected to freeze-drying (lyophilization) using a LyoQuest (Telstar, Wertheim – Germany). The resulting pellet was weighed and subsequently resuspended in 95% ethanol to achieve a final concentration of 12.5 μg/μl.

HPLC analysis (high performance liquid chromatography)

The HPLC separation was performed on a Beckman Model 7725i 5 mm Supelcosil LC-318 column (25 cm 4.6 mm i.d.) (Supelco). Solvent A was water +0.1% TFA and Solvent B was ethanol. A gradient from 0 to 85% solvent B within 110 minutes was used as the mobile phase. The flow rate was 1.2 ml/Min and detection was performed with a UV detector at 260 nm.

We employed electrospray ionisation tandem mass spectrometry (ESI-MS/MS) using a QTOF MicroTM system (Micromass, UK), operating both negative and positive ion modes, with sample cone voltage finely tuned to 40 volts (Piccoli et al., 1994).

The extract *in toto* and the fractions obtained by HPLC analysis were characterised by CapLC-ESI-MS/MS analysis.

CapLC-ESI-MS/MS analysis

CapLC-ESI-QTOF-MS/MS analysis was conducted using a CapLC system (Waters Corp., Milford, MA, USA) coupled with a QTOF Micro system (Waters Corp., Manchester, UK).

Samples were separated using a nanoease TM Symmetry300TM C18 column (75 μm × 150 mm; 3.5 μm) (Waters, Milford, MA). The mobile phases consisted of solvent A (98% water, 2% acetonitrile, 0.01% formic acid) and solvent B (100% acetonitrile with 0.01% formic acid). The gradient profile was as follows: 0–3 minutes, 5% B; 3–10 minutes, 5–15% B; 10–20 minutes, 15–20% B; 20–25 minutes, 20–30% B; 25–30 minutes, 30–35% B. Flow rate was 5 μl/Min, and the injection volume was 1 μl. Tandem mass spectrometry experiments were performed in both positive and negative ESI ionisation modes, with data acquisition in the range of 100–1600 Da.

Capillary voltages were set at 3200 V (positive mode) and 2800 V (negative mode), with a sample cone voltage of 40 V and a desolvation temperature of 90 °C. The survey scan mode encompassed an MS range from 200 to 1600 *m/z*, triggering MS to MS/MS transitions based on ion intensity, with an MS/MS range from 100 to 1800 *m/z*. Collision energies were adjusted according to ion charge states using Argon as the collision gas. Instrument control was managed using Waters Masslynx 4.1 software.

ESI-MS analysis was performed in both positive and negative ion modes to confirm the identity of the constituents in the *G. mangostana* extract. Following concentration, each phase was reconstituted using HPLC-grade acetonitrile, achieving a concentration of approximately 1 mg/l, with a 1 μl injection volume. Triplicate analyses were conducted in CapLC/QTOF-MS/MS mode. Tandem MS provided molecular weight information for compounds, and fragment ion data facilitated structural identification. HPLC-ESI-MS experiments were carried out in

positive and negative ion modes for further confirmation of the compounds assigned as peaks 1–10, following the previously described conditions. 4.1 software.

Cell culture and treatment conditions

Cells were grown at 37 °C in an atmosphere of 95% air and 5% CO_2. Human promonocytic U937 cells and human T-cell leukaemia line Jurkat were cultured in RPMI-1640 medium supplemented with antibiotics (50 units/ml penicillin and 50 µg/ml streptomycin), 1.2 mM glutamine, and 10% fetal bovine serum. Human Umbilical Vein Human umbilical vein endothelial cells (HUVEC) were maintained in M199 medium containing antibiotics, 1.4 mM glutamine, 10% fetal bovine serum and 50 µg/ml endothelial cell growth factor.

PBMCs from adult healthy donor were obtained by centrifugation of PBS-diluted (1:1) blood samples over a Ficoll-Hypaque gradient following the manufacturer's instructions and cultured in RPMI-1640 supplemented with FBS 10% and phitohemagglutinin.

For experiments, U937, Jurkat, HUVEC cells and PBLs were resuspended at a density of 2×10^5 cells/ml in 60 mm x 15 mm culture plastic dishes. In order to detect the effect of the alcoholic extract, cells were treated in a complete culture medium with different concentrations of ME (5, 9, 12.5, and 25 µg/ml) and incubated for 6, 24, and 48 hours. The control samples were treated with the equivalent amount of vehicle ethanol (a maximum of 0.2% in the culture medium was used).

