RESEARCH ARTICLE

Phytochemical Characterization of Methanolic Extract of *Zingiber officinale* using Gas Chromatography-Mass Spectrometry: An *In-vitro* Study

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Received: 27th May, 2025; Revised: 25th Jun, 2025; Accepted: 18th Jul, 2025; Available Online: 25th Sep, 2025

ABSTRACT

Background: Oral cancer is the sixth most common cancer worldwide and among the third in the Indian Sub-continent. *Zingiber officinale* Roscoe, commonly known as ginger, is generally used as an accompaniment herb or a food spice. Various *in vitro* and *in vivo* studies have demonstrated that ginger is an active compound in both the prevention and treatment of prostate, breast, ovarian, and colorectal cancers. However, the effects of ginger on oral pre-malignant conditions are still not known.

Objectives: To conduct a phytochemical analysis of the methanolic extract of *Zingiber officinale* by Gas Chromatography-Mass Spectrometry [GC-MS] and to identify its potential anti-cancer compounds.

Materials and methods: *Zingiber officinale* Roscoe (100 g) was cleaned, dried, and powdered. A Soxhlet extraction was performed using 500 mL of methanol at a temperature of approximately 60°C for 6 hours to investigate various phytochemical components. The extract was then subjected to GC-MS using the Clarus 680 GC to identify the quantitative constituents

Results: Individual compounds were analysed by GC-MS. The primary potent component identified was 6- shagoal at a peak area percentage of 35.91 %, and Zingerone, a phenolic compound with 28.16% of peak area %.

Conclusions: The existence of many secondary metabolites in the preliminary and GC-MS analysis of *Zingiber officinale* justifies the prevalence of phytochemicals with anti-cancer, anti-inflammatory, and other properties, like anti-angiogenic, neuroprotective properties.

Keywords: *Zingiber,* GC-MS, Phytotherapy, Pre-Cancer, chemoprevention.

How to cite this article: Angeline Deepthi, N Rakesh, Gracelin Ranjitha E, Deepu George Mathew, V Shaheen Shamsuddin, Sajeena Ashraf. Phytochemical Characterization of Methanolic Extract of *Zingiber officinale* using Gas Chromatography-Mass Spectrometry: An *In-vitro* Study. International Journal of Drug Delivery Technology. 2025;15(3):991-95. doi: 10.25258/ijddt.15.3.12

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cancer is derived from the word "crab", which means uncontrolled growth of cells. Oral cancer (mouth cancer) is the third most common type of cancer found in the Indian subcontinent, being the 6th in the world[1]. Several studies on the Asian population showed that they have a significantly reduced risk of diverse cancer types compared to the Western world[2,3]. This can be attributed to a wide range of dietary products like ginger, garlic, curcumin, green tea, etc. Phytotherapy, a branch of medicine, deals with the use of plants with their products in preventing and treating several diseases[4]. Since time immemorial, a lot of traditional Indian medicinal systems, such as Ayurveda, Siddha, Unani, naturopathy, etc., have been practicing phytomedicine to treat various conditions. The World Health Organization (WHO) reports the usage of more than 2500 plants in the treatment of various ailments[5]. Hence, there is a growing interest in identifying chemopreventive and therapeutic anti-cancer agents in plant-based products.

Zingiber officinale Roscoe is the generic terminology for ginger, which is commonly used as an accompaniment herb or as a spice in food considered one of the primary medicinal plants in Chinese, Ayurvedic, and Greek medicine, its use dates back over 2,000 years[6]. Various studies have demonstrated anti-inflammatory properties of ginger that mandate its usage in rheumatic disorders due to

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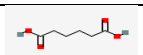
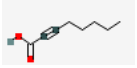
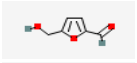
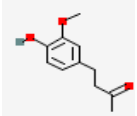
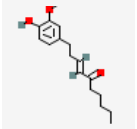
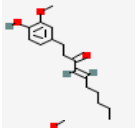
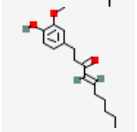
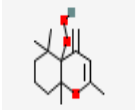
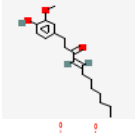
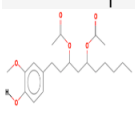
Table 1: Quantitative and qualitative phytochemical constituents of the methanolic extract of ginger

