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Establishing Gerger (*Eruca sativa*) Leaves as Functional Food by GC-MS and *In-vitro* Anti-lipid Peroxidation Assays

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Abstract Conventional food that safeguards against chronic illnesses is known as a functional food. Establishing functional foods starts with phytoconstituent analysis and *in-vitro* characterization of health benefits. *Eruca sativa,* popularly known as gerger or jarjeer in Saudi Arabia, is an annual edible shrub cultivated worldwide. Gerger leaves are consumed raw in salads and have additional health benefits. This study investigated the phytochemical profile of aqueous decoction of gerger leaves of the Saudi origin by GC-MS assay. We also performed *in-vitro* anti-lipid peroxidation and total antioxidant capacity assays using gerger decoction. Twenty-seven chemical compounds belonging to seven classes constituted the gerger decoction: organic siloxanes (39.75%), organic silyl esters (18.28%), phenolics (17.87%), aromatic and aliphatic esters (10.48%), terpenoids (7.09%), heterocycles (3.83%), and sulfur compounds (2.70%). This study reported the presence of compounds mentioned above for the first time in gerger leaves. The decoction method was efficient in the extraction of heat-stable terpenoids like astaxanthin (2.23%), cilonasterol (1.48%), ingol-12-acetate (0.4%), and phytol (2.98%). The *in-vitro* anti-lipid peroxidation study demonstrated the ability of gerger decoction to inhibit hepatic lipid peroxidation in a significantly dose-dependent (150 to 400 µg/ml) manner compared to quercetin. A dose of 400 µg/ml of gerger decoction resulted in 68.46 ± 0.01% inhibition of oxidation of hepatic lipids. The total antioxidant capacity of gerger leaves reported as the IC_{50} of the decoction was 217.90 ± 2.2 µg/ml and also statistically significant. The *in-vitro* models suggested the antioxidant mechanism of gerger was by hydrogen atom transfer and reduction of metal ions. The study substantiated that gerger is a functional food besides established the phytochemical profile contributing to the antioxidant activity. Given that the gerger decoction has a high silicon content and antioxidants, attempt to determine the bioavailability and to identify the molecular targets are essential to overcome bone disorders and oxidative stress.

Keywords: *antioxidant, functional food, gerger, GC-MS, lipid peroxidation*

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1. Introduction

The term functional food refers to a natural food or food constituent or synthetic food or modified food [1] that forms a part of a regular diet, when consumed at reasonable quantities provide nutritional benefits [2] and provide protection against chronic ailments through anti-hyperglycemic, antihypertensive, antioxidant, antimicrobial properties [3,4] to name a few. A nutraceutical marginally different from a functional food is an ingredient separated from food and purified, available as a pharmaceutical product to provide health benefits [2,5]. There are regional regulatory requirements, [6] to claim food as a functional food. The absolute requirement in the evaluation of a potential functional food is to prove its safety and efficacy [7]. Nutraceutical development is governed by stringent legal guidelines that require the support of clinical studies to substantiate claims [8]. A classical method for evaluation of a functional food primarily involves the analysis of phytoconstituents, and preliminary *in-vitro* studies to scientifically validate a preventive or therapeutic effect [9]. Therefore, our understanding is that a study on functional food can lead to the successful development of a nutraceutical when investigated based on chemical constituents and their contribution towards the food's health improvement profile, followed by molecular target-based studies.

Plants have a long history of being an indispensable source of human food. From cereals like rice, wheat, oats to fruits, vegetables, and green leaves are the vital foods providing the necessary balanced diet [10] to people all over the world. All of the foods mentioned above are also examples of functional foods [11]. A green leafy vegetable of the plant family Brassicaceae called as *Eruca sativa*, known by its popular synonyms arugula, gerger, jarjeer, rocket leaves, and taramira [12] was the plant food investigated in this study. We used the synonym gerger to refer to *Eruca sativa*. Gerger leaves are used uncooked in salads. Cooked leaves, flowers, and sprouted seeds of gerger also contribute to the vegan dishes [13]. Gerger leaves possess a characteristic pungent, bitter taste, and pepper-like flavor [14]. Regular consumption of gerger resulted in the cultivation in large quantities worldwide and has commercial value [15]. In Saudi Arabia, it is a cultivated crop and also commercially available. Efforts to chemically characterize the phytonutrients and phytochemicals of medicinal value from different solvent extracts of the whole gerger plant and parts of the plant are continuous and endorsed by scientists in the field of food, agriculture, and pharmaceutical research.