To comparatively assess extracts' cytotoxic effects, U937 cells were treated with 4 µg/ml of Fractions A, B, and G, and 12.5 µg/ml of the total mango extract (ME). The cell viability was subsequently evaluated using the MTT assay at 24 and 48 hours post-treatment to determine the percentage of viable cells compared to untreated control cells.

Cell proliferation and cell viability assays

The cytotoxic activity of ME was initially determined by cell proliferation analysis using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays (Ghasemi et al., 2021; Sestili et al., 2002). MTT was added (50 µg/ml) to each sample, and the cells were further incubated for 1 hour at 37 °C. Subsequently, they were washed and dissolved in 2 ml of dimethyl sulfoxide (DMSO). The formation of the blue formazan was measured spectrophotometrically at 570 nm. Results are expressed as 'MTT reducing activity' presented as a percentage of the control. The impact of ME on the viability of U937, Jurkat, and HUVEC cells was analyzed using a trypan blue exclusion method (presented only for the experiments performed with 12,5 µg/ml of the extract, exposed for three different times, i.e. 6, 24, and, 48 hours).

For the trypan blue exclusion assay, an aliquot of the cell suspension was diluted 1:1 with 0.4% trypan blue, and the cells were counted using a hemocytometer. The results are expressed as 'percent viable cells,' representing the percentage of viable (unstained) cells in the treated samples compared to the untreated samples.

Non denaturing fast-halo assay (FHA)

The non denaturing FHA (Sestili et al., 2006) allows to detect selectively the double-strand DNA breaks, typical of apoptosis. Briefly, cells were resuspended at a 4.0×10^4/ml density in PBS containing 5 mM EDTA; subsequently, 25 µl of each cell suspension was mixed with an equal volume of 2% low melting point agarose gel and immediately placed on microscope slides previously coated with 1% agarose. After complete gelation on ice, the coverslips were removed. The slides were incubated in lysis buffer (0.15 M NaOH, 0.1 M NaH_2PO_4, 1 mM EDTA, Triton X100 1% v/v, pH 9.6) for 10 minutes followed by incubation with 0.1 mg RNase (bovine pancreas Type 1A)/ml PBS (pH 7.4) for 15 minutes.

Ethidium bromide was then introduced directly into the solution during the last 5 minutes of incubation. The ethidium bromide-labelled DNA was visualised using a Leica DML/DFC300F fluorescence microscope. Captured images were saved and processed using image analysis software (Scion Image). To evaluate apoptotic events, a minimum of 100 cells per sample were counted, and the results were calculated as the percentage of haloed-apoptotic cells, defined as the number of apoptotic cells divided by the total number of cells, multiplied by 100.

Statistical analysis

In our experiments, each concentration of ME was tested in at least three independent experimental runs, with each experiment performed in duplicate.

Statistical analysis was performed using one-way ANOVA to compare the effects of ME concentrations on cell viability at each time point. Significant differences were assessed using Tukey's honest significant difference test for post-hoc comparisons among different treatment groups. All statistical tests were conducted at a significance level of $P < 0.05$, and analyses were carried out using GraphPad Prism software.

IC50 values were calculated using the ED50PlusV10 software, freely available at https://www.sciencegateway.org/protocols/cellbio/drug/data/ed50v10.xls

Results

Phytochemicals analysis of ME

Table 1 summarises the data obtained by MS for molecules detected from *G. mangostana* extract. The choice of positive and negative modes aimed to facilitate the identification of xanthones. In all MS spectra, the predominant feature was the $[M \pm H]^{\pm}$ molecular ion, accompanied by distinct characteristic losses. In the positive ion mode, the loss of 56 Da was employed as an indicator of the number of prenyl units. Figure 1 shows the negative MS/MS spectrum obtained for Garcinone E. LC-MS analysis confirmed the presence of the following compounds: β-mangostin, 9-hydroxycalabaxanthone, α-mangostin, γ-mangostin, garcinone E, 1,5,8-trihydroxy- 3-methoxy-2-(3-methylbut-2 enyl)xanthone, 2,7-di-(methyl-but-2-enyl)-1,3,8-trihydroxy-4-methylxanthone, 8-deoxygartanin, gartanin and A, 3-isomangostin hydrate (Fig. 1; Table 1).

