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Comprehensive *in vitro* study of the bioactive components and pharmacological interactions of *Zingiber officinale* rhizome extracts

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ABSTRACT

Introduction: *Ginger [Zingiber officinale] is profusely employ as spice in culinary preparations and as a vital ingredient in folk medicine throughout the world. Phytocomponents such as gingerols present in ginger rhizomes exhibit a mitigating effect against cellular oxidation and reduce age-related oxidative stress markers.*

Objectives: *This investigation determines the phytochemical composition, antioxidant ability and acetylcholinesterase inhibitory activity of ginger rhizome extracts prepared using eight different solvents.*

Methodology: *The phytochemical screening was performed using simple qualitative tests. Antioxidant potential of these extracts was studied using DPPH and ABTS assays. Acetylcholinesterase inhibitory activity was analyzed using the Ellman method. GC-MS was performed on the most potent extract.*

Results: *The phytochemical screening confirmed presence of bioactive components, flavonoids and phenols, among others, in all the extracts. DPPH and ABTS analysis revealed that ethanolic extracts had higher antioxidant potential. Also, the acetylcholinesterase inhibitory activity was enhanced in ethanolic extracts, specially 90% ethanol extract. Owing to the superior outcomes of the assays for 90% ethanol extract, it was subjected to gas chromatography-mass spectrometry (GC-MS) technique. A total of 35 compounds were identified in the extract. The main compounds detected were: 6-shogaol (1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one), zingiberene (1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,)]*), and [S]-6-gingerol ((5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one).*

Conclusion: *The bioactive compounds detected and reported to show antioxidant and neuroprotective, and acetylcholinesterase inhibitory properties. These results reiterate the immense beneficial properties of ginger rhizome, which reinforces its use in medicinal practices and its potential in combating diseases related to oxidative stress.*

Keywords: *Ginger rhizome, phytochemicals, antioxidants, acetylcholinesterase inhibition, GC-MS.*

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1. Introduction

For centuries, medicinal plants are widely used in healthcare practices and to manage and treat several diseases [1]. Tribal populations across several countries around the world have been known to use these plants for curing diseases [2]. In the past decade, there has been a significant increase in the consumption of medicinal plant products [3]. The use of these natural products is referred to as alternate medicine, and is more prevalent among older populations [4]. This is primarily because chronic diseases are more common in older individuals, which do not demand extensive use of intricate conventional drug therapy [5]. The bioactive phytochemical compounds present in the medicinal plants exert influence on specific physiological processes in the body which are beneficial to health [6]. Lack of desired efficacy of conventional drug usage and associated toxicity, coupled with its inaccessibility to the rural folk and underprivileged populace has kindled a renewed interest in the medicinal properties of herbs and spices [7]. Ginger [*Zingiber officinale*] is tropical herb from the family *Zingiberaceae* [8].

The underground rhizome of the plant has a firm and striated structure, and is the primary part utilized in traditional healthcare systems and as a spice in multiple cuisines globally [9]. Many countries in the Indian subcontinent have had the knowledge of its nutritional value for over 2000 years. Due to its beneficial properties, ginger rhizomes have been historically used in Ayurvedic and Chinese medicinal practices [10]. India is largest producer, where it is cultivated in most of the states [11]. Fresh and green ginger or dry ginger and split dry are three forms of ginger in which the rhizomes are commercialized [12]. Ginger is among food groups classified as functional foods due to their bioactive components which have mitigating effect against cell oxidation [13]. Various analytical processes have led to the identification of at least 115 constituents in fresh and dry ginger [14]. At least 14 bioactive compounds have been reported from the fractionation of ginger [15]. Oleoresins, vitamins, waxes, carbohydrates, fats, and minerals are other also found in ginger [16].

Traditional medicinal remedies use fresh ginger juice for the treatment of cough and asthma, as it is a potent cough suppressant. The antioxidants in ginger protect the gastric mucosa by combating ulcerogenic agents and prevent the formation of ulcers [17]. Nausea associated with chemotherapy is also relieved by using ginger [18]. The anti-tumor properties of compounds found in ginger can be attributed to their antioxidant activity, as well as their ability to induce apoptosis, reduce proliferation, arrest the cell cycle, and inhibit key signaling pathways [19]. Gingerols are potent anti-inflammatory compounds which exert their ameliorative influence by inhibiting the biosynthesis of prostaglandin and leukotriene [20]. Ginger extracts help in reducing cholesterol levels by inhibiting the production of cholesterol, and lead to an increase in serum HDL-cholesterol [21]. Consumption of dried ginger is known to reduce blood sugar levels and lower triglyceride levels [17]. Ginger enhances cellular metabolic activity by increasing blood circulation which helps to relieve cramps and tension [22].