Gerger leaf is an edible source rich in vitamin A, C, and K, and also has high levels of proteins, calcium, magnesium, iron, sulfur, and potassium [16]. Glucosinolates, flavanols, and flavanol glycosides are commonly present in gerger leaves. Glucosinolates, volatile isothiocyanates, and indole compounds are the chief constituents that provide gerger its flavor, besides contributing to the anti-carcinogenic activity [14,17,18]. Flavanol glycosides of gerger leaf improved the well-being of the cardiovascular system [19], possess anticancer activity [20], and the fatty acids of gerger leaves were able to induce adipogenic activity, thereby implicated in diabetes [21]. The methanol extract of gerger leaves was able to exhibit *in-vivo* antioxidant activity in rats [22], and the ethanolic extract improved the reproduction in male rats [23], also show *in-vitro* free radical scavenging activity [24]. The essential oil extracted from gerger leaves contain erucin, an isothiocyanate derivative shown to exhibit anticancer, antibacterial, and free radical scavenging activities [25,26]. Gerger leaves are also useful as anti-ulcer, astringent, antiphlogistic, diuretic, and laxative [16].

Water is a non-toxic and household solvent that justifies its use as a solvent for the extraction of foods. All available antioxidant studies evaluated the *in-vitro* free radical scavenging activities of either methanol or ethanol extract of gerger leaves. Moreover, the Saudi variety of gerger has not been investigated so far for its biological properties. Hence, we aimed to evaluate the *in-vitro* anti-lipid peroxidation and total antioxidant activity of aqueous decoction of gerger leaves and also attempted for the phytochemical characterization of the decoction by gas chromatography-mass spectrometry (GC-MS) analysis.

2. Materials and Methods

Gerger plants collected from a farm in the Jazan region, Saudi Arabia, during January 2020, was authenticated by Dr. Remesh Moochikkal, Assistant Professor, Herbarium, Department of Biology, Jazan University, Jazan, Saudi Arabia. A voucher specimen is available in the herbarium with the certificate number, JAZUH 1225.

Thermo Scientific (United States of America) GC-MS equipped with Thermo Scientific AS 3000 autosampler, Thermo Scientific trace ultra GC oven and Thermo Scientific MS, ISQ detector, and a Thermo Scientific TR column, 5MS, dimensions: internal diameter of 30 m x film thickness of 0.25 mm was effective for separation of the components.

Shimadzu (Japan) UV-1800 240V Ultraviolet-visible (UV-visible) spectrophotometer was used for measuring absorbances in *in-vitro* studies.

In-vitro lipid peroxidation studies involved centrifugation in Sigma (Germany) 3-30K refrigerated benchtop centrifuge.

Samples and tissue solutions were prepared or homogenized using Homogenizer overhead stirrer (Germany) IKA T25 Digital ULTRA TURRAX (rpm * 1000).

Leaf decoction had distilled water as the solvent. Ascorbic acid, ammonium molybdate, concentrated sulfuric acid, disodium hydrogen phosphate, ferrous chloride, methanol, n-butanol, potassium chloride, potassium dihydrogen phosphate, sodium chloride, quercetin, sodium phosphate, and thiobarbituric acid were commercially available. All chemicals used were of analytical grade from Sigma-Aldrich, Saudi Arabia purchased from a local dealer, Jeddah, Saudi Arabia.

2.1. Preparation of Decoction of Gerger Leaves

Weighed 250g of fresh gerger leaves into a blender and gave a pulse rotation for one second. Added 1000ml of water to the cut leaves in a beaker and boiled on a water bath for twenty minutes [27]. Allowed the solution to cool to room temperature, followed by filtration through Whatman filter paper of 45mm diameter and grade 1:11 µm. Pressed the marc using a steel spatula. The green-colored thick decoction obtained was evaporated on a boiling water bath to yield a dry powder. The obtained powder was stored in an airtight container protected from direct sunlight until further use. We carried out GC-MS analysis immediately after the preparation of dried decoction and completed the i*n-vitro* studies within a month of preparation of the dried decoction.

2.2. GC-MS Assay of Gerger Leaves Decoction

The dried decoction was dissolved in methanol at a proportion of 1:10v/v for GC-MS analysis. The carrier gas Helium was allowed to flow continuously at a rate of 1.2 ml/min. A 2 µL volume of the extract dissolved in methanol was injected into the injector adopting a splitless method, which enabled the entire vaporized sample in the injector to enter the column. The injector chamber was at 270°C, and the oven had an initial temperature of 40°C for 1 minute. The oven temperature was gradually increased at a rate of 5°C/min while maintaining the heat at 70°C for 5 minutes, 140°C for 5 minutes, 200°C for 5 minutes, 250°C for 5 minutes and finally to 270°C for 5 minutes. The oven finally had a temperature of 270°C for the rest of the chromatographic run. The mass spectrum of

compounds was obtained using electron ionization (EI) source in the mass spectrometer spanning a range of mass of 60-800 atomic mass units with a scan time of 0.6 minutes. The temperature of the capillary of the transfer of ions in the mass spectrometer was 290°C. The source of ions was at a temperature of 270°C, having 70 eV of energy of ionization. The Xcalibur software was used to interpret the mass spectra. The complete fragmentation patterns of ions for the compounds in the mass spectra in comparison with the characteristic standard mass spectra of similar compounds in the standard libraries, namely NIST, MAINLIB, and REPLIB hyphenated with the mass spectrometer confirmed the presence of the concerned phytochemicals. The percentage of each constituent was measured based on the peak area. We reported the phytoconstituents only when the SI (match factor) and RSI (reverse match factor) values were above the threshold values of 900 on a comparison between the measured spectrum and the reference spectrum, provided the probability of occurrence was also above 90%.

