Phytochemical Screening, Physiochemical and GC-MS Analysis of Ginger Oil

By

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A Dissertation

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

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November 2013
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Date of Examination: 02/11/2013
بسم الله الرحمن الرحيم

وَجَزَاهُمْ بِمَا صَبَرُوا جَنَّةً وَحَرِيرًا (12) مُتَّكِئِينَ فِيهَا عَلَى الْأَرَائِكِ ۖ لَا يَرَوْنَ فِيهَا شَمْسًا وَلَا زَمْهَرِيرًا (13) وَكَانَتْ عَلَيْهِمْ ظَالَاتُهَا وَذُلِّلَتْ قُطُوفُهَا تَذْلِيلًا (14) وَمُطَافٌ عَلَيْهِمْ بَاتِيَةً مِنْ فِضَّةٍ وَأَكْوَابٍ كَانَتْ قَوَارِيرَا كَانَ مِزَاجُهَا زَنْجَبِيلًا (17) عَيْنًا فِيهَا تُسَمَّى سَلْسَبِيلًا (18)

{

(الآيات من 12-18 سورة الإنسان)
Acknowledgement

Special dedication to my family members that always inspire, love and stand beside me,

My supervisor,

Dr. Asim Halfawi

My beloved friends especially the one who always help me,

Mofeed

My fellow colleagues,

Batch 3

For all your love, care, support, and believe in me.

Thank you so much.
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Abstract
Ginger or ginger root is the rhizome of the plant *Zingiber officinale* consumed as a delicacy, spice and medicine. It lends its name to its genus and family (*Zingiberaceae*). The main objective of the present study was focused on identification and quantification of chemical constituents present in the essential oil of ginger by GC-MS methods, the physical and chemical properties of the oil. The plant sample was taken from Omdurman market and extracted by Soxhlet apparatus, phytochemical screening of the ginger extract showed the presence of Flavonoids, Tannins, Saponin, Coumarin, Carbohydrates and sterols. Essential oil of the ginger was investigated by GC-MS, total 21 chemical constituents were found by gas chromatography and mass spectrometry (GC-MS) analysis. The physical and chemical properties of the ginger oil were examined and showed acceptable results when compared to the previous studies over the world. Based on this results obtained from this study, it is recommended that Solvent extraction combined with GC-MS has been shown to be a valuable tool for the analysis of ginger constituents and can provide a useful guide to components variation.
ملخص الدراسة

الزنجبيل أو جذر الزنجبيل هو ريزوم نبات الزنجبيل يستخدم كمشهى، توابل، أو دواء. يستعير إسمه من إسم جنسه وعائلته. وقد تركز الهدف الرئيسي من هذه الدراسة في تحديد وتقدير المكونات الكيميائية الموجودة في زيت الزنجبيل عن طريق كروماتوجرافيا الغاز ومطياف الكتلة والخواص الفيزيائية والكيميائية لزيت. تم أخذ عينة النبات من سوق أم درمان واستخلصت بواسطة جهاز سوكسليت وقد أظهر المسح الكيميائي للمستخلص وجود الفلافونويدات، التانينات، الصابونيات، الكومارين، الكاربوهيدرات والإستيرولات. تم إخضاع الزيوت الأساسية المستخلصة من الزنجبيل للتحليل بواسطة كروماتوجرافيا الغاز ومطياف الكتلة. هكذا، مجموع 21 مكوناً كيميائياً وجدت بواسطة التحليل بكروماتوجرافيا الغاز ومطياف الكتلة. الخصائص الفيزيائية والكيميائية لزيت الزنجبيل تم فحصها وأظهرت نتائج مشابهة بالمقارنة مع الدراسات السابقة في أنحاء العالم. بناءً على النتائج المتحصل عليها من هذه الدراسة وجد أن الاستخلاص بالمذيبات جيداً إلى جانب مع كروماتوجرافيا الغاز ومطياف الكتلة تكون أداة قيمة لتحليل مكونات الزنجبيل ويمكن أن توفر دليلاً مفيدة لإختلاف المكون.
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Abbreviation

LD50................................................................. Lethal dose, 50%

EOs ................................................................. Essential Oils

FID................................................................. Flame Ionization Detector
Chapter one

Introduction and Literature Review
Chapter one

1.1 Introduction and Literature Review

Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value; which forms one of the oldest sciences yet it is only in recent years that modern science has started paying attention to the properties of spices.