The structural identification of these compounds was carried out by spectroscopic analyses and comparison with published data (Azebaze et al., 2009; Gopalakrishnan and Balaganesan, 2000; Gutierrez-Orozco and Failla, 2013; Han et al., 2009; Ji et al., 2007; John et al., 2022; Kumar et al., 2022; Mahabusarakam et al., 1987a, 1987b; Nguyen et al., 2003; Nunna et al., 2022; Parveen and Khan, 1988; Sakai et al., 1993; Suksamrarn et al., 2006).

The elucidation of the structures of the newly identified compounds will be undertaken in subsequent studies.

Effect of ME on the growth of human cell lines

The initial investigation focused on assessing the antiproliferative and cytotoxic effects of ME using the U937 leukaemic cell line. To determine the concentration of ME required to inhibit cell growth, cells were exposed to various ME concentrations (5, 9, 12.5, and 25 µg/ml), over different time intervals: 6, 24, and 48 hours (Fig. 2).

The MTT assay demonstrated a dose-dependent and time-dependent inhibition of U937 cell MTT reducing capacity upon the introduction of ME into the culture medium (Fig. 2). The IC50 for ME against U937 cells was found to be 12.5 µg/ml. To further characterise the cytotoxic capacity, ME at a concentration of 12.5 µg/ml was examined at four different time points (0, 6, 24, and 48 hours), as illustrated in Figure 3A.

Table 1
Principal compounds detected from *G. mangostana* extract.

	R_1	R_2	R_3	R_4	R_5	R_6	R_7
β-mangostin	O		- OH		O	-	-
9-hydroxycalabaxanthone	O		- OH	R_5	O R_4	-	-
1,5,8-trihydroxy-3-methoxy-2-(3 methylbut-2-enyl) Xanthone	-	- OH	- OH		-	-	- OH
2,7-di-(methyl-but-2-enyl)-1,3,8- trihydroxy-4-methylxanthone		- OH	- OH		O	-	$-CH_3$
α-mangostin	O		- OH		- OH	- OH	-
γ-mangostin	- OH		- OH		- OH	-	-
garcinone E	- OH		- OH		- OH	-	
8-deoxygartanin		- OH	-	-	-	- OH	
gartanin		- OH	- OH	-	-	- OH	
3-isomangostin hydtrate	O	HO	- OH	R_5	O R_4	-	-

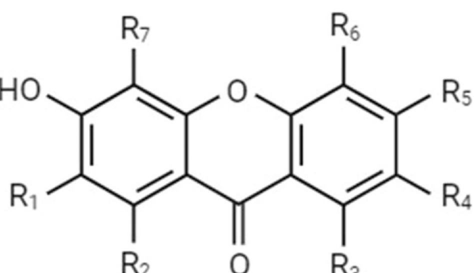

Fig. 1. Pharmacophore of xanthones.

In order to confirm MTT assay results, the viability of U937 cells was evaluated after treatment with 12.5 μg/ml ME for 6, 24, and 48 hours using the trypan blue exclusion assay (Fig. 3). Using this assay, the treatment of U937 cells with ME at 12.5 μg/ml for 24 hours caused a 60–70% decrease of viable cells compared to control.

For comparative purposes, the cytotoxicity of 12.5 μg/ml ME on U937 resulted similar to that elicited by Etoposide (1 μg/ml), (Kitagawa et al., 2010), a highly cytotoxic anticancer drug included as a positive standard in our experimental system (Fig. 3C).

MTT and trypan blue exclusion assays yielded consistent results when 12.5 μg/ml of ME was applied during 6, 24, and 48 hours in the context of both another leukaemic cell line (Jurkat) or non-tumour cell lines such as HUVEC (Fig. 4).

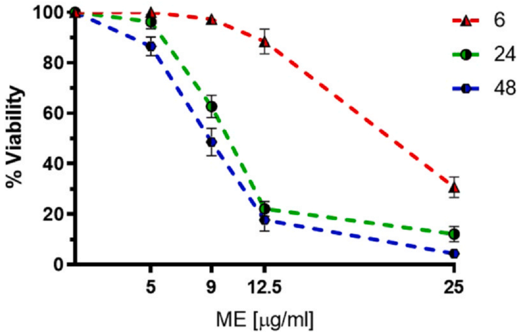

Fig. 2. Effect of increasing ME concentrations on U937 cells. Cells were exposed to increasing ME concentrations over different time periods (6 H, 24 H, 48 H) and immediately assayed for cytotoxicity using the MTT test. Data points are the means ± S.E.M. of three independent experiments performed in duplicate. ME, mango extract; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide.