2.3. *In-vitro* Antioxidant Studies

We focussed our attention on the evaluation of the anti-lipid peroxidation capacity of gerger leaves because lipid peroxidation is a crucial biomarker of oxidative stress [28]. Most of the plants synthesize small molecules like flavonoids, phenolic acids, carotenoids, and tannins as secondary metabolites as a part of their self-defense, and these phytochemicals possess immense antioxidant capability [29]. Therefore, we also proceeded to verify the total antioxidant activity of gerger leaves.

2.3.1. Anti-lipid Peroxidation Assay

Hepatic oxidative stress is related to severe dysregulation of metabolic homeostasis and liver injury [30]. Lipid peroxidation of polyunsaturated fatty acids is the leading cause of oxidative stress in cells [31]. Hence, we decided to evaluate the inhibitory capability of gerger on hepatic lipid peroxidation.

A chief biomarker for lipid peroxidation is malondialdehyde [28]. The aldehyde, which is the final product of lipid peroxidation, can be estimated through a colorimetric assay based on the formation of a pink-colored chromophore substance on reaction with thiobarbituric acid [32]. The method followed here was the thiobarbituric acid-reactive substance assay [33].

Fresh bovine liver purchased from local meat sellers served as the source of lipids for the assay. The stock solution of dried decoction of gerger at a concentration of 1mg/1ml was prepared in distilled water, and the reference standard quercetin was diluted with a mixture of water and methanol. The assay procedure initially involved the preparation of 10% v/v liver homogenate in saline buffered with phosphates to provide 7.4 pH, after that centrifuged for ten minutes at 2880 x g of relative centrifugal force at 4°C. To the supernatant (500 µL) obtained, saline buffered with phosphates, 100 µL; 0.04 M concentration solution of ferrous chloride, 50 µL; 0.1 mM solution of ascorbic acid, 50 µL was added to induce lipid peroxidation, further incubated with different concentrations (100 – 500 µL) of the dried gerger decoction or quercetin for one hour at 37°C. Also, added 0.6% w/v thiobarbituric acid dissolved in sodium hydroxide of 1M strength, 2 ml; distilled water, 0.9 ml. Besides, heat the mixture for thirty minutes to enhance the reaction between the reagent and the biochemical intermediates of lipid metabolism. Boiling was accomplished using a boiling water bath. The mixture was cooled, and 5 ml of n-butanol was added with vigorous shaking. After centrifuging the resultant mixture for thirty minutes at 2888 x g, at 4°C, separated the n-butanol layer and the quantity of the pink chromophore formed was determined at a maximum wavelength of 532 nm.

Results were the mean of three replicate measurements reported as mean ± SD (standard deviation). We calculated the % inhibition of lipid peroxidation as in equation (1) where Abs = absorbance and control is the reagent blank without the plant decoction or standard.

$$\%\ inhibition = \frac{\left(Abs_{control} - Abs\ s_{ample}\right)}{Abs_{control}} X100. \quad (1)$$

2.3.2. Total Antioxidant Capacity Assay

The total antioxidant capacity of the dried gerger decoction was determined using the phosphomolybdenum method (*33*). The assessment of total antioxidant capacity depends on the presence of antioxidant compounds in the decoction that can reduce Mo (VI) present in the reagent to Mo (V). The reduced molybdenum forms a green color phosphomolybdate complex, enabling a quantitative spectrophotometric determination. In short, 100 to 500 µL of the sample decoction solution or the standard ascorbic acid solution mixed with 3 ml of phosphomolybdate reagent (prepared by dissolving 4 mM ammonium molybdate in 06 M sulfuric acid containing 28 mM sodium phosphate) was incubated for 90 minutes at 95°C. The absorbance of the cooled mixture containing the resultant green color complex was measured at 692 nm against a reagent blank. Three independent experiments for each concentration of the sample and standard solutions were carried out.

Results were the mean of three independent measurements reported as mean ± SD (standard deviation). Equation (2) was used to calculate the total antioxidant capacity in %. The total antioxidant capacity of gerger decoction was reported as the concentration required to inhibit 50% oxidation, i.e., IC_{50}. The IC_{50} values of the decoction and ascorbic acid were obtained by plotting the log of the total antioxidant activity in % against the concentration in mcg/ml.

$$\%\ antioxidant\ capacity = \left(1 - Abs_{sample} - Abs_{control}\right) X100. \quad (2)$$

2.3.3. Statistical Analysis

The anti-lipid peroxidation and total antioxidant capacity of the extracts were compared with the experimental values obtained for solvent control and standard compounds to determine the statistically significant difference between the groups by means of multiple comparisons through one-way ANOVA. Also, applied a subsequent Dunnett's test. The difference was accepted as significant when less than 0.05 was the P-value.