S. No.	Phytochemical constituents	Methanol extract	Quantitative (mg/ml)
1.	Alkaloids: Hager's test	Present	11.2mg/ml
2.	Phenolic compound: $FeCl_3$ test	Present	0.12mg/ml
3.	Flavonoids: Lead acetate test	Present	0.052mg/ml
4.	Tannins: $FeCl_3$ test	Absent	-
5.	Saponins: Foam test	Absent	-
6.	Terpenoids: Horizon test	Present	10.6mg/ml
7.	Carbohydrates: Benedict's test	Absent	-
8.	Steroids: Salkowski's test	Present	-
9.	Phlobatannins: Acidic test	Present	-
10.	Glycosides: Keller killiani test	Absent	-

Table 2: Phytochemical constituents of methanolic extract of BY GC-MS spectra

S. No.	Compound name	Mol Formula	Mol. Weight (g/mol)	Rt (min)	Peak area (%)	Chemical Structure
1.	Hexanal	C6H12O	100	3.228	5.46	
2.	4H-pyran-4one2,3dihydro-3,5-dihydroxy-6-methyl	C6H8O4	144	7.304	4.17	
3.	5-Hydroxy methyl Furfural	C6H6O3	126	8.104	5.76	
4.	2-Butanone 4-(4-hydroxy-3-methoxy phenyl)	C11H14O3	194	12.051	28.16	
5.	(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	C17H24O3	276	16.276	5.70	
6.	2,5,5,8aTetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroxyperoxide	C14H22O3	238	13.301	3.45	
7.	6-Shagaol	C17H24O3	276	16.906	35.91	
8.	3-Decanone,1-(4-hydroxy-3-methoxy phenyl)-5-methoxy	C18H28O4	308	17.332	5.23	
9.	8-Shagaol	C19H28O3	304	19.231	2.85	
10.	(3R.5S)-1-(4-Hydroxy-3-methoxy phenyl)dcane-3,5-diyl diacetate	C21H32O6	380	19.306	3.30	

its ability to inhibit cyclooxygenase[7,8]. Both *in vitro* and *in vivo*, this plant has shown potent antioxidant action, and additive antimicrobial, antifungal, and antiviral actions[8,9]. It contains bioactive compounds, such as gingerol and shogaol, which are shown to contribute to these effects.

Multiple studies have proved that ginger can be used as an active component in the prevention and treatment of prostate, breast, ovarian, and colorectal cancers[10,11]. Usually recognised as a safe medicinal plant, there are some cases of gastric irritation and intestinal cramps reported to have been seen with the use of ginger.

In any phytochemical research, meticulous standardization as well as validation of the individual pharmacological effects remain crucial in ensuring the safe delivery and efficiency of herbal medicines[12]. Plants do naturally possess many phytochemical components that are responsible for their individual medicinal properties. Phytochemical profiling of various secondary metabolites is required to ascertain their pharmacological actions. Pharmacological uses can be assessed primarily by profiling the phytochemical metabolites, which are secondary. Gas Chromatography–Mass Spectrometry (GC-MS) is one of

Table 3: Pharmacological activity and nature of identified phytochemical constituents in *Zingiber officinale*[20]

S. No.	Compound name	Nature of compound	Pharmacological activity
1.	Hexanal	Hexyl aldehyde	Odorant, flavoring agent, Conversion of gingerol to shogaol
2.	4H-pyran-4one2,3dihydro-3,5-dihydroxy-6-methyl	Pyranone	Artificial seasoner, increased radiation sensitivity in human cervical cancer cells
3.	5-Hydroxy methyl Furfural	Aryl-aldehyde	Odorant, flavoring agent
4.	Zingerone	Phenol	Anti-bacterial activity, antiproliferative, apoptotic induction, reactive oxygen species mediated in cervical cancer cells
5.	(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one-(6)/Isoshogaol	Phenol	Programmed cell death in oral cancer induces cytotoxic effects. Also inhibits the uncoordinated growth of cancer cells
6.	2,5,5,8aTetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroxyperoxide	Dihydrochromene	Antioxidant, anti-inflammatory, and antiapoptotic processes
7.	6-Shagaol	Poly Phenolic compound	Anti-cancer activity, reactive oxygen species production, and autophagy induction
8.	3-Decanone,1-(4-hydroxy-3-methoxy phenyl)-5-methoxy phenyl)	Ketone and phenolic ether	Anti-inflammatory, anti-tumor
9.	8-Shagaol	Phenolic compound	Anti-inflammatory, anti-emetic, anti-cancer properties
10.	(3R.5S)-1-(4-Hydroxy-3-methoxy phenyl)dcane-3,5-diyl diacetate	Fatty alcohol ester	anticancer, anti-inflammatory, anti-oxidative stress, and improves cardiovascular metabolism effects

the many advanced techniques to find the volatile phytochemical compounds of the available herbal plant samples[13].

