

Original Research Article

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Production of Ginger (*Zingiber officinale* Roscoe) Essential Oil by Hydrodistillation and Evaluation of its Antimicrobial, Anti-inflammatory and Cytotoxic Activities against HepG2 Cells

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ABSTRACT

Ginger (*Zingiber officinale* Roscoe) is an important culinary and medicinal plant containing volatile constituents with reported biological activities. The present study was undertaken to produce ginger essential oil by hydrodistillation and to evaluate its antimicrobial, in vitro anti-inflammatory and cytotoxic activities. Dried ginger rhizomes were subjected to hydrodistillation using a Clevenger-type apparatus. Antimicrobial activity was evaluated by agar disc diffusion against *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* using 10, 20 and 30 µL of essential oil per disc. Anti-inflammatory potential was assessed using the human red blood cell (HRBC) membrane stabilization assay, while cytotoxicity against HepG2 cells was assessed by the MTT assay at 15–240 µg/mL. The essential oil produced concentration-dependent inhibition zones against the four bacterial test organisms. At 30 µL/disc, inhibition zones were 22 mm for *M. luteus*, 20 mm for *S. aureus*, 40 mm for *P. aeruginosa* and 32 mm for *E. coli*, whereas no inhibition was observed against *C. albicans*. HRBC membrane protection increased from 14.86% at 5 µg/mL to 72.97% at 20 µg/mL. HepG2 cell viability decreased from 99.1% at 15 µg/mL to 51.5% at 240 µg/mL. These findings indicate that ginger essential oil possesses measurable antibacterial, membrane-stabilizing and antiproliferative activities under the tested in vitro conditions. Further work involving chemical profiling, minimum inhibitory concentration determination, appropriate statistical analysis, non-malignant cell controls and mechanistic assays is required to substantiate these preliminary observations.

Keywords

Zingiber officinale;
essential oil; hydro
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antimicrobial activity;
HRBC membrane
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Introduction

Medicinal plants remain important sources of biologically active secondary metabolites, including phenolic compounds and terpenoids. Ginger (*Zingiber officinale* Roscoe; family Zingiberaceae) is one of the most widely consumed spices and has a long history of use in traditional medicine for digestive, respiratory and inflammatory complaints (Ali *et al.*, 2008). The pungent principles of ginger are mainly associated with gingerols and shogaols, whereas the volatile fraction contains monoterpenes and sesquiterpenes that contribute to its characteristic aroma and biological properties (Sivasothy *et al.*, 2011).

Essential oils are complex mixtures of lipophilic volatile constituents and have been investigated for antibacterial, antifungal, antioxidant, anti-inflammatory and antitumour activities. Disruption of microbial membrane integrity is considered one important mechanism underlying the antimicrobial activity of many essential oils (Burt, 2004; Bakkali *et al.*, 2008). Ginger-derived essential oils have also been investigated in experimental inflammatory models and against selected microorganisms, including *Streptococcus mutans* (Funk *et al.*, 2016; Mostafa, 2018). In addition, plant essential oils and ginger-derived constituents have attracted interest as potential sources of anticancer agents (Bayala *et al.*, 2014; Mahomoodally *et al.*, 2021).

Hydrodistillation using a Clevenger-type apparatus is a practical method for recovering volatile oil from aromatic plant material. Hepatocellular carcinoma remains an important cancer burden, and HepG2 cells are widely used as an *in vitro* model for preliminary cytotoxicity screening. Therefore, the present study was designed to produce ginger essential oil by hydrodistillation and to investigate its antimicrobial activity against selected bacterial and fungal organisms, its HRBC membrane-stabilizing activity as an *in vitro* indicator of anti-inflammatory potential, and its cytotoxic effect against HepG2 cells.

Materials and Methods

Plant material and authentication

Rhizomes of *Z. officinale* were purchased from a local market in Chennai, Tamil Nadu, India, in February and authenticated at HeteroGene Biotech Pvt. Ltd., Chennai.

The rhizomes were air-dried at approximately 40 °C and evenly crushed to increase the surface area before extraction.

Extraction of ginger essential oil

One hundred grams of dried and crushed ginger rhizome were placed in a distillation flask containing 300 mL of distilled water (1:3, w/v). The material was gently heated to generate steam through the plant material. The vapour was condensed and hydrodistillation was continued for 2–4 h until no further visible oil separation occurred. The recovered oil was separated from the hydrosol using a separating funnel and stored in airtight amber glass bottles in a cool, dry place until analysis.