3. Results and Discussions

The yield of dried aqueous decoction was 1.3 %w/w. Figure 1 was the GC-MS chromatogram of aqueous decoction of gerger leaves obtained as the result of analysis performed, as mentioned under section 2.2.

The GC-MS spectrum revealed the presence of twenty-seven organic compounds in the aqueous decoction of gerger. Table 1 represents the GC-MS peak characters corresponding to the nomenclature of the phytochemical compound identified and its molecular formula. The peak area in Table 1 corresponds to the percentage of the phytochemical constituent present in the dried decoction of gerger leaves. The study reported for the first-time heat-stable siloxanes, silyl esters, terpenoids, heterocyclic compounds, phenolic compounds, and esters from gerger.

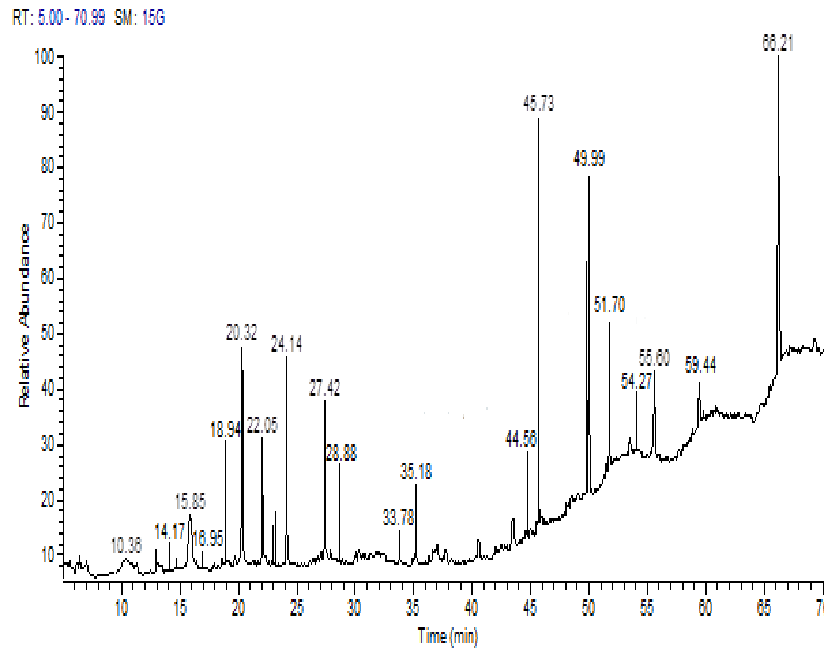


Figure 1. GC-MS Chromatogram of aqueous decoction of gerger leaves

Table 1. Interpretation of GC-MS Spectrum of Aqueous Decoction of Gerger Leaves of Saudi origin

Peak Characters		Compound Name	Mol.Formula
RT (min)	Area (%)		
10.36	1.53	Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{13}H_{22}O_3Si_2$
12.95	0.47	Benzoic acid, ethyl ester	$C_9H_{10}O_2$
14.17	2.39	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$
14.83	1.11	Thieno[3,2-c]pyridine	C_7H_5NS
15.85	2.55	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$
16.95	0.30	Pipradrol	$C_{18}H_{21}NO$
18.94	1.98	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	$C_{12}H_{24}O_3$
20.32	4.37	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$
20.74	0.40	Ingol 12-acetate	$C_{22}H_{32}O_7$
22.05	6.71	Phenol,2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$
22.36	1.59	Dithiocarbamate, S-methyl-,N-(2-methyl-3-oxobutyl)-	$C_7H_{13}NOS_2$
23.02	0.40	2,4-Imidazolidinedione, 5-[3,4-bis [(trimethylsilyl) oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-	$C_{25}H_{40}N_2O_4Si_3$
23.37	1.04	6-hydroxy-3-(2-nitro-1-phenylethyl)-3,4-dihydro-1H-naphthalen-2-one	$C_{18}H_{17}NO_4$
24.14	3.39	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$
27.42	2.62	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$
28.88	3.04	á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-,cyclic butylboronate	$C_{17}H_{37}BO_6Si_2$
33.78	2.23	Astaxanthin	$C_{40}H_{52}O_4$
35.18	2.98	Phytol	$C_{20}H_{40}O$
44.56	1.14	2(1H)-Isoquinolinecarboximidamide, 3,4-dihydro-	$C_{10}H_{13}N_3$
45.73	8.03	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$
49.73	6.73	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$
49.99	20.49	Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$
51.70	4.19	Heptasiloxane,hexadecamethyl-	$C_{16}H_{48}O_6Si_7$
54.27	6.58	1-monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$
55.60	2.14	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$
59.44	1.48	Cilonasterol	$C_{29}H_{50}O$
66.21	10.12	Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl]maleate	$C_{38}H_{56}O_6$