Among other beneficial properties, ginger also exerts immunity boosting influence by exhibiting antimicrobial action and antioxidant capacity [23]. The antimicrobial properties of ginger are due its phenolic compounds which are effective in managing certain bacterial, viral, and fungal diseases [24, 25]. The antioxidant activity of ginger offers health benefits by alleviating symptoms in diseases associated with oxidative stress [26]. Age-related oxidative stress markers have been reported to be reduced by the consumption of ginger [27]. The polyphenol compounds example of 6-gingerol and derivatives present in ginger rhizomes have high free radical scavenging ability [28]. 6-gingerol were recorded to dose-dependently obstruct nitric oxide production which is a reactive nitrogen species that influences signal transduction, causing DNA damage [29]. There is clinical evidence that bioactive compounds potentially prevent age-related diseases by decreasing risk factors related to aging [30].

Ginger shows immense promise in its role in health management. Further studies are required to fill the research gap in understanding mechanism of action and safety of beneficial phytocomponents present in the rhizomes. The present study aims to analyze the diversity of the phytochemicals present in ginger extracts and to determine antioxidant potential and acetylcholinesterase inhibitory assay.

2. Materials and Methods

2.1 Reagents required

Petroleum ether, methanol, acetone, 70% ethanol, 80% ethanol, 90% ethanol, 95% ethanol and 100% ethanol, Wagner's reagent, Molisch's reagent, concentrated sulfuric acid, glacial acetic acid, iron(III) chloride solution, hydrochloric acid solution, ninhydrin solution, chloroform, acetic anhydride, glacial ethanoic acid, copper sulphate solution, DPPH solution, ABTS solution, acetylcholine chloride, DNTB, EDTA, phosphate buffer saline.

2.2 Collection of plant sample and preparation of plant extract

Zingiber officinale rhizomes were collected from a farm in the Kodagu region of Karnataka state. The authentication of the ginger rhizome samples was done by Dr. V. Rama Rao, Research Officer (Botany), CARI, Bangalore; with accession number RRCBI-mus205. The rhizomes were washed and cut into small pieces before being subjected to drying in hot air oven @ 60 °C for 3 days.

Rhizomes were pulverized and subjected to Soxhlet extraction. Eight different apparatus were prepared using eight different solvents - methanol, petroleum ether, acetone, 70% ethanol, 80% ethanol, 90% ethanol, 95% ethanol and 100% ethanol, respectively. The concentrated extracts obtained from rotary evaporation were then used to perform different assays.

2.3 Preliminary phytochemical screening

The phytochemicals in the plant extracts were analyzed using a simple screening method to determine their presence or absence. The screening methods were as follows.

2.3.1 Test for alkaloids (Wagner's reagent test)
2.3.2 Test for carbohydrates (Molisch's test)
2.3.3 Test for cardiac glycosides (Keller-Kiliani's test)
2.3.4 Test for flavonoids (Alkaline reagent test)
2.3.5 Test for phenols (Ferric chloride test)
2.3.6 Test for phlobatannins (Precipitate test)
2.3.7 Test for amino acids and proteins
2.3.8 Test for saponins (Foam test)
2.3.9 Test for sterols (Liebermann-Burchard's test)
2.3.10 Test for tannins (Braymer's test)
2.3.11 Test for terpenoids (Salkowski's test)
2.3.12 Test for quinones
2.3.13 Test for oxalates
2.3.14 Test for fats and oils
2.4 Antioxidant assays

2.4.1 DPPH assay - The free radical scavenging capacity (inhibition percentage) of the extracts from the various samples was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) [31]. Six samples were prepared at various concentrations (ranging from 100 to 500 µg). Methanol was added to bring the volume of each sample to 0.1 mL, followed by the addition of 3 mL of DPPH solution (adjusted to an absorbance of 1). The test tubes were incubated in the dark for 15 minutes. Methanol served as the solvent, and the optical density (OD) was measured at a wavelength of 517 nm using a spectrophotometer. Subsequently, the inhibition percentage was calculated.

Percentage inhibition = [Control OD − (Sample OD/Control OD)] × 100

2.4.2 ABTS assay - The free radical-scavenging capacity of various plant extracts was measured using the stable radical ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)) [32]. Six samples at various concentrations (ranging from 5 to 25 µg) were prepared using an oxidizing agent (potassium persulfate, 2.45 mM). The volume in each test tube was adjusted to 0.1 ml with methanol, and then 3 ml of ABTS solution (with an absorbance value of 1) was added. The tubes were incubated in the dark for 30 minutes. Methanol served as the control (blank), and absorbance was measured at a wavelength of 734 nm using a spectrophotometer. Finally, the percentage of activity was calculated.

Percentage inhibition = [Control OD − (Sample OD/Control OD)] × 100

2.5 Acetylcholinesterase inhibition assay

The acetylcholinesterase (AChE) inhibition assay was performed using a modified Ellman method [33].

