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RESEARCH ARTICLE

Antiproliferative Activity of Lemongrass and Rosemary Essential Oils Against Colorectal and Gastric Cancer Cell Lines an in vitro Study

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Abstract

Introduction: Essential oils from medicinal plants, such as lemongrass and rosemary, contain a variety of bioactive compounds. Their potential to target cancer cells makes them promising candidates for adjunctive therapy in gastrointestinal cancers. To evaluate the antiproliferative effects of lemongrass and rosemary oils, and their combined essential oils, on gastrointestinal cancer cell lines (CaCo-2 and AGS) compared to normal human dermal fibroblasts (HdFn). **Materials and Methods:** HdFn, CaCo-2, and AGS cells were subjected to escalating concentrations (12.5–400 µg/ml) of single or combined essential oils for 24 hours. The MTT assay was used to assess cell viability, and the results were analyzed to determine concentration-dependent effects and patterns of selectivity. **Results:** Both essential oils reduced cancer cell viability in a dose-dependent manner, with CaCo-2 and AGS cells showing significantly greater sensitivity than HdFn fibroblasts. At a concentration of 400 µg/ml, cancer cell viability decreased to approximately 40–50%, compared to about 70% in HdFn cells. Lower concentrations-maintained fibroblast viability for over 90% of the time. The oils performed better together in inhibiting cell growth, particularly in CaCo-2 cells, suggesting they may work more effectively together. **Conclusion:** lemongrass and rosemary essential oils exhibit selective antiproliferative effects against gastrointestinal cancer cells, while preserving the viability and integrity of normal fibroblasts. Their combined increased efficacy suggests their potential development as complementary natural anticancer agents. Further studies into the biological and biochemical mechanisms are warranted.

Keywords: Lemongrass essential oil- Rosemary essential oil- Antiproliferative activity- Gastrointestinal cancer

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Introduction

Because of their abundance of bioactive phytochemicals, such as phenolics, aldehydes, and terpenes, herbal essential oils have become intriguing anticancer prospects. These substances show promise in suppressing metastasis, inducing apoptosis, inhibiting the development of cancer cells, and modifying oxidative stress pathways. Essential oils from plants like oregano, clove, turmeric, rosemary (Rosmarinus officinalis), and lemongrass (Cymbopogon citratus) have drawn a lot of attention lately. Molecules like citral, carvacrol, rosmarinic acid, and eugenol, which interact with important cellular targets involved in proliferation and survival, are frequently associated with their anticancer potential [1, 2]. Some references are cited to illustrate general molecular and inflammatory mechanisms relevant to cancer biology rather than disease-specific clinical contexts [3-5].

At present, the management of gastrointestinal cancers utilizes multimodal approaches that integrate surgical intervention with systemic chemotherapy regimens (including 5-fluorouracil, oxaliplatin, and irinotecan), targeted therapies (such as HER2 and EGFR inhibitors), and, more recently, immune checkpoint inhibitors. In this therapeutic context, plant-derived essential oils are not recommended as substitutes for conventional treatment but may function as adjunctive therapies to improve the chemosensitivity of tumor cells or reduce treatment-related toxicity. This study is designed as an exploratory in vitro investigation to determine if lemongrass and rosemary

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essential oils exhibit adequate antiproliferative activity to warrant further assessment as potential complementary agents in neoadjuvant, adjuvant, or palliative treatment contexts.

The essential oils of rosemary and lemongrass are abundant in phenolic compounds and bioactive terpenes that demonstrated definite antiproliferative effects in cancer models. It has been demonstrated that lemongrass oil, which is primarily composed of citral and related monoterpenes, inhibits the growth of colon, lung, cervical, and other cancer cell lines by inducing apoptosis, stopping the cell cycle, and altering signaling pathways like p53 and Src/STAT3 [6]. Conversely, carnosic acid, carnosol, and rosmarinic acid found in rosemary have antioxidant, pro-apoptotic, and anti-angiogenic properties that result in decreased viability and increased cell death in breast, oral, and other carcinoma cells [7].