This study aims to extract the methanolic extract of fresh ginger (*Zingiber officinale*), perform a preliminary phytochemical screening, and subsequently characterize its bioactive constituents using GC-MS analysis to identify potential anti-cancer compounds.

METHODOLOGY

The ginger samples were procured from Velayani Agricultural Farm, Trivandrum, and were identified and authenticated by a botanist. *Zingiber officinale* Roscoe (100 g) was cleaned, dried, and powdered. A Soxhlet extraction was performed using 500 mL of methanol at a temperature of approximately 60°C for 6 hours to investigate various phytochemical components. Quantitative analysis was done by GC-MS. The gas chromatogram showed the eluted concentration of compounds as retention time (RT). The chemical components detected are shown as chromatogram peaks. The height of the peaks represented the eluted concentrations of the chemical components. A graphical representation of a compound by ion distribution of their mass and charge ratio (m/z) is Mass spectrometry. This is crucial in the identification of individual chemical structures and corresponding characters. The quantitative presence of the phytochemical is expressed in mg/ml.

RESULTS

The phytochemical analysis of the Methanol extract of ginger is shown in Tables 1, 2, and 3. It revealed the existence of phytochemicals such as alkaloids (11.2mg/mL), phenolic compounds (0.12mg/mL), terpenoids (10.6mg/mL), flavonoids (0.052mg/mL), steroids, and phlobatannins.

IN the GC-MS analysis, 10 secondary metabolites were identified, out of which 6-Shagoal gave peak area% of 35.91 %, and zingerone gave the highest peak area% with 28.16% [Table 2].

DISCUSSION

The present study identified a range of bioactive phytochemicals, among which Zingerone, a phenolic compound with the highest peak area (37.41%), emerged as the primary potent component. Zingerone is recognized for its multi-target pharmacological properties, particularly its anti-inflammatory, antioxidant, and anti-cancer effects. Previous studies have shown that Zingerone significantly inhibits the invasive and metastatic potential of human oral squamous cell carcinoma (OSCC) cell lines by modulating key signaling pathways involved in cell proliferation and apoptosis[14]. Zingerone nanoparticles have further demonstrated enhanced efficacy in reducing OSCC cell viability and motility, possibly via suppression of the Akt signaling pathway, underscoring their therapeutic promise in oral cancer management[15].

Another potent compound identified was (3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-diyl diacetate, a fatty alcohol ester with a peak area of 3.30%. This compound has been reported to exhibit anti-cancer, anti-inflammatory, and anti-oxidative stress activities, along

with benefits for cardiovascular metabolism, suggesting its potential as a multifunctional therapeutic agent[16].

Several other identified compounds, such as 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, have shown promise in increasing radiation sensitivity in cervical cancer cells[17], while 5-hydroxymethylfurfural is noted for its antioxidant and flavoring properties[18]. Furthermore, 6-shogaol and 8-shogaol, polyphenolic compounds, are well-documented for their anti-cancer, anti-inflammatory, and autophagy-inducing properties, particularly in colon and breast cancer models[19].

The presence of phenolic ketones and dihydrochromene derivatives, such as 3-decanone derivatives and 2,5,5,8a-tetramethyl-chromenyl hydroxyperoxide, reinforces the chemopreventive potential of the extract, given their roles in PI3K/Akt, MAPK, and cell cycle-apoptosis pathway modulation[4]. Collectively, these findings align with prior literature emphasizing the polypharmacological nature of phytochemicals in targeting multiple cancer-related pathways (Table 3).

These identified compounds collectively point toward a synergistic effect that could be harnessed in developing plant-based therapeutics for managing OSCC and other malignancies.

CONCLUSION

The GC-MS analysis of the methanolic extract of *Zingiber officinale* revealed approximately ten bioactive compounds, many of which are known for their anti-inflammatory, anti-tumor, and antioxidant activities. The relatively high peak area percentages of these compounds suggest a significant contribution to the observed pharmacological properties of *Zingiber officinale*, particularly its anti-inflammatory and anti-tumor effects. Studies on prevention of early cancer with the usage of these phytochemical products on genetically susceptible individuals, high-risk pre-cancer patients, can be tried. Further tests and analysis are needed to assess both the efficacy and safety of this common Indian kitchen spice in various ailments.

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