Analysis of the GC-MS chromatogram indicated that the phytochemicals belong to seven different classes of organic compounds, as in Table 2. Information regarding the uses of compounds represented in Table 3 was retrieved from the PubChem database (www. pubchem.ncbi.nlm.nih.gov). In the case of non-availability of information on the pharmaceutical uses, information was obtained from Dr. Duke's phytochemical and ethnobotanical databases (www.phytochem.nal.usda.gov). When there was no available data regarding their health benefits, we have declared as "Activity unknown," besides confirming their presence in other medicinal plants.

Table 2. Phytochemicals in Gerger Leaves Decoction

Phytochemical class	Content %
Organic siloxanes	39.75
Organic silyl esters	18.28
Phenolic compounds	17.87
Aromatic and aliphatic esters	10.48
Terpenoids	7.09
Heterocyclic compounds	3.83
Sulfur compounds	2.70

Table 4 shows the structures of the seven organic siloxanes isolated from gerger leaves. Octasiloxane,1,1,3, 3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-(20.49%) contributed to a higher proportion than other siloxanes. A study indicated the antimicrobial activity of octasiloxane and also confirmed its presence in herbs [34]. Siloxanes generally were reported to exhibit significant antimicrobial and antioxidant properties.

The second major group of constituents was the silyl esters. Table 5 provides the structures of organic silyl esters of gerger. These esters containing a trimethylsilyl group linked to sugars, heterocyclic compounds, and phenyl rings reported elsewhere in plants exerted antimicrobial, anticancer, and anti-inflammatory activities.

Activities of 2,4-Imidazolidinedione, 5-[3,4-bis [(trimethylsilyl) oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)- and á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-,cyclic butylboronate were not proven. However, reports have confirmed their presence in medicinal plants [35] and seaweeds [36].

It is interesting to note that the gerer leaves contain a high amount of silicon, and as they are consumed raw, gerger may be a rich source of dietary silicon. Silicates are the dietary form of silicon that provides health benefits like improvement of bone density, regeneration of collagen, innate immunity, regulation of inflammatory responses, decreases the risk of atherosclerosis, and also strengthens hair, skin, and nail [37]. Given the high silicon content, the bioavailability of siloxanes and silyl esters of gerger needs further investigation. Also, standardization of gerger leaves concerning its silica compounds and recommendations for the quantity of gerger leaves consumption are required.

Table 3. Health Benefits of Gerger Leaf Constituents

Compound Name	Pharmaceutical Use
Benzoic acid, 4-[(trimethylsilyl)oxy] trimethylsilyl ester	Antimicrobial
Benzoic acid, ethyl ester	Food additive, flavoring agent*, antibacterial
Dasycarpidan-1-methanol, acetate (ester)	Anti-inflammatory, antimicrobial
Thieno[3,2-c]pyridine	Antiplatelet therapy
Cyclohexasiloxane, dodecamethyl-	In cosmetics, food products*, antibacterial
Pipradrol	Antifatigue, antidepressant, antiobesity*
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	Food and drug colorant*
Cycloheptasiloxane, tetradecamethyl-	Antifungal
Ingol 12-acetate	Anti-inflammatory
Phenol,2,4-bis(1,1-dimethylethyl)-	Antioxidant
Dithiocarbamate, S-methyl-,N-(2-methyl-3-oxobutyl)-	Anti-inflammatory [37]
2,4-Imidazolidinedione, 5-[3,4-bis [(trimethylsilyl) oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-	Activity unknown
6-hydroxy-3-(2-nitro-1-phenylethyl)-3,4-dihydro-1H-naphthalen-2-one	Activity unknown
Cyclooctasiloxane, hexadecamethyl-	Antimicrobial
Cyclononasiloxane, octadecamethyl-	Antifungal
á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-,cyclic butylboronate	Activity unknown
Astaxanthin	The carotenoid pigment in nutraceutical, cosmetics, food and feeds*, antioxidant
Phytol	Food additive as flavoring agent*, antioxidant
2(1H)-Isoquinolinecarboximidamide, 3,4-dihydro-	Antihypertensive
1,2-Benzenedicarboxylic acid, diisooctyl ester	Food additive*, antimicrobial
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	Anticancer, anti-inflammatory
Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7,9,9,11,11,13,13,15,15-hexadecamethyl-	Antimicrobial [34]
Heptasiloxane,hexadecamethyl-	Antimicrobial
1-monolinoleoylglycerol trimethylsilyl ether	Antimicrobial
Cyclodecasiloxane, eicosamethyl-	Antimicrobial, antioxidant, hepatoprotective
Cilonasterol	Antidiabetic, hypolipidemic
Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl]maleate	Activity unknown

* Retrieved from PubChem. Others were retrieved from Dr. Duke's phytochemical and ethnobotanical databases.