Natural oils, especially essential oils high in phenolic compounds and terpenes, have been proven in vitro to dramatically lower the viability and multiplication of cancer cells in gastrointestinal tumors. Researchers have observed dose-dependent antiproliferative effects, increased apoptosis, cell-cycle arrest, and modulation of oxidative stress following exposure to plant oils such as Eucalyptus globulus, Teucrium ramosissimum, and mixed essential-oil formulations using colon and gastric cancer cell lines like HCT-116, HT-29, and AGS [8]. Many essential oils selectively harm tumor cells while sparing healthy cells, and some can even make cancer cells more susceptible to TRAIL-induced apoptosis or traditional chemotherapeutic drugs, according to recent research on colorectal cancer. Before proceeding to animal models or clinical trials, in-vitro evaluation of natural oils offers a useful platform to screen for antiproliferative activity, compute IC•[1] values, and investigate processes such ROS production, mitochondrial malfunction, and control of Bax/Bcl-2 balance [9].

Essential oils are intricate blends of bioactive substances, primarily terpenes, phenylpropanoids, and their derivatives, which are becoming more widely acknowledged as potentially useful instruments in cancer treatment. Important compounds including citral and geraniol from lemongrass, carnosic acid and carnosol from rosemary, and eugenol from clove have demonstrated the capacity to stop the growth of cancer cells, induce apoptosis, interfere with mitochondrial function, and regulate oxidative stress [6]. These substances affect several signaling pathways, such as AMPK, Nestin, p53, NF-κB, and MAPKs, which are essential for cell survival, inflammation, and metastatic behavior, according to recent studies [3-5].

New research shows that essential oils or isolated compounds can make standard chemotherapy work better by making tumor cells more sensitive, getting around multidrug resistance transporters, or protecting normal tissues from oxidative stress [10]. Simultaneously, nano- and micro-delivery systems are being engineered to enhance the stability, solubility, and tumor specificity of these highly volatile compounds, especially for colon, breast, and liver cancers. These findings collectively establish the bioactive compounds in essential oils as a significant reservoir of multitarget anticancer agents, appropriate for investigation as adjunct or supplementary alternatives to standard cancer treatments [11].

Plant extracts have many secondary metabolites, like flavonoids, alkaloids, terpenoids, phenolic acids, and essential oil compounds, that are very toxic to cancer cells and stop them from growing. These bioactive molecules disrupt essential cellular pathways by triggering apoptosis, increasing the production of reactive oxygen species (ROS), altering mitochondrial membrane potential, and modulating critical proteins such as Bax, Bcl-2, p53, and caspases. Many extracts also stop the cell cycle, usually at the G0/G1 or G2/M phases, which stops tumors from growing out of control. Recent in vitro studies demonstrate that plant-derived compounds can influence signaling networks, including PI3K/Akt, MAPK, NF-κB, and Wnt/β-catenin, which are pivotal in tumor progression, angiogenesis, and metastasis. Some extracts also make cancer cells more sensitive to chemotherapy, lower drug resistance, and only attack cancer cells while leaving healthy tissues alone. This multi-targeted behavior shows that plant extracts are a great source of naturally occurring anticancer agents and good candidates for integrative cancer therapy [12-14].

Materials and Methods

Cell Lines and Reagents

We obtained CaCo-2, AGS, and HdFn cells from the Department of Pharmacology at the University of Malaya. These cells were used for all our research in passages 5–20. We frequently used phase-contrast microscopy to check cell shape and ensure that epithelial (CaCo-2, AGS) and fibroblastic (HdFn) phenotypes were normal. We used a PCR-based test to check for mycoplasma contamination in the cultures periodically, and these tests were always negative throughout the study. This study did not incorporate short tandem repeat (STR) profiling or comprehensive functional differentiation assays for CaCo-2 cells, including alkaline phosphatase activity or CDX2 expression. However, these will be used in future studies to confirm the authenticity and differentiation status of the cell line.