To assess whether ME cytotoxicity led to apoptosis, non-denaturing Halo Assay was performed on U937 cells treated with 12.5 µg/ml of ME for 6, 24, and 48 hours. The results showed extensive secondary DNA fragmentation that under the FHA conditions utilised herein is indicative of apoptotic nucleolysis (Sestili et al., 2006) at 24 and 48 hours time points, but not at 6 hours exposure (Fig. 5).

Characterisation of ME single fractions

ME was separated into several fractions using HPLC in order to evaluate the total cytotoxic effect of the whole extract versus the single molecules. Three fractions, named A, B, G, and ME (whole extract) have been characterised. The three fractions were chemically characterised, and each was found to contain a distinct set of compounds (Table 2). Fraction A consisted of 8-Deoxygartanin, 1,5,8-Trihydroxy-3-Methoxy-2-(3-Methylbut-2-enyl) Xanthone, γ-Mangostin, 3-Isomangostin Hydrate, Garcinone E and α-Mangostin. Fraction B contained 2,7-Di-(Methyl-but-2-enyl)-1,3,8-Trihydroxy-4-Methylxanthone, α-Mangostin, β-Mangostin, and 3,6-Dihydroxy-1-(3-Hydroxy-3-Methylbutyl)-2,8-Dimethoxy-7-(3-Methylbut-2-enyl) 9-MXanthen-9-one. Fraction G was comprised of Garcinone E, α-Mangostin, 9-Hydroxy Calabaxanthone, 2,7-Di-(Methyl-but-2-enyl)-1,3,8-Trihydroxy-4-Methylxanthone and either γ-Mangostin or Gartanin, alongside 8-Deoxygartanin.

A phytocomplex is a complex mixture composed of a wide variety of organic compounds, including polyphenols, alkaloids, and various other constituents, alongside inorganic substances. The biological effects attributed to a phytocomplex may arise from the synergistic interactions among all of its constituents.

In this study, our focus was on the organic components, and we assessed the cytotoxic and antiproliferative activities of each individual fraction, comparing them to the antiproliferative activity of the extract (Fig. 6). Interestingly the cytotoxic effect of each of the three fractions *per se* was far lower as compared to that of the entire ME.

Discussion

In the present investigation, it has been demonstrated that an ethanolic extract derived from the pericarp of *G. mangostana* (ME) possesses significant anti-proliferative and cytotoxic properties toward leukaemic cell lines. The results highlight the extract's selective cytotoxicity, which preferentially targets malignant cells without affecting non-malignant cells, such as lymphocytes and Human Umbilical Vein Endothelial Cells (HUVEC). Furthermore, the integrated extract displayed enhanced cytotoxic effects on U937 cells relative to its isolated fractions. This observation suggests that the heightened efficacy of the whole extract can be attributed to the synergistic interactions among its constituents, which affect multiple molecular pathways involved in cellular proliferation and survival.

While existing literature has reported data regarding the anti-proliferative properties of individual components within this extract against cancer cells, there has been limited comprehensive investigation into the synergistic effects of its various compounds and the impact of ME on healthy human cells. Nonetheless, this aspect holds paramount significance, as a substantial proportion of contemporary anti-proliferative drugs employed in cancer therapy induce significant side effects by harming healthy cells.

Furthermore, assessments through the fast halo assay and propidium-calcein staining demonstrated that apoptotic events induce anti-proliferative and cytotoxic effects, corroborating findings from earlier studies (Matsumoto et al., 2003), where 72 hours xanthones treatment induced apoptosis in HL60 leukaemic cells. Many antiproliferative and cytotoxic drugs effectively target diseased cells; however, they also