Table 6 exhibits the structures of aromatic and aliphatic esters separated from gerger. Phenolic compounds that were present in gerger decoction are shown in Table 7. Table 8 represents the structures of terpenoids obtained from the gerger. Structures in Table 9 were that of the heterocyclic compounds isolated from gerger leaves. Structures of sulfur-containing compounds are shown in Table 10. Phenolics and terpenoids, well known for their antioxidant activities, contributed to a considerable proportion of the gerger leaf's phytochemical profile. Dithiocarbamate, S-methyl-, N-(2-methyl-3-oxobutyl)- was recently reported from aerial parts of the tulsi plant and possessed anti-inflammatory property [38].

The decoction process can extract phytochemicals that are stable to heat and lipophilic because of higher temperatures employed than other extraction methods (27). Therefore, we understood that siloxanes, silyl esters, phenols, terpenoids, and heterocyclic compounds reported here were heat-stable. For example, all of the well-known terpenoids in the decoction like astaxanthin (tetraterpenoid), clionasterol (γ-sitosterol, tetracyclic triterpenoid), ingol-12-acetate (diterpenoid), and phytol (acyclic diterpenoid) are heat-stable and possess hydrophobic chemical structures.

Table 4. Structures of Organic Siloxanes from Gerger

Cyclohexasiloxane, dodecamethyl-
Cycloheptasiloxane, tetradecamethyl-
Cyclooctasiloxane, hexadecamethyl-
Cyclononasiloxane, octadecamethyl-
Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7,9,9,11,11,13,13,15,15-hexadecamethyl-
Heptasiloxane,hexadecamethyl-
Cyclodecasiloxane, eicosamethyl-

Table 5. Structures of Silyl Esters from Gerger

Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester
2,4-Imidazolidinedione, 5-[3,4-bis [(trimethylsilyl) oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-
á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-,cyclic butylboronate
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-
1-monolinoleoylglycerol trimethylsilyl ether

Table 6. Structures of Aromatic and Aliphatic Esters of Gerger

Benzoic acid, ethyl ester
1,2-Benzenedicarboxylic acid, diisooctyl ester
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester

Despite regular consumption of raw gerger leaves in Saudi Arabia, there were no studies reported regarding their nutritional benefits or health benefits. Moreover, gerger is a commercial crop cultivated around the kingdom of Saudi Arabia. Hence, we proceeded to verify the antioxidant potential of the decoction of gerger leaves.

Table 7. Structures of Phenolic Compounds from Gerger

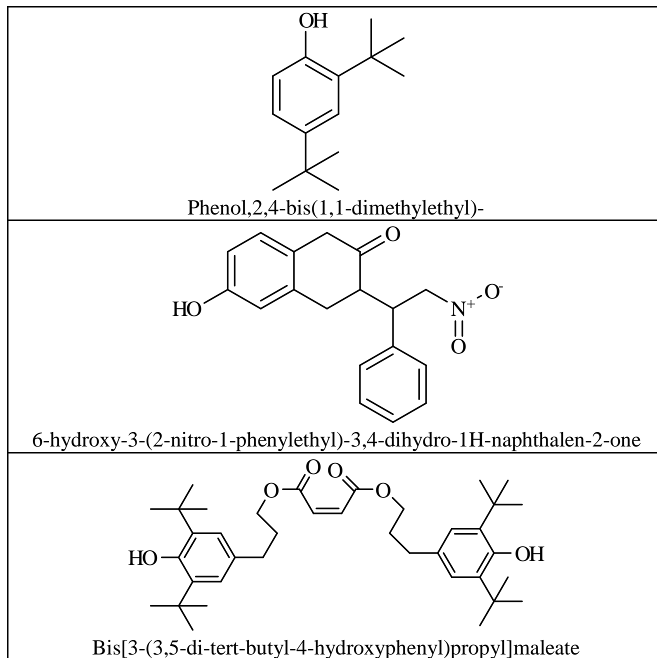
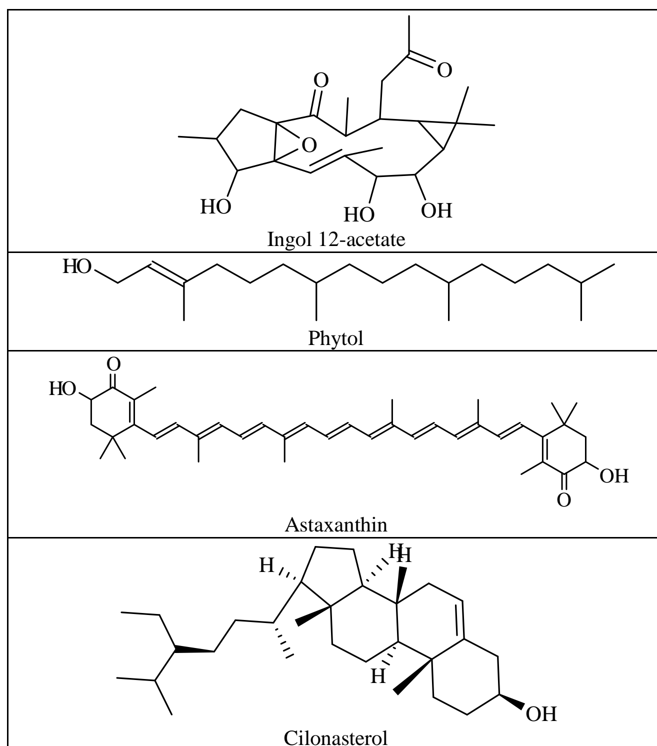