The reaction mixture contained the enzyme, acetylcholine chloride (substrate) at a concentration of 100 mM, 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) at 0.4 mg/mL, ethylenediaminetetraacetic acid (EDTA) at 0.4 mg/mL, and the samples to be analyzed at various concentrations (100–500 µg). The samples were incubated at 37°C for 10 minutes. Thiocholine, produced by the action of acetylcholinesterase, reacted with DTNB to form a compound that imparted a yellow color to the solution. Color intensity correlated with enzymatic activity, which was measured by determining the optical density at a wavelength of 412 nm. Subsequently, the percentage of inhibition was calculated.

Percentage inhibition = [Control OD − (Sample OD/Control OD)] × 100

2.6 GC-MS analysis of 90% ethanol extract of ginger rhizome

Gas chromatography-mass spectrometry (GC-MS) can be used to identify and quantify volatile and heat-sensitive substances, provided they can withstand the rigorous separation conditions within a gas chromatograph. The method yields a spectrum showing all the components detected in the sample. The process begins by injecting a sample into the inlet of the gas chromatograph. This is followed by the evaporation and separation of individual compounds one by one. These compounds produce unique peaks which are then identified by a mass spectrometer.

In the present study, 90% ethanol extract of ginger rhizomes was analysed using a Shimadzu GC-2010 gas chromatograph connected to an AOC-20i injector, coupled to an MS-QP2020 mass spectrometer. An electron ionization system with an ionization energy of 70 eV was used. Helium served as the carrier gas at a constant flow rate of 1.20 mL/min. The fused-silica capillary column was maintained at 60 °C, using helium as the carrier gas at a constant pressure of 72.8 kPa. A 1 µL sample was injected using the "split" technique with a split ratio of 20. The injectors and ion sources were set at 220 °C. The oven temperature was programmed to increase from an initial value of 60 °C to a final value of 240 °C at a linear rate of 3 °C/min. Mass spectra obtained via GC-MS were interpreted by consulting the National Institute of Standards and Technology (NIST) database. The relative peak areas of unknown compounds were compared to known compounds stored in the NIST library.

Statistical analysis

Data from the three parallel measurements are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using GraphPad Prism software (version 5.0). Significant differences between means were determined using Tukey's HSD (Honestly Significant Difference) post hoc test ($p < 0.05$).

3. Results

3.1 Preliminary phytochemical screening

Simple qualitative tests performed on eight different ginger rhizome extracts revealed the presence of an array of phytochemicals (Table 1). Carbohydrates, flavonoids, tannins, oxalates, and fats and oils were found in all the extracts. Alkaloids, cardiac glycosides, and phenols were detected in most of the extracts. However, other classes such as phlobatannins, saponins, sterols, terpenoids and quinones were absent in all the extracts.

Table 1: Phytochemical screening of eight extracts of Zingiber officinale rhizome.

Qualitative Tests	Methanol	Petroleum Ether	Acetone	Ethanol 100%	Ethanol 95%	Ethanol 90%	Ethanol 80%	Ethanol 70%
Alkaloids	+	+	+	+	+	+	+	-
Carbohydrates	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	-
Flavonoids	+	+	+	+	+	+	+	+
Phenols	+	-	+	+	+	+	+	-
Phlobatannins	-	-	-	-	-	-	-	-
Amino acids and proteins	+	-	-	-	-	-	-	+
Saponins	-	-	-	-	-	-	-	-
Sterols	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-	-	-
Quinones	-	-	-	-	-	-	-	-
Oxalates	+	+	+	+	+	+	+	+
Fats and oils	+	+	+	+	+	+	+	+

Note: *In Table 1, (+) denotes presence of phytochemical and (-) denotes absence of phytochemical.*

3.2 DPPH assay

The DPPH method is widely used to assess the ability of a compound to scavenge free radicals or donate hydrogen atoms, as well as to evaluate its antioxidant potential. The results of antioxidant activity are interpreted based on the IC50 (half-maximal inhibitory concentration) value, which refers to the efficacy of a drug or substance in inhibiting a specific biological process or activity by 50%. In this context, this parameter refers to the substrate concentration that causes a 50% decrease in DPPH activity (staining). Gallic acid was used as a reference standard and exhibited an IC_{50} value of 0.617 mg/mL. The free radical scavenging activity of the eight different extracts is demonstrated in **Table 2**. Among the extracts, lowest IC_{50} value was obtained by 90% ethanolic extract of ginger rhizome at 0.018 mg/mL followed by 70% ethanol extract at 0.031 mg/mL, 95% ethanol extract at 0.038 mg/mL, and the methanolic extract at 0.053 mg/mL. Other extracts showed higher values. IC_{50} values of 100% ethanol extract, petroleum ether extract, acetone extract, and 80% ethanol extract were 0.057 mg/mL, 0.108 mg/mL, 0.119 mg/mL, and 0.216 mg/mL, respectively. The results suggest that ethanolic extracts of ginger rhizome exhibited higher antioxidant activity.