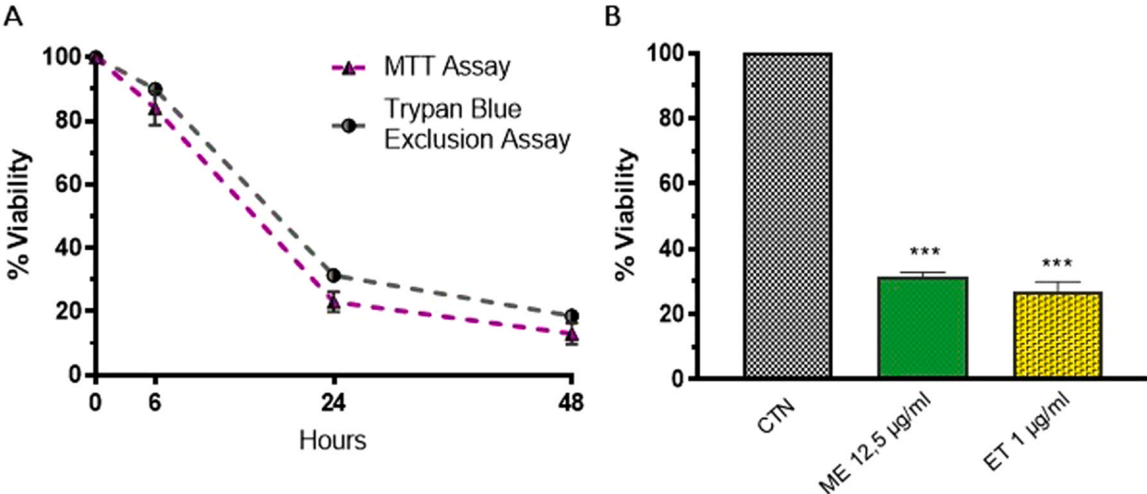

Fig. 3. Cytotoxic effect of ME as assayed with MTT or Trypan blue exclusion assays. (A) Cytotoxic effect of ME at 12.5 µg/ml after different times of continuous incubation as assessed with the MTT or Trypan blue exclusion assays. The percentage of U937 viable cells was evaluated after treatment with 12,5 µg/ml ME. (B) Comparison of the cytotoxic effects of 24 H exposure of U937 cells to ME (12.5 µg/ml) or etoposide (1 µg/ml) as determined with the Trypan blue exclusion assay. Values are the means of three to four independent experiments perfomed in duplicate ± S.E.M. The statistically significant differences (**$P < 0.01$, and ***$P < 0.001$ vs Control) were determined by means of the one-way ANOVA method followed by Bonferroni's post-hoc test. ME, mango extract; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide.

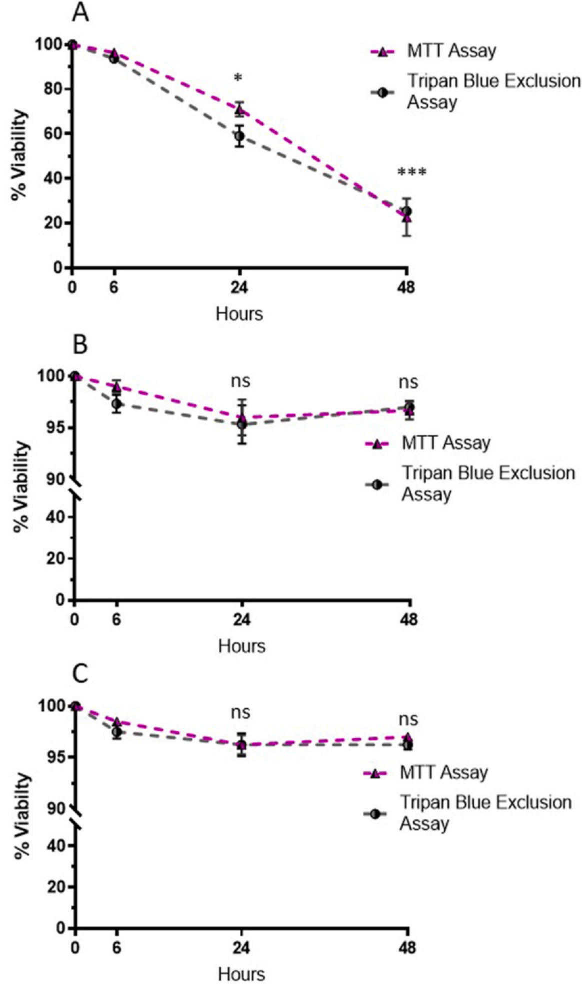

Fig. 4. Cytotoxic effect of ME on Jurkat, HUVEC cells or peripheral blood leucocytes. The effect of 6, 24, and 48 H exposure to 12.5 μg/ml ME was determined on Jurkat (A), HUVEC (B) or peripheral blood leucocytes (C) with MTT or trypan blue assays. Values are the means ± S.E.M. of three to four independent experiments performed in duplicate. Statistically significant differences (*$P < 0.05$, and ***$P < 0.001$ vs Control) were determined by means of the one-way ANOVA. HUVEC, human umbilical vein endothelial cells; ME, mango extract; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; ns, non significant.

induce damage to healthy tissues (Chen and Chen, 2007).