Cell Culture

The University of Malaya's Department of Pharmacology gave us CaCo-2, AGS, and HdFn cells. We used them from passage 5 to passage 20. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. We used phase-contrast microscopy to see how the cells looked and a PCR-based test to see if there was any mycoplasma contamination in the cultures (all tests came back negative). We used a haemocytometer to count the cells and put them in 96-well plates, as shown before the study [15].

Preparation of Essential Oil Treatments

To get final concentrations of 25 to 400 µg/mL, essential oils were mixed with culture medium. The concentration of DMSO in all wells was kept below 0.5% at all times [16].

The producer sent commercial food-grade essential oils of *Cymbopogon citratus* and *Rosmarinus officinalis*, which were kept in airtight vials that were amber-colored and stored at 4 °C until they were needed. Over the course of the study, oils were used to slow down the process of deterioration. People thought of the oils as crude essential oils instead of pure parts.

MTT antiproliferative effects Assay

After treatment, each well got 10 µL of MTT solution (5 mg/mL in PBS) and was kept at 37 °C for 4 hours. We used DMSO to break up formazan crystals and a microplate reader to check absorbance at 575 nm. There were untreated cells and DMSO vehicle controls on each plate (final DMSO ≤0.5%). Wells with only medium were used as blanks. All tests were done in two wells and at least three times by different people [17].

Statistical Analysis

The data are shown as mean ± SD from at least three independent experiments, each done three times. We employed the Shapiro–Wilk test to ascertain the normality of the data distribution and Levene's test to evaluate the equality of variances. When the assumptions were met, one-way ANOVA was used for several comparisons, and then Duncan's multiple range test was used. If the assumptions were not satisfied, non-parametric tests were employed instead. A p-value of 0.05 or lower was considered statistically significant [18]. We used linear interpolation of dose–response data from three independent experiments to get IC_{50} values. If the 50% inhibition threshold wasn't reached in the tested concentration range, we made it clear that the values were extrapolated.

Results

Effect of lemongrass essential oil on HdFn and Caco-2 cells

When HdFn and Caco-2 cells were treated with lemongrass essential oil at increasing concentrations (12.5–400 µg/ml) for 24 hours, cell viability decreased significantly with increasing concentration. This effect was more pronounced in the Caco-2 colon cancer cell lineage than in normal HdFn fibroblasts. At the highest dose (400 µg/ml), the number of viable cells decreased to 73.03 ± 1.33% in HdFn cells and 49.96 ± 1.04% in Caco-2 cells, compared to the untreated control group (100%). On the other hand, lower concentrations (12.5–25 µg/ml) resulted in a slight decrease in cell viability, with HdFn cells remaining viable at 97.34–98.77% and Caco-2 cells remaining viable at 93.90–95.91%. Caco-2 cells were consistently more sensitive than HdFn cells in the tests. Cell viability remained at 83.72 ± 3.83% in HdFn at a concentration of 200 µg/ml, but decreased to 62.42 ± 3.49% in Caco-2. Even at a concentration of 100 µg/ml, HdFn cell viability remained above 87%, while Caco-2 cell viability decreased to approximately 75.39 ± 2.57%. These results indicate a decrease in the IC_{50} of Caco-2 cells (between 200 and 400 µg/ml). On the other hand, HdFn cells did not achieve 50% inhibition at any of the tested concentrations, suggesting that the drug is more effective against cancer cells (Table 1).

Effect of lemongrass essential oil on HdFn and AGS cells

When HdFn and AGS cells were exposed to lemongrass essential oil, cell viability clearly decreased with increasing concentration (Table 2). As with the Caco-2 results, the AGS gastric cancer cell line was significantly more sensitive than normal HdFn fibroblasts at all concentrations. At the highest concentration (400 µg/ml), HdFn cells maintained a viability of 73.03 ± 1.33%, while AGS cell viability decreased significantly to 41.67 ± 1.41%, indicating the most pronounced antiproliferative effect observed in this experiment. At intermediate concentrations (100–200 µg/ml), HdFn cell viability remained relatively high (83.71–87.11%), while AGS cell viability showed a more significant decrease (50.35% at 200 µg/ml and 65.32% at 100 µg/ml). Low concentrations (12.5–25 µg/ml) had little effect on HdFn cells, with cell viability remaining above 97%. In contrast, AGS cells showed a slight decrease (86.19–95.18%). The calculated IC_{50} value for AGS cells was lower than that for HdFn cells, just as it was for Caco-2 cells. This indicates that the drug is selectively toxic to gastric cancer cells.