Table 2: All values are Mean ± SD. Percentage inhibition values of eight extracts of Zingiber officinale rhizome in DPPH assay. Mean values along a column with similar superscript are not significantly different by Tukey's HSD test (p < 0.05)

Test samples	Concentration (µg/mL)				
	100	200	300	400	500
Gallic acid	7.80 ± 0.80^{a}	14.10 ± 0.60^{a}	24.90 ± 0.30^{a}	33.00 ± 0.50^{a}	39.70 ± 0.10^{a}
Methanol	50.80 ± 0.40^{b}	66.10 ± 0.50^{b}	74.10 ± 0.45^{b}	83.00 ± 0.83^{b}	87.50 ± 0.30^{b}
Petroleum ether	50.50 ± 0.50^{b}	56.70 ± 0.41^{c}	68.40 ± 1.00^{c}	83.70 ± 0.95^{b}	88.90 ± 0.55^{b}
Acetone	45.50 ± 1.05^{c}	57.70 ± 1.01^{c}	74.60 ± 0.55^{b}	85.70 ± 1.01^{c}	87.80 ± 1.50^{b}
Ethanol 100%	50.50 ± 0.60^{b}	65.00 ± 0.50^{d}	77.50 ± 0.50^{d}	84.20 ± 0.60^{b}	88.40 ± 0.40^{b}
Ethanol 95%	54.10 ± 0.40^{d}	65.10 ± 0.55^{d}	77.50 ± 0.20^{d}	86.30 ± 0.35^{c}	90.90 ± 0.10^{c}
Ethanol 90%	53.80 ± 0.35^{d}	67.10 ± 0.30^{b}	74.10 ± 0.40^{b}	81.00 ± 0.90^{d}	86.90 ± 0.30^{d}
Ethanol 80%	34.20 ± 0.10^{e}	52.10 ± 0.15^{e}	60.60 ± 0.40^{e}	68.80 ± 0.60^{e}	78.70 ± 0.40^{e}
Ethanol 70%	50.70 ± 0.50^{b}	69.40 ± 0.30^{f}	79.80 ± 0.26^{f}	83.90 ± 0.15^{b}	89.40 ± 0.30^{b}

3.3 ABTS assay

The IC_{50} of the experimental standard, gallic acid was found to be 0.173 mg/mL. The free radical scavenging activity of the eight different extracts is demonstrated in Table 3. The IC_{50} value of 90% ethanol extract is the lowest at 0.007 mg/mL, consistent with the outcome of DPPH assay; followed by that of the methanol extract at 0.016 mg/mL, 100% ethanol extract at 0.017 mg/mL, and 80% ethanol extract at 0.019 mg/mL. Higher values were obtained in the case of other extracts – 95% ethanol extract at 0.023 mg/mL, 70% ethanol extract at 0.030 mg/mL, petroleum ether extract at 0.035 mg/mL, and acetone extract at 0.049 mg/mL. These results indicate that ethanolic and methanolic extracts have better free radical scavenging activity, with 90% ethanol extract being the most efficient.

Table 3: All values are Mean ± SD. Percentage inhibition values of eight extracts of Zingiber officinale rhizome in ABTS assay. Mean values along a column with similar superscript are not significantly different by Tukey's HSD test (p < 0.05)

Test samples	Concentration (µg/mL)				
	5	10	15	20	25
Gallic acid	36.00 ± 2.00^{a}	54.00 ± 0.61^{a}	73.90 ± 0.52^{a}	88.00 ± 0.86^{a}	98.40 ± 0.59^{a}
Methanol	21.50 ± 0.30^{b}	33.50 ± 0.45^{b}	46.60 ± 0.40^{b}	58.50 ± 0.62^{b}	75.00 ± 0.47^{b}
Petroleum ether	20.50 ± 0.40^{b}	23.70 ± 0.52^{c}	30.50 ± 0.46^{c}	35.60 ± 0.41^{c}	38.80 ± 0.50^{c}
Acetone	30.70 ± 0.35^{c}	32.60 ± 0.45^{b}	35.30 ± 0.26^{d}	37.40 ± 0.25^{c}	38.50 ± 0.40^{c}
Ethanol 100%	20.50 ± 0.46^{b}	31.60 ± 0.68^{b}	44.50 ± 0.50^{e}	59.00 ± 0.51^{d}	65.70 ± 0.30^{d}
Ethanol 95%	6.50 ± 0.45^{d}	15.50 ± 0.35^{d}	24.70 ± 0.40^{f}	42.60 ± 0.45^{e}	57.50 ± 0.45^{e}
Ethanol 90%	40.90 ± 0.23^{e}	57.60 ± 0.36^{e}	67.30 ± 0.26^{g}	72.70 ± 0.35^{f}	82.50 ± 0.45^{f}
Ethanol 80%	20.50 ± 0.46^{b}	32.60 ± 0.35^{b}	41.10 ± 0.85^{h}	48.80 ± 0.50^{g}	61.50 ± 0.45^{g}
Ethanol 70%	2.67 ± 0.35^{f}	8.93 ± 0.50^{f}	21.60 ± 0.47^{i}	32.70 ± 0.35^{h}	37.20 ± 0.40^{c}