Medicinal and spice plants are renewable raw materials, their production is an alternative to the over production of traditional crops in agriculture. They also have an increasing economic importance. Spices can be defined as any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms that contribute flavor, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages. [1]

Although as natural substances spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects; spices as medicine should be used judiciously. [2]

This is because a substance being derived from a plant does not mean it is always harmless and one drug used for one ailment could actually be detrimental to the treatment of another. The latest finding suggests that the chemicals present in spices can be allergens, carcinogens, and mutagens. [2, 3]
Essential oils (EOs) also called volatile or ethereal oils; are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs. The term ‘essential oil’ is thought to derive from the name coined in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim; he named the effective component of a drug Quinta essentia. An estimated 3000 EOs are known, of which about 300 are commercially important—destined chiefly for the flavours and fragrances market. [4]

Essential oils have become an integral part of everyday life. They are used in a great variety of ways: as food flavorings, as feed additives, as flavoring agents by the cigarette industry, and in the compounding of cosmetics and perfumes. Furthermore, they are used in air fresheners and deodorizers as well as in all branches of medicine such as in pharmacy, balneology, massage, and homeopathy. A more specialized area will be in the fields of aromatherapy and aromachology. In recent years, the importance of essential oils as biocides and insect repellents has led to a more detailed study of their antimicrobial potential. Essential oils are also good natural sources of substances with commercial potential as starting materials for chemical synthesis. [4]

The health benefits of ginger root oil can be attributed to its digestive, carminative, expectorant, antiseptic, analgesic, anti inflammatory, stimulating and aphrodisiac properties. These benefits of ginger oil include its ability to treat stomach problems, nausea, heart strokes, indigestion, inflammations, respiratory problems, menstrual disorders. [4]
Ginger, a very useful herb plant, is said to be originated from India, China and Java, yet is also native to Africa and the West Indies. It is grown throughout the tropical areas of the world and also commonly found in South East Asia especially in Indo-Malaysia. The main producer of ginger is Jamaica. Ginger is scientifically named as *Zingiber officinale Roscoe*. On 1807, an English botanist, William Roscoe (1753-1831) named the plant as *Zingiber officinale* in his publication. [6] The ginger family is a tropical group especially abundant in Indo-Malaysia, consisting of more than 1200 plant species in 53 genera. The genus *Zingiber* includes about 85 species of aromatic herbs from East Asia and tropical Australia. The name of the genus, *Zingiber*, derives from a Sanskrit word denoting "horn-shaped," in reference to the protrusions on the rhizome.

A numeral of commercial variety of ginger exists. Nigerian Ginger is darker in color, minute size and more pungent taste. Bangladeshi Ginger is habitually larger, well scraped, contains more starch and breaks with a shorter fracture. African Ginger is darker in color, more pungent in taste and less flavor than Jamaica Ginger. Ginger plant is propagated by rhizome cuttings each bearing a bud. The pieces of rhizome are planted in holes during March and April in a well-drained clayey soil. In December or January rhizomes are unruffled. Ginger requires a warm and humid atmosphere. A well-distributed rainfall is required for its cultivation. If the area is getting fewer rainfalls, the crop needs habitual irrigation. [9]

### 1.2 Taxonomy

**Ginger (Zingiber Officinale)**

**Classification**

Kingdom: *Plantae*
1.3 Morphology
The ginger plant is an erect perennial growing from one to three feet in height. The stem is surrounded by the sheathing bases of the two-ranked leaves. A club-like spike of yellowish, purple-lipped flowers have showy greenish yellow bracts beneath. Unfortunately, ginger rarely flowers in cultivation. The ginger of commerce consists of the thick scaly rhizomes (underground stems) of the plant. They branch with thick thumb-like protrusions, thus individual divisions of the rhizome are known as "hands.". Rhizomes are 7-15 cm long and 1-1.5 cm broad and laterally compressed. The branches arise obliquely from the rhizome are about 1-3 cm long and terminate in depress scars or in undeveloped buds. The outer surface is buff-colored and longitudinally striated or fibrous. Fractured surface shows a narrow cortex, a well marked endodermis and a wide stele. [9]