Antimicrobial activity

Micrococcus luteus, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* were used as test organisms. Colonies were grown in nutrient broth until turbid, and suspensions were adjusted with 0.9% saline to the turbidity of a 0.5 McFarland standard. Antimicrobial activity was evaluated by the agar disc diffusion method (Bauer *et al.*, 1966). The inoculum was spread uniformly over Mueller–Hinton agar plates. Sterile Whatman No. 1 discs (6 mm) were loaded with 10, 20 or 30 µL of ginger essential oil, air-dried and placed on the inoculated agar surface. Imipenem (IPM) discs were used as the reference antibiotic. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured in millimetres. The assay was performed in triplicate.

HRBC membrane stabilization assay

Blood from a healthy volunteer who had not taken non-steroidal anti-inflammatory drugs for two weeks was mixed with an equal volume of Alsever's solution and centrifuged at 3000 rpm. The packed erythrocytes were washed with isosaline and prepared as a 10% suspension. The reaction mixture contained the test oil at the specified concentration, 1 mL phosphate buffer, 2 mL hyposaline and 0.5 mL HRBC suspension. The mixture was incubated at 37 °C for 30 min and centrifuged. Haemoglobin released into the supernatant was measured spectrophotometrically at 560 nm. Indomethacin was used as the reference drug (Gandhidasan *et al.*, 1991). Percentage protection against haemolysis was calculated as: $[(Ah - At)/(Ah - Ab)] \times 100$, where Ah is the

absorbance of the haemolysis control, At is the absorbance of the test sample and Ab is the absorbance of the untreated blank.

Cytotoxicity assay

HepG2 cells were exposed to ginger essential oil at concentrations of 15, 30, 60, 120 and 240 µg/mL, with untreated cells serving as the control. MTT solution was added and the cells were incubated for 1–4 h to permit viable cells to reduce the tetrazolium salt to purple formazan (Mosmann, 1983). The formazan product was solubilized and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as (mean absorbance of treated cells / mean absorbance of control cells) × 100. Each treatment was performed in triplicate and values were reported as mean values.

Results and Discussion

Antimicrobial activity

Ginger essential oil produced measurable inhibition zones against all four bacterial organisms tested. The inhibition zones increased with increasing oil volume from 10 to 30 µL/disc. *P. aeruginosa* showed the largest zone of inhibition, increasing from 20 to 40 mm, followed by *E. coli* (18–32 mm), *M. luteus* (3–22 mm) and *S. aureus* (10–20 mm). No inhibition zone was observed for *C. albicans* at the tested oil volumes.

At 30 µL/disc, the zone produced against *P. aeruginosa* (40 mm) was numerically larger than that produced by the imipenem reference disc (26 mm), whereas the zones against *M. luteus*, *S. aureus* and *E. coli* were smaller than the corresponding reference zones.

Because disc diffusion is affected by the diffusion characteristics of essential oils, these zone sizes should not be interpreted as direct measures of relative potency against the antibiotic reference.

HRBC membrane stabilization activity

The membrane-stabilizing activity of ginger essential oil increased with concentration. Protection against haemolysis increased from 14.86% at 5 µg/mL to 32.43%, 50.00% and 72.97% at 10, 15 and 20 µg/mL, respectively. The absorbance decreased from 0.68 to 0.25

over the same concentration range.

Cytotoxic activity against HepG2 cells

HepG2 cell viability decreased progressively with increasing concentrations of ginger essential oil. Viability was 99.1% at 15 µg/mL, 85.5% at 30 µg/mL, 74.4% at 60 µg/mL and 62.7% at 120 µg/mL, decreasing to 51.5% at 240 µg/mL. Thus, under the tested conditions, approximately 50% viability was reached only at the highest concentration tested, and an exact IC₅₀ could not be established from the supplied concentration range.