Table 8. Structures of Terpenoids of Gerger



The anti-lipid peroxidation potential of gerger decoction was commendable as understood from Table 11; the results of *in-vitro* lipid peroxidation assay.

Gerger decoction was able to inhibit liver lipid peroxidation in the *in-vitro* model. The decoction exhibited a significant inhibition at concentrations of 250 µg/ml to 400 µg/ml. In the procedure, the gerger decoction was added to the liver homogenate after induction of lipid peroxidation, which revealed that the gerger leaves could reduce the process of lipid peroxidation in the liver. GC-MS analysis revealed the presence of antioxidant chemicals like phytol, astaxanthin, ingol-12-acetate, clionasterol, and phenolic compounds along with other silicates that contributed to the anti-lipid peroxidation activity of gerger decoction. There was a dose-dependent increase in the percentage inhibition of hepatic peroxidation observed with the dried decoction of gerger leaves. Besides, the mechanism of inhibition of lipid peroxidation in the *in-vitro* model was by the transfer of hydrogen atom (*29*), which suggests that the constituents of the gerger decoction also act in a similar model.

Table 9. Structures of Heterocyclic Compounds of Gerger

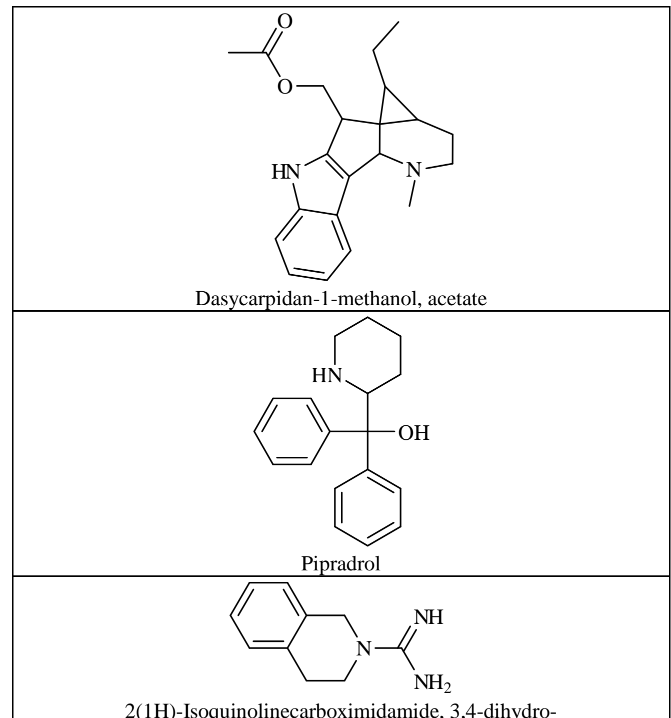


Table 10. Structures of Sulphur Compounds from Gerger

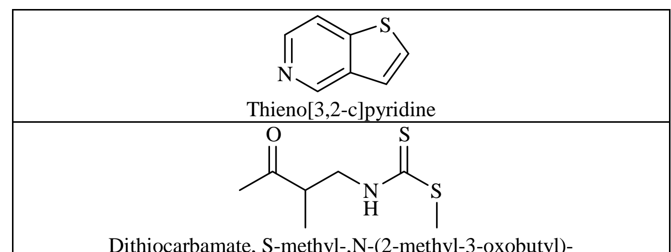


Table 11. Results of *In-vitro* Lipid Peroxidation Assay

Concentration (µg/ml)	% inhibition (mean ± SD) Gerger decoction	% inhibition (mean ± SD) Quercetin
150	17.12 ± 0.20	49.99 ± 0.03
200	31.98 ± 0.01	55.36 ± 0.06
250	43.02 ± 0.09*	61.09 ± 0.02
300	51.59 ± 0.03*	72.11 ± 0.01
350	59.99 ± 0.02*	82.07 ± 0.01
400	68.46 ± 0.01*	91.26 ± 0.01

* $P < 0.05$ compared to quercetin.