1.4 Physical properties of Ginger oils
The color of ginger essential oil varies from pale yellow to profound amber hue. It is a thick liquid whose viscosity differs from average to watery. The essential oil obtained from the ginger roots possesses a potent, zesty, pungent, peppery and warm fragrance that resembles the smell of real ginger. This oil has the capability to set off as well as reinstate self-determination or freewill. [7]
1.5 Chemical properties of ginger oil

1.5.1 Acid value
The acid value $I_A$ is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. [18]

1.5.2 Saponification value
The saponification value $I_S$ is the number of mg of potassium hydroxide required to neutralize the fatty acid resulting from hydrolysis of 1 g oil or fat. [18]

1.5.3 Ester value
The ester value $I_E$ is the number that expresses in milligrams the quantity of potassium hydroxide required to saponify the esters present in 1 g of the oil. It is calculated from the saponification value $I_S$ and the acid value $I_A$. [18]

1.6 Gas Chromatography-Mass Spectrometry
Mass spectrometry (MS) can be defined as the study of systems through the formation of gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge ratios (m/z) and relative abundances. The analyte may be ionized thermally, by an electric field or by impacting energetic electrons, ions, or photons. [16]
During the past decade, there has been a tremendous growth in popularity of mass spectrometers as a tool for both, routine analytical experiments and fundamental research. This is due to a number of features including relatively low cost, simplicity of design and extremely fast data acquisition rates. Although the sample is destroyed by the mass spectrometer, the technique is very sensitive and only low amounts of material are used in the analysis. In addition, the potential of combined
gas chromatography-mass spectrometry (GC-MS) for determining volatile compounds, contained in very complex flavor and fragrance samples, is well known. The subsequent introduction of powerful data acquisition and processing systems, including automated library search techniques, ensured that the information content of the large quantities of data generated by GC-MS instruments was fully exploited. The most frequent and simple identification method in GC-MS consists of the comparison of the acquired unknown mass spectra with those contained in a reference MS library. [16]

A mass spectrometer produces an enormous amount of data, especially in combination with chromatographic sample inlets. Over the years, many approaches for analysis of GC-MS data have been proposed using various algorithms, many of which are quite sophisticated, in efforts to detect, identify, and quantify all of the chromatographic peaks. Library search algorithms are commonly provided with mass spectrometer data systems with the purpose to assist in the identification of unknown compounds.

The sample is normally introduced as a vapour on to the chromatographic column. On the column, the solubility of each component in the gas phase is dependent on its vapour pressure, which is in turn a function of the column temperature and the affinity between the compound and the stationary phase. Differences in vapour pressure cause the molecules of each component to partition between the mobile gas phase and the stationary phase. In fact, as the molecules are continually moving rapidly between the two phases, it is the difference in residence time in each phase that affects the partition. [16]

Every time a molecule enters the gas phase it is swept towards the detector by the carrier gas flow. Consequently, compounds having different physical and chemical properties will arrive at the detector at different times. The stationary phase can be
a solid or a liquid coating an inert solid support, this gives rise to two forms of gas chromatography; gas-solid (GSC) and gas-liquid chromatography (GLC) respectively. [16]

1.7 Chemical composition

The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1-3% of its weight. The major active ingredients in ginger oil are the sesquiterpenes: bisapolene, zingiberene, and zingiberol, the concentrations of active ingredients vary with growing conditions [3]. Ginger’s active ingredients have a variety of physiologic effects. For example, the gingerols have analgesic, sedative, antipyretic and antibacterial effects in vitro and in animals. [9]