Crucially, our findings indicate that ME's cytotoxicity selectively targets cancer cells, sparing healthy cells, namely HUVEC cells and *ex vivo* leucocytes. This selectivity warrants further exploration into its mechanisms and implications.

The pericarp of *G. mangostana* contains various compounds with documented cytotoxic activity against cancer cells. The synergy among these compounds likely contributes to the enhanced effects observed in our study. This relationship between the constituents of the extract and their combined impact warrants a more in-depth discussion and an extensive review of relevant literature.

For example, α-Mangostin has demonstrated anti-cancer effects, including apoptosis induction in cancer cells, as shown in previous research. Additionally, the compound has been associated with the modulation of ROS levels and protective effects against drug-induced damage. The multifaceted mechanisms of α-mangostin's action suggest its dependency on the delicate balance within cells. Its actions are orchestrated through intricate regulatory processes and interactions among various cellular components (Galadari et al., 2017; Pérez-Rojas et al., 2016).

Interestingly, while many xanthones are known to elevate ROS levels to a point detrimental to cancer cell survival, α-mangostin has been reported to decrease ROS levels while still inducing apoptosis in specific cancer cell lines. This underscores the context-dependent modulation of ROS levels in the context of α-mangostin's antitumor effects. A study also demonstrated a reduction in nuclear factor κB (NFκB) and signal transducer and activator of transcription 3 (STAT3) activation following α-mangostin treatment (Chiarugi and Fiaschi, 2007; Hafeez et al., 2014; Li and Engelhardt, 2006; Storz, 2002).

Furthermore, gartanin has been associated with the induction of apoptosis and endoplasmic reticulum (ER) stress responses in prostate cancer cells. Gartanin treatment was linked to an increase in ER stress markers, which play pivotal roles in the UPR. This Garcinia compound also reduced androgen receptor (AR) levels in prostate cancer, suggesting its potential as an adjuvant agent for prostate carcinoma (Li et al., 2016).

The intricate landscape of α-mangostin and gartanin's effects on cancer cells unfolds within the broader context of prostate cancer research. The AR, a key regulator in prostate cancer progression, is closely related to gartanin's effects, including the reduction of PSA levels. This underlines the interconnectedness of AR and PSA in prostate cancer development (Jariwala et al., 2007; Li et al., 2016).

Our findings reveal the selective cytotoxicity of ME towards cancer cells while sparing healthy cells. The synergy among the compounds in ME enhances its cytotoxic effects, and α-mangostin and gartanin, as key components, exhibit multifaceted mechanisms that warrant further investigation. The context-dependent modulation of ROS levels, induction of apoptosis, and impact on cancer-related signalling pathways underscore the complexity of these substances' actions.

In line with these observations, we draw attention to the well-documented mechanisms of action of alpha-mangostin, a compound closely related to the constituents of ME. Previous research has shown that alpha-mangostin induces apoptosis in leukaemic cells (e.g., U937 and Jurkat) and potentially other cancer cell types, as evidenced by DNA ladder formation, nuclear condensation, and activation of caspases, specifically caspase-9 and caspase-3. These findings support the notion that ME may similarly engage the caspase-9/caspase-3 pathway to trigger apoptosis in these specific cancer cell populations, contributing to its selective cytotoxicity (Matsumoto et al., 2003, 2004).

Furthermore, alpha-mangostin has been shown to disrupt mitochondrial function in these specific cell types, leading to mitochondrial swelling, release of cytochrome c and AIF, decreased mitochondrial membrane potential (DWm), accumulation of reactive oxygen species (ROS), and reduced intracellular ATP levels. These mitochondrial dysfunctions are considered pivotal steps in the apoptotic cascade, and they have been observed in the context of leukaemic cells, prostate carcinoma cells, and potentially other relevant cancer cell types.

The temporal sequence of these events suggests that mitochondrial dysfunction precedes caspase activation, highlighting its importance in alpha-mangostin-induced apoptosis across these specific cancer cell populations.

Drawing from this parallel, it is plausible that the molecular effects of alpha-mangostin, which are well-documented and encompass the disruption of mitochondrial function, may at least partially rationalise our experimental observations regarding the potent anti-leukaemic effects of ME concerning apoptosis and DNA fragmentation. This mechanistic insight is likely operative also in our conditions and offers valuable support for the hypothesis that ME's constituents might exert their cytotoxic actions by modulating mitochondrial function in these specific cancer cell types.