Table 1. Antiproliferative Activity of Lemongrass Essential Oil on HdFn and Caco-2 Cells

Cymbopgon citratus	HdFn		CaCo-2	
Conc.	mean	SD	mean	SD
400	73.03267	1.334742	49.96133	1.037073
200	83.719	3.828863	62.423	3.488055
100	87.114	3.64933	75.38567	2.56831
50	95.409	0.371747	85.571	3.996765
25	97.338	1.060539	93.904	0.582331
12.5	98.76533	0.770288	95.91067	0.65849

Table 2. Antiproliferative Activity of Cymbopogon Citratus on HdFn and AGS Gastric Cancer Cells

Cymbopgon citratus	HdFn		AGS	
Conc.	mean	SD	mean	SD
400	73.03267	1.334742	41.66667	1.407995
200	83.719	3.828863	50.34733	2.027975
100	87.114	3.64933	65.31633	3.131786
50	95.409	0.371747	76.81333	2.058791
25	97.338	1.060539	86.18833	2.297944
12.5	98.76533	0.770288	95.17733	0.176647

Table 3. Antiproliferative Activity of Rosmarinus Officinalis Oil on HdFn and Caco-2 Cells

Rosmarinus officinalis	HdFn		CaCo-2	
Conc.	mean	SD	mean	SD
400	70.795	2.224263	50.23133	1.364566
200	76.312	1.456125	56.36567	0.834962
100	87.423	1.505977	64.54467	2.190914
50	93.827	0.176757	73.457	2.257317
25	95.679	0.467654	86.69	1.557015
12.5	97.068	0.406335	94.48267	0.467819

Table 4. Antiproliferative Activity of Rosmarinus Officinalis Oil on HdFn and AGS Cells

Rosmarinus officinalis	HdFn		AGS	
Conc.	mean	SD	mean	SD
400	70.795	2.224263	51.08	1.011401
200	76.312	1.456125	62.73133	2.391989
100	87.423	1.505977	76.35033	1.885172
50	93.827	0.176757	89.04333	1.874784
25	95.679	0.467654	94.48333	0.267313
12.5	97.068	0.406335	73.418	38.52867

Effect of Rosemary oil on HdFn and Caco-2 cells

Rosemary oil had strong antiproliferative effects, with inhibition reaching 50.23 ± 1.36 at 400 µg/ml and 94.48 ± 0.46 at 12.5 µg/ml. Compared to CaCo-2 cells, HdFn fibroblasts survived longer at all concentrations. However, at lower concentrations, the bioavailability increased significantly, reaching 94.48 ± 0.46 at a concentration of 12.5 µg/ml (Table 3).

Effect of Rosemary oil on AGS and HDFn cells

When rosemary essential oil was used to treat gastric squamous cell carcinoma (ASC) cells, it clearly and dose-dependently reduced cell viability. At the highest tested concentration (400 µg/ml), AGS viability decreased to 51.08 ± 1.01% of the reference value. It then gradually increased to 62.73 ± 2.39%, 76.35 ± 1.89%, and 89.04 ± 1.87% at 200, 100, and 50 µg/ml, respectively. At lower concentrations (25 and 12.5 µg/ml), AGS cells exhibited relatively high viability (94.48 ± 0.27% and 73.42 ± 38.53%, respectively). However, the large standard deviation at 12.5 µg/ml indicates a more varied response closer to the threshold of biological activity. Conversely, normal human dermal fibroblasts (HDFn) showed less sensitivity to Rosemary essential oil at equivalent concentrations. At 400 µg/ml, HDFn viability was 70.80 ± 2.22%, and it steadily increased to 76.31 ± 1.46%, 87.42 ± 1.51%, 93.83 ± 0.18%, 95.68 ± 0.47%, and 97.07 ± 0.41% at 200, 100, 50, 25, and 12.5 µg/ml, respectively. Overall, AGS cells were more affected by the antiproliferative effects of rosemary essential oil than HDFn cells at all tested concentrations. This suggests that rosemary essential oil is more potent against gastric cancer cells (Table 4).