The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaols and gingerols, volatile oils that composed one to 3% of the weight of fresh ginger. In laboratory animals, the gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative, antipyretic and antibacterial properties. Ginger oil has been shown to prevent skin cancer in mice and a study at the University of Michigan demonstrated that gingerols can kill ovarian cancer cells [3]. [6]-Gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy- 3-decanone) is the major pungent principle of ginger. The chemo preventive potentials of [6]-gingerol present a promising future alternative to expensive and toxic therapeutic agents. Ginger contains up to 3% of a fragrant essential oil whose main constituents are sesquiterpenoids, with (-)-zingiberene as the main component. Smaller amounts of other sesquiterpenoids (β-sesquiphellandrene, bisabolene and farnesene) and a small monoterpenoid fraction (β-phelladrene, cineol, and citral) have also been identified [9]. The pungent taste of ginger is due to nonvolatile phenylpropanoid-derived compounds, particularly gingerols and shogaols, which
are formed from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process; this compound is less pungent and has a spicy-sweet aroma. Ginger is also a minor chemical irritant, and because of this was used as a horse suppository by pre-World War I mounted regiments for feaguing. Ginger has a sialagogue action, stimulating the production of saliva, which makes swallowing easier. The oil is sparingly soluble in 95 percent alcohol but is generally soluble in 90 percent alcohol. [9]

![Gingerol](image1)

![Paradol](image2)

![Shagaol](image3)

![Zingerone](image4)

**Figure 1.1**: Non-Volatile Pungent Components of Ginger. [9]
1.8 Traditional uses

Ginger is extensively used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines. In India the fresh and dried roots were measured distinct medicinal products. Fresh ginger has been used for cold-induced disease, nausea, asthma, cough, colic, heart palpitation, swellings, dyspepsia, loss of appetite, and rheumatism. In short, it is used for the same purposes as in ancient China. In nineteenth century India, one English writer observed that a popular preparation for cough and asthma consisted of the juice of fresh ginger with a little juice of fresh garlic, mixed with honey. A glue of powdered dried ginger was applied to the temples to mitigate headache. To dispel nausea, fresh ginger was mixed with a little honey, topped off with a nip of burnt peacock feathers. One modern government health guide in India suggests 1-2 teaspoons of ginger juice with honey as a cough suppressant. Ginger is as popular a home remedy in India today, as it was 2,000 years ago. The rhizomes of ginger are used as spice in food and beverages and in traditional medicine as carminative, antipyrexia and treatment of waist pain rheumatism and bronchitis. It is used for the treatment of gastrointestinal disorders and piles. [3, 5]

1.9 Nutritional importance

Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% Carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. The composition varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions. Ginger (Zingiber officinale Roscoe) has been used as a spice for over 2000 years. Its roots and the obtained extracts contain polyphenol compounds ([6]-gingerol and its derivatives),
which have a high antioxidant activity. Although the digestion stimulating effect of this spice became known a long time ago, the stimulating effect on peptic juices, such as gastric juice, bile, pancreatic and intestinal juices, was discovered later. [11] Bile acids play a major role in the uptake of fats and each upset in the metabolism of fats would impede food digestion as a whole, because the fatty particles cover the other food elements and make them inaccessible for the action of the digestive enzymes. Lipase is the other key factor which plays a vital role in fat digestion. When ginger was included in animal diets, it was found that there was a considerable increase in the pancreatic and intestine lipase. [11]

1.10 Anti-oxidant activity

The antioxidant properties of [6]-gingerol which is very effective agent for anticipation of ultra violet B (UVB)-induced reactive oxygen species production and COX-2 idiom, and a promising therapeutic agent against UVB- induced skin disorders, has been studied both in-vitro & in-vivo. It also has a protective role to toxicity and lethality against some agent like carbon-tetra chloride, cisplatin etc. [10]

1.11 Antimicrobial activity

Some reports are available on the antimicrobial property of the volatile oil from the rhizomes of ginger. The essential oil from ginger was studied for antimicrobial activity against Aspergillus niger, Saccharomyces cerevisiae, Mycoderma sp., Lactobacillus acidophilus and Bacillus cereus, as determined by paper agar diffusion method [14]. Another study reports on the bioassay-guided isolation of antifungal compounds from an African land race of ginger, Zingiber officinale Roscoe, and the identification of 6, 8 and 10-ingerols and 6-ingerdiol as the main antifungal principles. The compounds were active against 13 human pathogens at
concentrations of <1 mg/mL, the gingerol content of the African land race was at least 3 times higher than that of typical commercial cultivars of ginger. A survey of the literature reveals that there are no reports on the antimicrobial properties of the fresh ginger oil on the selected microorganisms. So this study was carried out. [14]