3.4 Acetylcholinesterase inhibition assay

The principle of this assay involves the inhibition of acetylcholinesterase (AChE) activity in order to conserve acetylcholine. The results of this assay are interpreted in terms of IC_{50}, which in this case is the effectiveness of the extracts to inhibit the activity of hydrolysis of acetylcholine by 50%. The IC_{50} of the experimental standard, donepezil was found to be 0.760 mg/mL. All the eight extracts contained some level of inhibitory activity against AChE. The percentage inhibition of the eight different extracts is demonstrated in **Table 4**. A low IC_{50} value is indicative of good inhibition of the enzyme. Among all the extracts, the highest inhibitory activity was displayed by 90% ethanol extract with an IC_{50} value of 2.05 mg/mL followed by that of 80% ethanol extract at 2.59 mg/mL, 95% ethanol extract at 2.86 mg/mL, and the petroleum ether extract at 4.62 mg/mL. Other extracts showed moderate activity with methanol extract having an IC_{50} value of 5.30 mg/mL followed by acetone extract at 6.13 mg/mL, 100% ethanol extract at 6.34 mg/mL, and 70% ethanol extract at 9.25 mg/mL. These results suggest that 90% ethanol is a better solvent to extract more active compounds with possible AChE inhibitory activity. These findings are concurrent with the outcomes of DPPH and ABTS assays, implying that 90% ethanolic extract has superior antioxidant activity and acetylcholinesterase inhibiting ability.

Table 4: All values are Mean ± SD. Percentage inhibition values of eight extracts of Zingiber officinale rhizome in Acetylcholinesterase inhibition assay. Mean values along a column with similar superscript are not significantly different by Tukey's HSD test (p < 0.05)

Test samples	Concentration (µg/mL)				
	100	200	300	400	500
Donepezil	5.96 ± 0.17^{a}	12.70 ± 0.08^{a}	18.50 ± 0.15^{a}	25.20 ± 0.22^{a}	33.10 ± 0.30^{a}
Methanol	1.85 ± 0.35^{b}	2.51 ± 0.17^{b}	3.23 ± 0.43^{b}	4.61 ± 0.31^{b}	5.21 ± 0.08^{b}
Petroleum ether	1.95 ± 0.26^{b}	2.51 ± 0.08^{b}	3.56 ± 0.22^{b}	4.91 ± 0.17^{b}	5.86 ± 0.15^{c}
Acetone	1.10 ± 0.22^{c}	1.95 ± 0.15^{c}	2.81 ± 0.08^{c}	3.46 ± 0.26^{c}	4.26 ± 0.17^{d}
Ethanol 100%	0.85 ± 0.08^{c}	1.70 ± 0.17^{c}	2.11 ± 0.15^{c}	3.06 ± 0.22^{c}	3.61 ± 0.30^{e}
Ethanol 95%	1.30 ± 0.18^{c}	3.51 ± 0.09^{d}	5.11 ± 0.26^{d}	6.42 ± 0.34^{d}	8.17 ± 0.43^{f}
Ethanol 90%	1.70 ± 0.17^{d}	3.71 ± 0.08^{d}	6.42 ± 0.17^{e}	9.17 ± 0.27^{e}	11.60 ± 0.34^{g}
Ethanol 80%	1.20 ± 0.25^{c}	3.21 ± 0.17^{d}	5.06 ± 0.08^{d}	6.82 ± 0.17^{d}	8.77 ± 0.43^{f}
Ethanol 70%	0.60 ± 0.15^{e}	1.05 ± 0.15^{e}	1.75 ± 0.17^{f}	2.21 ± 0.87^{f}	2.61 ± 0.17^{h}

3.5 GC-MS analysis of 90% ethanol extract of ginger rhizome

The GC-MS analysis allowed identification and quantification of 90% ethanol soluble bioactive principles from ginger rhizome. The spectral curve, peak retention time, peak area, composition percentage, and name of the components of the extract are presented in Figure 1 and Table 5. The TIC reveals that a total of 35 compounds were identified. The most abundant compounds detected were 6-shogaol (11.83%), zingiberene (11.50%), 6-gingerol (11.40%), butan-2-one, 4-(3-hydroxy-2- methoxyphenyl)- (8.14%) and β-sesquiphellandrene (7.29%), represented in Table 6. Several other compounds were present in moderate amounts. This reinforces the natural diversity of polyphenolic and other classes of compounds, such as flavonoids, terpenes, phenolic acids, tannins, carbohydrates, and fatty acids, present in ginger rhizomes. The positive pharmacological interactions of the ginger rhizomes can be attributed to this array of beneficial substrates present in the rhizomes.