The present study demonstrates that ginger essential oil obtained by hydro distillation exhibited measurable antibacterial activity against both Gram-positive and Gram-negative bacteria. The increase in inhibition zone with increasing oil volume indicates a concentration-dependent response under the disc diffusion conditions used. *P. aeruginosa* showed the largest inhibition zone, followed by *E. coli*, *M. luteus* and *S. aureus*. Similar antibacterial activity has been reported for ginger essential oils obtained from different plant materials and geographical sources (Sivasothy *et al.*, 2011; Mostafa, 2018). The greater zones observed for the two Gram-negative organisms in this experiment should be interpreted cautiously. Although Gram-negative bacteria possess an outer membrane that can restrict penetration of hydrophobic molecules, small lipophilic components of essential oils may interact with and disrupt microbial membranes (Nikaido, 2003; Burt, 2004). Essential-oil diffusion through agar is also variable, and zone diameter is influenced by physicochemical properties as well as antimicrobial activity. Consequently, inhibition zones should not be used to directly compare the potency of the oil between organisms or with an antibiotic disc. Minimum inhibitory concentration and minimum bactericidal concentration assays would provide more quantitative measures (Balouiri *et al.*, 2016).

No inhibition of *C. albicans* was observed at the tested oil volumes. This result indicates that the tested preparation and exposure conditions did not produce a detectable antifungal effect in this assay. Because essential-oil activity can vary substantially with chemical composition, strain characteristics and concentration, broth microdilution and chemical profiling would be useful for determining whether the absence of activity reflects intrinsic tolerance or insufficient exposure.

Table.1 Zone of inhibition (mm) of ginger essential oil against the test organisms. IPM, imipenem; – no zone of inhibition.

Test organism	10 µL	20 µL	30 µL	IPM	Response
<i>Micrococcus luteus</i>	3	18	22	36	Sensitive
<i>Staphylococcus aureus</i>	10	15	20	28	Sensitive
<i>Pseudomonas aeruginosa</i>	20	30	40	26	Sensitive
<i>Escherichia coli</i>	18	25	32	40	Sensitive
<i>Candida albicans</i>	–	–	–	12	Resistant

Table.2 Effect of ginger essential oil on HRBC membrane stabilization. Untreated blank OD = 0.05; haemolysis control OD = 0.79.

Concentration (µg/mL)	Absorbance (OD)	Protection against haemolysis (%)
5	0.68	14.86
10	0.55	32.43
15	0.42	50.00
20	0.25	72.97

Table.3 Viability of HepG2 cells after treatment with ginger essential oil. Values are means of three replicates.

Concentration (µg/mL)	Mean absorbance (570 nm)	Cell viability (%)
Control	0.966	100.0
15	0.958	99.1
30	0.826	85.5
60	0.719	74.4
120	0.606	62.7
240	0.498	51.5

Figure.1 Representative agar disc diffusion plates showing inhibition produced by ginger essential oil against selected bacterial isolates such as *Pseudomonas aeruginosa* and *Escherichia coli*.

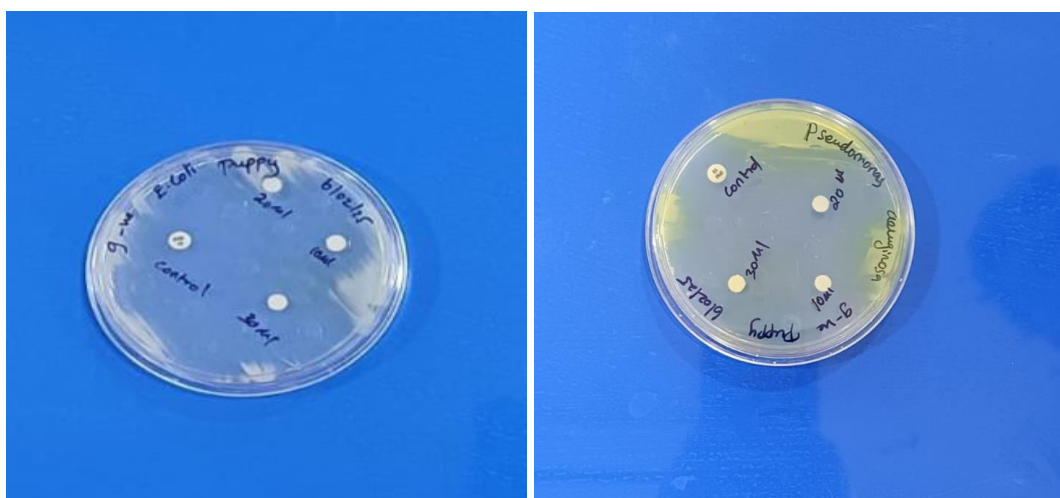
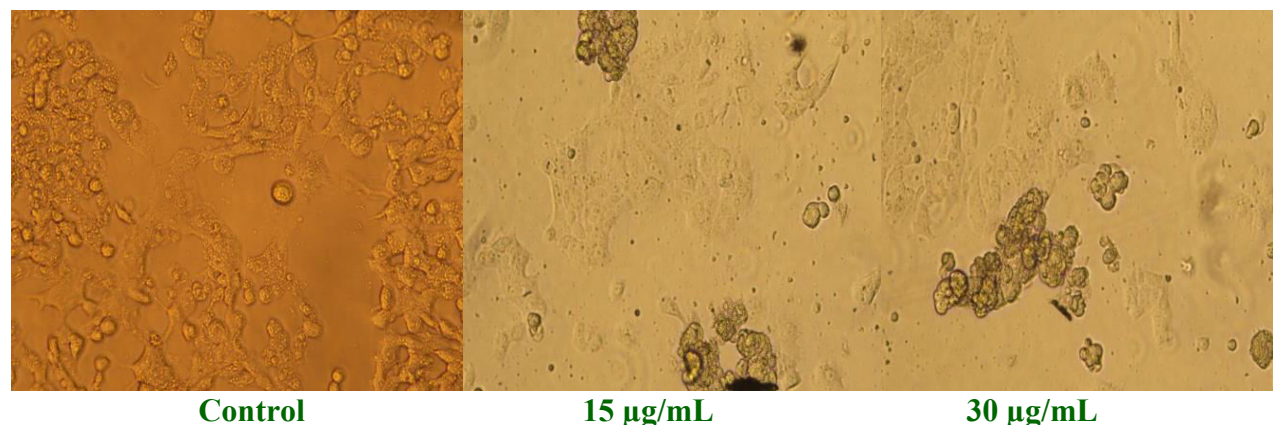