Although alpha-mangostin mitochondrial targeting might play a pivotal role in ME cell death, it is plausible that the extract's distinct constituents induce multiple converging effects that collectively culminate in ME actual toxicity. To this end, the existence of a multifactorial mechanism is mirrored by the finding that the single A, B and

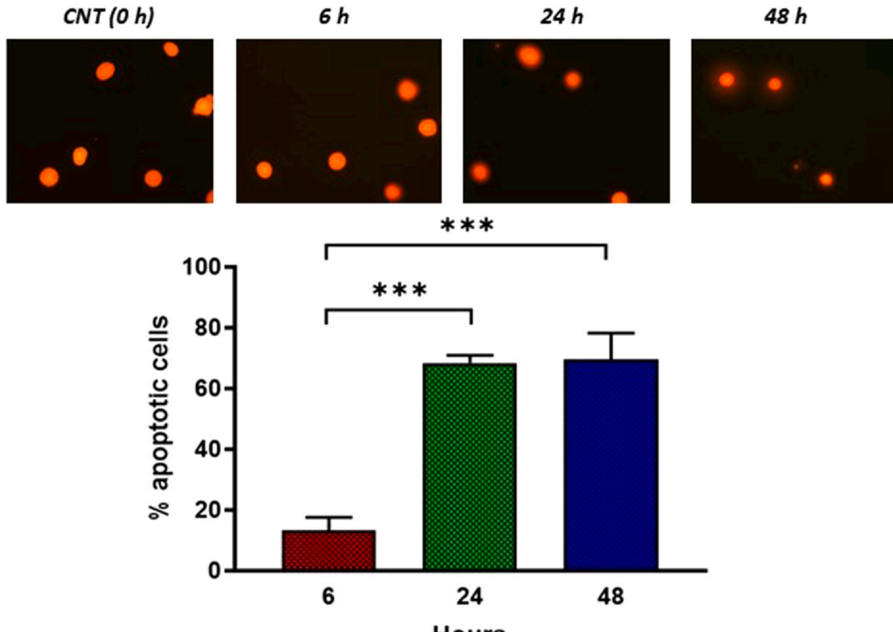

Fig. 5. The percentage of apoptotic U937 cells as a function of increasing ME exposure times. Cells were exposed to 12.5 µg/ml ME for 6, 24, and 48 H and immediately assayed for secondary DNA fragmentation with FHA operating at pH 9.6. Values are the means ± S.E.M. of three to four independent experiments performed in duplicate. Statistically significant differences (*** $P < 0.001$ vs 6 H) were determined by means of the one-way ANOVA method. FHA, fast-halo assays; ME, mango extract.

Table 2
Known chemical compounds identified in fractions A, B, and G.

Fraction	Peak	Compound	Reference
Fraction A	$[M-H]^-$ = 379.16	8-deoxygartanin	(Ji et al., 2007)
	$[M-H]^-$ = 341.10	1,5,8-thrihydroxy – 3-methoxy – 2-(3 methylbut – 2-enyl) xanthone	(Parveen and Khan, 1988)
	$[M-H]^-$ = 395.15	γ-mangostin o gartanin	(Ji et al., 2007)
	$[M + H_2O-H]^-$ = 427.16	3-isomangostin hydrate	(Han et al., 2009)
	$[M-H]^-$ = 409.16	α mangostin	(Ji et al., 2007)
	$[M-H]^-$ = 463.21	Garcinone E	(Sakai et al., 1993)
Fraction B	[M-H]- = 393.17	2,7-di-(methyl-but – 2-enyl) – 1,3,8-trihydroxy – 4-methylxanthone	(Gopalakrishnan and Balaganesan, 2000)
	$[M-H]^-$ = 409.16	α-mangostin	(Ji et al., 2007)
	$[M-H]^-$ = 423.18	β-mangostin	(Ji et al., 2007)
	$[M-H]^-$ = 463.21	Garcinone E	
Fraction G	$[M-H]^-$ = 463.21	Garcinone E	(Sakai et al., 1993)
	$[M-H]^-$ = 409.16	α-mangostin	(Ji et al., 2007)
	$[M-H]^-$ = 407.15	9-hydroxy calabaxanthone	(Ji et al., 2007)
	$[M-H]^-$ = 395.15	γ-mangostin o gartanin	(Ji et al., 2007)
	[M-H]- = 393.17	2,7-di-(methyl-but – 2-enyl) – 1,3,8-trihydroxy – 4-methylxanthone	(Gopalakrishnan and Balaganesan, 2000)
	[M-H]- = 379.16	8-deoxygartanin	(Ji et al., 2007)