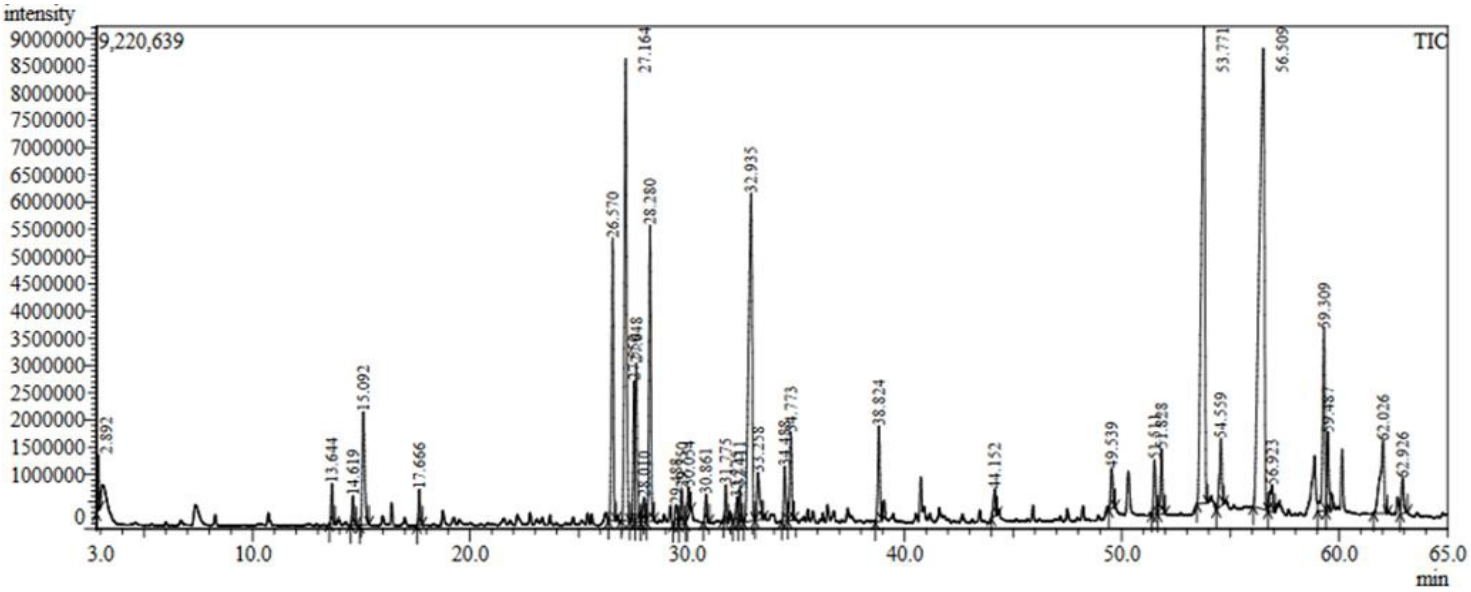
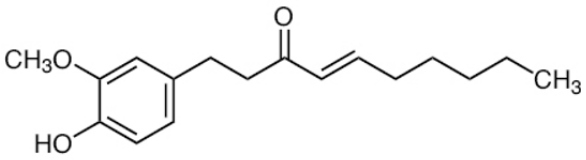
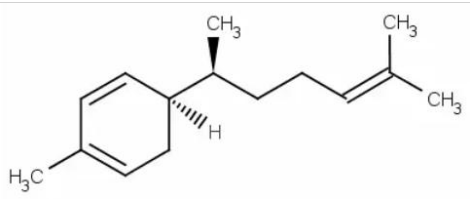
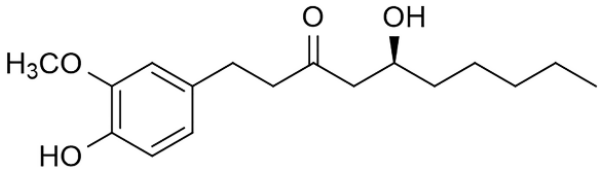
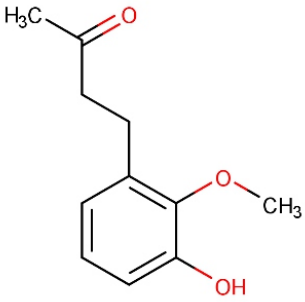
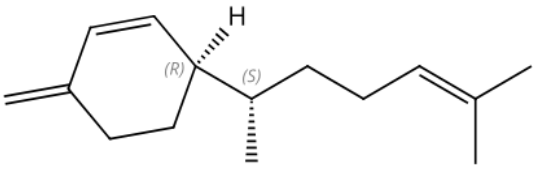


Figure 1: TIC of GC-MS analysis of 90% ethanol extract of Zingiber officinale rhizome

Table 5: Components identified in GC-MS profiling of 90% ethanol extract of Zingiber officinale rhizome.