Further investigation on the total antioxidant capacity of the gerger decoction by the phosphomolybdenum method also confirmed the potentiality as an antioxidant. Figure 2 shows the total antioxidant capacity of gerger decoction determined in percentage plotted against the screened concentrations. The IC_{50} of gerger decoction

obtained from Figure 2 was 217.90 µg/ml ± 2.2 µg/ml that was significant ($P < 0.05$) when compared to the IC_{50} of ascorbic acid, 74.91 µg/ml ± 1.5 µg/ml. The results indicated that the identified phytoconstituents in gerger leaf decoction function as reducing agents, which is one of the mechanisms of plant antioxidants, especially in this *in-vitro* model [39]. The complex mixture of phytoconstituents of gerger decoction exhibits a linear increase in the total antioxidant activity with the dose.

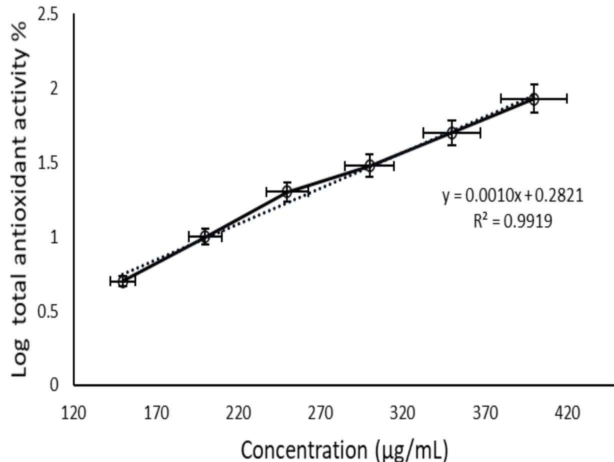


Figure 2. Total antioxidant capacity of gerger decoction

Antioxidants like resorcinol, catechol, and vanillin were present in soxhlet -methanol extract of gerger leaves marketed in Pakistan [40]. A report on the quantitative estimation of quercetin by reverse-phase HPLC in the methanol extract of gerger leaves from Saudi Arabia was also available [41]. Bulgarian and Italian gerger studied for the total phenolic content, and the antioxidant potential of ethanol extract suggested a higher antioxidant capacity in Bulgarian gerger than the Italian variety [24]. The method of extraction of food antioxidants affects the nature of compounds isolated, and the quality of the total antioxidant activity [42]. Compounds reported in the soxhlet aided methanol extract of gerger leaves were absent in the gerger leaf decoction reported in this study. The absence of water-soluble small molecule antioxidants like resorcinol, catechol, and vanillin in the gerger decoction was because of the high extraction temperatures. Nevertheless, the decoction method was proven effective in extracting oil-soluble antioxidants like astaxanthin, clionasterol, ingol-12-acetate, and phytol leading to functional inhibition of biological oxidation processes.

Oxidative stress plays a higher role in the pathophysiology of diseased states and metabolic disorders. Food plays a significant role in reducing oxidative stress. Food antioxidants mitigate the development of cancer, diabetes mellitus, hypertension, inflammation, and obesity [10,21,25]. Gerger leaves regularly consumed raw by Saudi people and also worldwide can have health benefits owing to the antioxidant potential. Gerger leaves being edible has been consumed since long, are safe in humans, but recommendations on the quantity of daily intake are necessary. The explored antioxidant potential of gerger leaves substantiated the claim that it is a functional food. This study serves as the basis for development of a nutraceutical from gerger water decoction.

Given that gerger is a functional food, we call for focussed efforts to characterize antioxidant biomarkers of gerger and to elucidate the molecular mechanisms of action by further *in-silico*, and *in-vitro* studies in correlation with *in-vivo* studies. Additionally, the nutritional value of the gerger leaves in terms of its silicon content needs further investigation. Extensive studies to evaluate the bioavailability of silicon compounds of gerger are necessary in order to exploit the role in bone development.

4. Conclusions

Leaves of *Eruca sativa,* commonly known as gerger and jarjeer in Saudi Arabia, exhibited significant *in-vitro* antioxidant activity. The explored phytochemical profile by GC-MS analysis of the aqueous decoction of gerger leaves confirmed the presence of twenty-seven compounds, classified into seven groups based on the chemical structure. The decoction was made up of a higher amount of organic siloxanes, organic silicates, and phenolic compounds, while minor constituents included aromatic and aliphatic esters, heterocyclic compounds, sulfur compounds, and terpenoids. The dried decoction of gerger leaves demonstrated a significant hepatic anti-lipid peroxidation activity besides a good total antioxidant activity. The study results substantiated that gerger leaf is a functional food. Focussed efforts are necessary for the identification and validation of the biological target, the determination of bioavailability of the phytonutrients, and phytoconstituents by combined *in-silico*, *in-vitro*, and *in-vivo* studies to combat oxidative stress-related chronic illnesses using gerger as a functional food.

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Statement of Competing Interests

The authors have no competing interests.

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