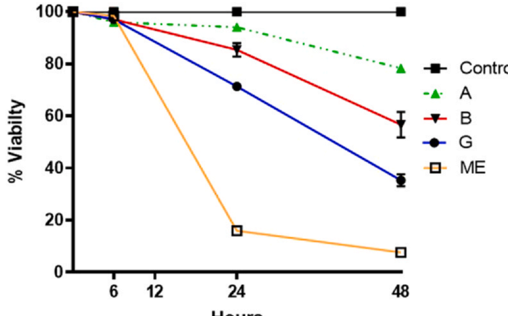

Fig. 6. Cytotoxic effect of different ME fractions on U937 cells. Cells were treated for 24 or 48 H. With 4 µg/ml of fractions A, B, G and 12.5 µg/ml of ME. Values are the means ± S.E.M. of three independent experiments perfomed in duplicate. Statistically significant differences (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs Control) were determined by means of the one-way ANOVA. ME, mango extract.

C fractions taken singularly showed a far lower cytotoxic activity than the whole extract, suggesting that the toxicity of the latter is the result of a synergistic interaction between the distinct constituents. Such an effect is typical of phytocomplexes, whose biological activities are more often the result of mutual interactions rather than dependent on a single, specific mechanism.

Conclusion

This study elucidates the phytochemical constituents of the *G. mangostana* extract, revealing a rich array of compounds with promising cytotoxic potential against leukaemic cells. While we have identified numerous compounds, the precise quantification of these constituents requires further investigation.

The extract displayed pronounced antiproliferative and cytotoxic capabilities, particularly evident in its actions on U937 and Jurkat leukaemic cell lines. Importantly, these effects were significantly attenuated in non-cancerous cells such as HUVECs and human leucocytes,

highlighting its selective cytotoxicity. ME extract appears to induce apoptosis selectively in cancer cells, a mode of cell demise pharmacologically relevant since it minimises collateral damage to healthy tissues. The comprehensive biological activities—antioxidant, antiproliferative, and proapoptotic—of the extracts can largely be ascribed to the cooperative interactions among their constituents, enhancing the overall therapeutic potential. Furthermore, our findings support the traditional use of *G. mangostana* in Asian traditional medicine, shedding light on the potential of its pericarp extract as a source of nutraceuticals or adjunctive therapy in cancer management. However, further research is necessary to fully validate its efficacy and safety for clinical applications.

The observed cytotoxicity against leukaemic cells aligns with previous studies highlighting the potential of individual compounds within the extract, such as α-Mangostin and gartanin, to induce apoptosis and modulate various cellular pathways. This synergistic action of the extract's constituents is a crucial factor in its enhanced effects, warranting further exploration of their intricate mechanisms. The extract's multifaceted mechanisms of action warrant comprehensive exploration, as they may offer insights into the development of novel anticancer therapies that minimise harm to healthy tissues.

In summary, this study contributes to our understanding of the cytotoxic potential of *G. mangostana* extract against leukaemic cells, emphasising its selectivity for cancer cells and its potential as a valuable component in the fight against cancer. Further research is imperative to unravel the molecular mechanisms underpinning the hypotheses generated from our experimental data.

Ethics approval

No human or animal experiments were performed in this research.

Author contributions

Cinzia Calcabrini: Validation, Investigation. **Asher Abdur Rehman:** Writing – review & editing. **Lucia Potenza:** Methodology, Investigation. **Alessia Bartolacci:** Writing – review & editing, Formal analysis. **Giovanni Piccoli:** Resources, Methodology. **Anna Maria Gioacchini:** Writing – original draft, Investigation. **Fabio Ferrini:** Writing – review & editing, Validation, Investigation, Formal analysis. **Matteo Micucci:** Writing – original draft, Validation. **Sabrina Donati Zeppa:** Writing – original draft, Methodology, Investigation. **Piero Sestili:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Vilberto Stocchi:** Supervision, Resources, Funding acquisition. **Giosuè Annibalini:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supporting material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.hermed.2024.100937.

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