Peak no.	R. Time	Area	Height (%)	Name of compound
1	2.892	4018301	1.38	2,3-Butanediol, [R-(R*,R*)]-
2	13.644	3994978	1.00	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-
3	14.619	3023822	0.71	α-Terpineol
4	15.092	16924223	2.80	Decanal
5	17.666	3519602	0.88	Citral
6	26.570	32669667	6.97	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
7	27.164	65199765	11.50	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-
8	27.550	14643379	3.48	α-Farnesene
9	27.648	16336186	3.93	β-Bisabolene
10	28.010	2916346	0.59	γ-Muurolene
11	28.280	33471355	7.29	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-
12	29.488	2635664	0.47	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-viny
13	29.750	3907875	0.87	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-
14	30.054	3314525	0.72	4-(1-Hydroxyallyl)-2-methoxyphenol
15	30.861	2897061	0.68	trans-Sesquisabinene hydrate
16	31.775	3575317	0.89	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)cyclohex-2-enol
17	32.275	3114904	0.59	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol
18	32.411	3664376	0.86	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)cyclohex-2-enol
19	32.935	64490578	8.14	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-
20	33.258	8011072	1.16	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methyl
21	34.488	6938793	1.35	trans-Sesquisabinene hydrate
22	34.773	10200684	2.18	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-
23	38.824	11324286	2.37	Caryophyllene oxide
24	44.152	3472167	0.65	n-Hexadecanoic acid
25	49.539	5125318	0.99	13-Octadecenal, (Z)-
26	51.511	6634281	1.32	(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one
27	51.828	8106182	1.60	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-
28	53.771	95475502	11.83	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one
29	54.559	9180200	1.65	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione
30	56.509	134564142	11.40	[6]-Gingerol
31	56.923	4624313	0.65	1-(3,4-Dimethoxyphenyl)-5-hydroxydecan-3-one
32	59.309	25824346	4.52	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one
33	59.487	9754842	1.96	(3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-diyl diacetate
34	62.026	17416477	1.78	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one
35	62.926	4765093	0.86	(E)-1-(4-Hydroxy-3-methoxyphenyl)tetradec-3-en-5-one

Table 6: Major compounds detected in GC-MS profiling of 90% ethanol extract of Zingiber officinale rhizome.

Name & peak no. of the compounds	Chemical structure	Pharmacological actions
6-shogaol (No. 28)		Anti-carcinogenic Antioxidative Anti-inflammatory Neuroprotective
Zingiberene (No. 7)		Antioxidative Anti-inflammatory Antiemetic Antibacterial Antiviral
6-Gingerol (No. 30)		Anti-inflammatory Antioxidative Antiemetic Anti-carcinogenic Cardiotonic Anti-pyretic
Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)- (No. 19)		Anti-inflammatory Antidiabetic Antilipolytic Antidiarrhoeic
β-Sesquiphellandrene (No. 11)		Antioxidant Antimicrobial Anti-inflammatory

4. Discussion

The practice of using medicinal plants for combating several illnesses is as old as mankind itself [34]. A 5000-year-old Sumerian clay slab-Nagpur, the oldest written evidence of the use of plant materials for preparation of drugs [35]. In recent times, an increasing number of people are redirecting their interest from allopathic medicine to natural remedies. Decreasing efficiency of synthetic drugs and the contraindications associated with them have raised concerns resulting in a renewed interest in the application of natural drugs in treatment protocols [35, 36].

Ginger (*Zingiber officinale*) been found to have antiinflammatory, antioxidant, antiapoptotic, antitumour, antipyretic, antiplatelet, anti-diabetic, cardiotonic, anti-clotting, acetylcholinesterase inhibitory, and analgesic properties [37]. Ginger contains a plethora of antioxidant phytocomponents that reduce free radical load. Oxidative stress plays a pivotal role in the development and progression of heart diseases, neurodegenerative diseases, cancer, and aging [38]. Therefore, ginger has been used as a potential preventive and ameliorative agent against these illnesses.