Note: According to the dose-response curves, the CI_{50} values for CaCo-2 and AGS cells treated with lemongrass and rosemary oils ranged between 200 and 400 µg/mL. However, the doses analyzed did not achieve 50% inhibition in HdFn cells.

Discussion

The current study demonstrated that lemongrass essential oil exhibits an antiproliferative effect against gastrointestinal cancer cells, with a moderate but distinct effect. Caco-2 cells showed increased sensitivity compared to normal HdFn fibroblasts. The reduced viability of Caco-2 cells at high concentrations (approximately 50% at 400 µg/ml) compared to HdFn cells (approximately 73% at the same dose) suggests a degree of tumor selectivity. This pattern is consistent with previous studies indicating that lemongrass extracts and essential oil inhibit the proliferation of colorectal cancer cell lines, such as Caco-2 and HCT116, primarily by inducing apoptosis and cell cycle arrest [19]. The observed activity is consistent with the known phytochemical composition of lemongrass oil, which is characterized by an abundance of citral (a mixture of neral and geranyl), as well as other monoterpenes such as geraniol and limonene. Recent in vitro and computer studies have demonstrated that citral-rich lemongrass essential oil can exhibit anticancer properties through various mechanisms, including mitochondrial dysfunction, oxidative stress, caspase activation, and modulation of p53-dependent pathways [6]. Formulation and delivery strategies are likely to be important for future applications, as the overall effect in our experiment required relatively high concentrations to achieve approximately 50% inhibition in Caco-2 cells. Recent research on essential oils has shown that nanoemulsions or polymer-based carriers can significantly increase antiproliferative effects and selectivity by enhancing solubility, stability, and cellular uptake [20].

lemongrass essential oil exhibits potent antiproliferative

Table 5. Estimated IC_{50}

Essential oil	Cell line	IC_{50} (µg/mL)	Note
Lemongrass (C. citratus)	CaCo-2	≈ 399 µg/mL	Linear interpolation (near the top of tested range)
Lemongrass (C. citratus)	AGS	≈ 208 µg/mL	Linear interpolation inside tested range
Rosemary (R. officinalis)	CaCo-2	≈ 408 µg/mL	Extrapolated beyond highest tested (400 µg/mL) — interpret with caution
Rosemary (R. officinalis)	AGS	≈ 419 µg/mL	Extrapolated — interpret with caution

effects against gastric cancer (AGS) cells, while significantly weakening its effect on normal HdFn cells as the results of this study. The substantial reduction in AGS viability at high concentrations is consistent with contemporary findings suggesting that compounds derived from lemongrass essential oil, specifically citral, geraniol, limonene, and beta-myrcene can effectively target gastric cancer cells through oxidative stress, mitochondrial dysfunction, and apoptosis signaling pathways. Recent research indicates that citral induces mitochondrial polarization, reactive oxygen species (ROS) accumulation, and activation of endogenous apoptosis in AGS cells [21]. Similarly, [22] reported that lemongrass extract inhibits gastric cancer proliferation by blocking NF-κB and PI3K/AKT survival pathways, consistent with the significant reduction in AGS cell viability observed in this study. These mechanistic insights may explain the increased susceptibility of AGS cells compared to HdFn cells, which exhibited high viability across all concentrations.