Figure.2 Representative HepG2 cell line.



The HRBC membrane stabilization assay showed a clear concentration-related increase in protection against haemolysis, reaching 72.97% at 20 µg/mL. Stabilization of erythrocyte membranes under hypotonic stress is commonly used as an indirect in vitro indicator of anti-inflammatory potential because erythrocyte membranes share some properties with lysosomal membranes (Gandhidasan *et al.*, 1991). The present result is compatible with previously reported anti-inflammatory activity of ginger essential oils in experimental models (Funk *et al.*, 2016). Nevertheless, HRBC stabilization is a screening assay rather than direct evidence of an anti-inflammatory mechanism. Enzyme inhibition, cytokine profiling and validated cellular inflammatory models would be needed for mechanistic confirmation.

The MTT assay showed a progressive decline in HepG2 metabolic viability with increasing oil concentration. Viability remained above 60% up to 120 µg/mL and decreased to 51.5% at 240 µg/mL. The data obtained therefore support a concentration-dependent cytotoxic response, but they do not establish a precise IC₅₀ because the tested range did not include concentrations sufficient to define the 50% inhibitory point with confidence. In addition, MTT primarily reflects cellular metabolic activity and does not identify the mode of cell death (Mosmann, 1983).

Several limitations should be considered when interpreting these findings. The chemical composition of the essential oil was not determined, so the observed activities cannot be attributed to specific volatile constituents. GC–MS profiling would help identify the major components and permit comparison with ginger oils from other geographical and botanical sources. The

antimicrobial work did not include MIC/MBC determinations, and the cytotoxicity study did not include a non-malignant cell line or a positive cytotoxic control. Future studies should incorporate these elements and evaluate apoptosis, oxidative stress and other mechanistic endpoints.

In conclusion, Ginger essential oil produced by hydro distillation showed antibacterial activity against *M. luteus*, *S. aureus*, *P. aeruginosa* and *E. coli* under the tested disc diffusion conditions, while no activity was detected against *C. albicans*. The oil also exhibited concentration-dependent HRBC membrane stabilization and reduced HepG2 cell viability at increasing concentrations, reaching 51.5% viability at 240 µg/mL. These findings support further investigation of ginger essential oil as a source of biologically active compounds. However, the present data are preliminary and do not establish therapeutic efficacy. Chemical characterization, MIC/MBC determination, appropriate statistical analysis, comparison with non-malignant cells and mechanistic studies are required before biological or therapeutic claims can be strengthened.

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Authors' Contributions

Meena M conceived and supervised the study, guided the experimental design and prepared the manuscript. Puppy S carried out the experimental work, collected and analysed the data and contributed to drafting. Both authors read and approved the final manuscript.

Data Availability

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

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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