All parts of the ginger plant exhibit health benefits. Ginger rhizomes were utilized for this study. To obtain maximum benefits from the plant product, the active ingredients need to be concentrated by carrying out extraction process. Hot-air oven drying has been the most favored drying method for lab-scale experimentation [39]. Ginger rhizomes can be effectively dried using hot air drying. Major bioactive compounds like gingerols, total phenolic content, total flavonoid content, and higher antioxidant potency are conserved in the drying process [40]. 13 kg of fresh ginger rhizomes were cleaned and subjected to hot air oven drying. The dried product was pulverized. The weight of the dried product was 850 g. This was utilized for the extraction process.

Selecting the appropriate extraction method is a crucial step in the qualitative and quantitative analysis of plant-derived bioactive compounds [41, 42]. Key factors influencing extraction efficiency include matrix properties (plant parts), solvent type, temperature, pressure, and process duration [43]. Eight different solvents were used for the Soxhlet extraction of ginger rhizome. Phytochemical analyses revealed the presence of bioactive substances—including flavonoids, phenolic compounds, and tannins—in most of the extracts.

Evaluations using DPPH and ABTS radicals were conducted to determine the free radical scavenging capacity of the ginger rhizome extracts. There is an inverse relationship between the IC50 value and antioxidant potential. Results from both tests consistently indicated that the 90% ethanolic extract exhibited the lowest IC50 value, signifying the highest antioxidant potential. The IC50 values for the 90% ethanolic ginger rhizome extract were 0.018 mg/mL in the DPPH assay and 0.007 mg/mL in the ABTS assay. Extracts obtained using ethanol at other concentrations also demonstrated high activity.

Several studies have indicated that the antioxidative and AChE inhibitory properties of the phytochemicals found in ginger conserve neurons and limit the progression of neurodegeneration [44]. There is evidence of association between AD and a cholinergic deficit in the post-mortem brain. Better quality of life in developed countries has led to a substantial increase in the older population, among whom AD is prevalent and characterized by a marked reduction in acetylcholine amount [45]. A major feature of an AD brain is the deficiency of acetylcholine at synapses of the cerebral cortex. Enzymatic activity of acetylcholinesterase breaks down acetylcholine into inactive choline and acetate [46, 47]. There is significant promise in management and treatment therapy aiming towards conservation of acetylcholine quantity by inhibition of AChE enzyme [48]. The outcomes of the acetylcholinesterase inhibition assay find 90% ethanolic extract of ginger rhizome to have the best AChE inhibitory potential, with an IC_{50} of 2.05 mg/mL. The results of all biochemical assays were found to be statistically significant using one-way ANOVA ($p < 0.05$).

Due to the superlative results of 90% ethanol ginger rhizome extract, GC-MS was performed on the extract. Up to 35 compounds were identified from the spectral curve. Compounds in the highest quantity were 6-shogaol, zingiberene and 6-gingerol. These compounds have been found to have beneficial biological interactions in living systems leading to therapeutic outcomes. In light of the obtained results of phytochemical screening, antioxidant assays, AChE inhibition assay, and GC-MS profiling, it can be reinforced that ginger rhizome extracts, especially 90% ethanolic extract, are a valuable treasure of natural pharmaceuticals. These considerations reiterate the significance of alternative medicine and encourage us to continue our search for therapeutic agents in plant materials like ginger rhizomes, to design holistic management approaches towards health disorders.

5. Conclusion

There is substantial documented evidence that insinuate that since ancient times, humans have searched for drugs in nature. Our ancestors have thoroughly utilized ginger rhizomes in herbal medicinal prescriptions by virtue of their rich composition of pharmacologically active compounds, such as minerals, flavonoids, and phenolic compounds. In this study, ginger rhizomes were dried in a hot air oven and subjected to Soxhlet extraction. Eight extracts were prepared using eight different solvents. Phytochemical screening, antioxidant assays, and AChE inhibitory assay were performed on all the extracts. It was discovered that all extracts were rich in beneficial phytochemicals and 90% ethanol extract of ginger rhizomes exhibited the highest free radical scavenging capacity and AChE inhibitory activity. GC-MS was performed on 90% ethanolic extract to reveal the abundance of pharmacotherapeutic agents such as 6-shogaol and 6-gingerol, among others.

These deliberations indicate that ginger is not only a valuable dietary supplement but also a potent pharmacological agent. It can be concluded that ginger rhizomes are a powerhouse of natural drugs that can be utilized to fight diseases and achieve holistic well-being. This research serves as a valuable addition to the inventory of existing research paradigms involving phytotherapy. Reservoirs of herbal medicines such as ginger rhizomes are a promising realm that needs to be tapped for their action against pathogenesis and their limited contraindications. Nevertheless, a more organized and pragmatic approach needs to be applied to future studies to facilitate further understanding of the empirical implications of the use of ginger and its plant parts.

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