The current results indicate that Rosemary oil exhibit selective anti-proliferation activity, which is consistent with previous research suggesting that oils rich in phytochemicals facilitate programmed cell death in cancer cells due to their altered oxidative state and mitochondrial instability [23, 24]. Rosemary oil contains carnosic acid, rosmarinic acid, and 1,8-cineole. These compounds are known for their cell-growth inhibition activity. The high antiproliferative effects observed in rosemary at low concentrations in CaCO-2 cells is consistent with findings that carnosic acid exhibits significant oxidative activity in cancer cells, inducing apoptosis even at low doses [25, 26]. Also, the increased viability of HdFn at low concentrations suggests that the evaluated essential oils displayed relative sparing of normal fibroblasts at lower concentrations; however, the therapeutic window is narrow at higher doses and translational relevance is limited without optimized delivery or clear in-vivo pharmacokinetic data, reinforcing their potential use as adjuvant cancer agents. This is consistent with recent in vitro toxicity studies showing that plant essential oils cause very little antiproliferative effects in normal human fibroblasts at low doses [27, 28].

In the current study, rosemary essential oil demonstrated an antiproliferative effect against gastric cancer (AGS) cells, with a relatively low toxicity to normal HDFn fibroblasts. At a concentration of 400 µg/ml, AGS cell viability decreased to approximately 50% of the reference level, while HDFn cells maintained a viability exceeding 70% at the same concentration. The slow increase in viability at low concentrations, particularly in HDFn cells, suggests a concentration-dependent effect and a certain level of selectivity for gastric cancer cells. This selective growth inhibition is promising for future anticancer applications, as it indicates that rosemary oil may specifically target cancer cells while preserving normal tissue. Our results are consistent with previous studies indicating the efficacy of rosemary extracts and essential oil in combating various gastrointestinal cancer models. Numerous laboratory studies have shown that rosemary preparations inhibit colon cancer cell proliferation and induce apoptosis, including in the case of Caco-2, HT-29, and SW480 cells, often exhibiting more pronounced effects with longer incubation periods [29]. Rosemary polyphenols, particularly carnosic acid, carnosol, and rosmarinic acid, are the main bioactive compounds responsible for these effects. These compounds achieve this by altering oxidative stress, halting the cell cycle, and activating endogenous apoptosis pathways [30]. In gastric cancer models, purified rosemary diterpenes (such as sageon) have been reported to inhibit cell viability and induce apoptosis, indicating the sensitivity of gastric tumor cells to rosemary-derived compounds [31]. The increased vulnerability of AGS cells compared to HDFn cells identified in our study confirms previous findings and suggests that rosemary oil possesses components capable of distinguishing between malignant and non-malignant cells.

Some studies suggest that the conditions tested, the combination of lemongrass and rosemary oils had stronger antiproliferative effects than either oil alone. However, a formal synergy analysis was not done. Therefore, these findings should be considered preliminary and will require validation through established methodologies, such as Bliss independence or isobologram analysis, in future investigations [32-34].

Previous research suggests that citral, carnosic acid, and similar compounds can induce apoptosis, oxidative stress, and modulate pathways such as p53, NF-κB, and PI3K/Akt; however, the present study did not directly evaluate these mechanisms. Therefore, the proposed pathways remain speculative within our experimental framework. Further research should include mechanistic validation using assays such as caspase-3 activation, ROS quantification, mitochondrial membrane potential assessment, and cell cycle profiling to elucidate the main pathways responsible for the observed antiproliferative effects [35, 36].

Research suggests that citral, carnosic acid, and similar compounds may induce apoptosis, oxidative stress, and the modulation of signaling pathways such as p53, NF-κB, and PI3K/Akt; however, these mechanisms were not directly assessed in the present study. As a result, mechanical interpretations remain speculative. Subsequent investigations will include annexin V/PI labeling, quantification of reactive oxygen species, mitochondrial membrane potential assays, cell-cycle analysis, and Western blotting for essential pathway proteins to clarify the principal mechanisms within our system [37, 38].

The estimated IC_{50} values indicate moderate antiproliferative efficacy, particularly for lemongrass essential oil in AGS cells. The high concentrations required indicate that this in-vitro study is still in its preliminary phase and necessitates formulation optimization or compound fractionation (Table 5).

The effective concentrations observed in this study (CI_{50} values ranging from 200 to 400 µg/mL) are relatively high and could exceed those achievable through conventional systemic administration of botanical preparations. The selectivity margin between cancer cells (≈40–50% viability) and normal fibroblasts (≈70% viability) at the highest dose indicates a limited treatment window.

These results demonstrate the challenges of translating clinical findings into practical applications, particularly regarding the volatility, metabolic instability, and poor absorption of essential oils in vivo. To achieve greater tumor selectivity and reduce damage to healthy tissue, advanced formulation techniques such as nanoemulsions, polymer carriers, or customized delivery systems may be necessary. Therefore, in vivo pharmacokinetic and efficacy studies are essential before any clinical determinations are made [39, 40].

A limitation of this study is the use of only two gastrointestinal cancer cell lines (CaCo-2 and AGS), which do not fully capture the molecular heterogeneity of colorectal and gastric cancers, including differences in microsatellite instability status, chromosomal instability, and Lauren classification. Additionally, human dermal fibroblasts (HdFn) were employed as a normative control to conduct an initial evaluation of antiproliferative effects selectivity; however, these cells do not precisely reflect the sensitivity of gastrointestinal epithelial tissues relevant to GI oncology. Future studies should incorporate normal colon and stomach epithelial cell lines, alongside cancer models representing diverse molecular subtypes, to improve the evaluation of tissue-specific toxicity and therapeutic relevance. The absence of STR authentication and evaluation of functional differentiation of CaCo-2 cells represents a limitation of the present study and will be addressed in future research. The primary deficiency of this study is its failure to employ gas chromatography-mass spectrometry (GC-MS) for the characterization of the essential oils utilized. Essential oils have different chemical compositions depending on where they come from and how they are made. To ensure the reproducibility of results and to correlate biological activity with specific components, subsequent investigations will include a comprehensive chemical analysis and the standardization of the utilized proportions [41].

Another issue with this study is that it didn't look at how the new drug worked compared to well-known chemotherapy drugs like oxaliplatin and 5-fluorouracil. Adding positive controls to future tests will help put the effectiveness of essential oils in the context of traditional cancer pharmacology.

This study also does not look at important parts of the tumor microenvironment, like low oxygen levels, how immune cells talk to each other, and how stromal cells send signals. It also doesn't look at how these things affect migration or invasion. In subsequent research, it will be essential to integrate three-dimensional culture systems, co-culture models, and assays that examine tumor propagation to more accurately represent its behavior in living organisms. The considerable variability observed at low doses in AGS cells (e.g., 12.5 µg/mL) may signify biological heterogeneity at the response threshold or experimental variability, requiring additional repetitions and kinetic analysis in subsequent studies.

In conclusion, this study provides initial in vitro evidence that lemongrass and rosemary essential oils exhibit dose-dependent antiproliferative effects in $CaCO_2$ and AGS gastrointestinal cancer cell lines, while showing relatively low toxicity to dermal fibroblasts. These findings suggest potential bioactivity; however, they are preliminary and do not establish the drug's safety or efficacy in humans. The observed selectivity is limited and requires validation in more relevant normal epithelial models. Extensive preclinical research is needed before considering any therapeutic use. This includes mechanistic studies, improved formulations, in vivo validation, and ultimately, clinical trials. The present findings constitute a basis for future research, rather than serving as evidence of clinical applicability.

Declarations

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Clinical trial registration

Not applicable.

Conflicts of interest / Competing interests

The authors declare that they have no conflicts of interest.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Authors' contributions

First author contributed to the conception and design of the study. First author performed the experiments and data collection. Second author contributed to data analysis and drafted the manuscript. Second author supervised the study and critically revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval

Not applicable.

(This study involved in-vitro experiments using established cell lines and did not include human participants or animal subjects)

Consent to participate

Not applicable.

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