1.12 Standard & adulteration

Ginger should contain minimum 10% of water soluble extractives, 4.5% Alcohol soluble extractives. It should offer maximum 6.0% of Total ash, 2.0% Acid insoluble ash and minimum 1.7% water soluble ash. Adulteration can be detected by routine microscopical examination. Powdered ginger may have been prepared from ‘wormy’ drug, and so attention should be paid to the absence of insect fragments. Adulteration may also take the form of the addition of ‘spent ginger’ which has been exhausted in the preparations of essence. This may be detected by the official standards for alcohol-soluble extractive, water soluble extractives, total ash and water soluble ash. [8]

1.13 Toxicity and contraindications

All herbal products carry the potential for contamination with other herbal products, pesticides, herbicides, heavy metals, and pharmaceuticals. This is particularly concerning for imports from developing countries. Allergic reactions can occur to any natural product in sensitive persons. Allergic reactions to ginger have been reported, but only as contact dermatitis in those with occupational exposure to spices. [13]

Potentially toxic compounds in ginger: None Acute toxicity: Aside from mild stomach upset in persons unaccustomed to spicy foods, ginger has no known acute toxicity at the usual doses consumed for dietary or medicinal purposes. Very large doses of 6 grams or more of ginger may lead to gastric irritation and loss of protective gastric mucosa. At normal doses (up to 2 grams daily); ginger does not
interfere with blood clotting or any individual coagulation parameter. The acute LD50 of ginger in rats is greater than 5 grams of ginger oil per kilogram of body weight.[8,12]

Chronic toxicity: None reported; no significant mutagenic or carcinogenic activity

Limitations during other illnesses or in patients with specific organ dysfunction: Unknown; none reported. Some herbalists advise against ginger for patients with cardiac conditions, gallstones or other biliary disease or patient with diabetes or hypoglycemia; however, there are no reports of adverse effects of ginger in patients eating it as part of their diet or as a dietary supplement. [15]

Interactions with other herbs or pharmaceuticals: Unknown; none reported. Some herbalists recommend avoiding use by patients taking anticoagulant medications; no adverse interactions have been reported. Safety during pregnancy, lactation and/or childhood: Unknown. Presumed safe based on its long history of use as food. Because of the reported uterotonic activity of a related species, Zingiber cassumunar, some herbalists recommend avoiding ginger during pregnancy. No adverse effects in pregnancy have been reported. [20, 21]
1.12 Objectives

1.12.1 General Objectives

The objective of this research to determine the physiochemical properties and analysis by GC-MS of Ginger oils (*Zingiber officinale Roscoe*).

1.12.2 Specific Objectives

- To extract the volatile oils of (*Zingiber officinale Roscoe*) by using organic solvents.
- To study physiochemical properties of *Zingiber officinale Roscoe*.
- To analyze the ginger oil by GC-MS.
Chapter two

Materials and Methods
Chapter two

Materials and methods

2.1. Materials

2.1.1. Plant material

The ginger roots were purchased from Omdurman market. The roots were ground into fine powder using an electric grinder. Then 500 grams of the powdered mass obtained was stored in clean sterile bottles at room temperature and used for the extractions.

2.1.2 Instruments and Apparatus

- Soxhlet apparatus.
- Shimadzu GC-MS model GC 2010.
- Refractometer (abbe 60/DR).
- Polarimeter (BR 02004 - England).
- Reflux apparatus.
- Density bottle.
- Burettes.
- Volumetric flasks.
- Conical flasks.
- Beakers.
- Pipettes.
- Analytical balance.
- Water bath.
- Heat mantle.
### 2.1.3 Chemicals

**Table 2:1** the chemicals used in the study

<table>
<thead>
<tr>
<th>Substance</th>
<th>Purity</th>
<th>Company</th>
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<tbody>
<tr>
<td>Ammonium chloride</td>
<td>99.5%</td>
<td>LOBA CHEMIE India</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>97.5%</td>
<td>CDH Laboratory Reagent</td>
</tr>
<tr>
<td>Ethanol</td>
<td>absolute</td>
<td>CL CHEM-LAB</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>60-70°C</td>
<td>NICE Laboratory Reagent</td>
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<tr>
<td>Phenolphthalein</td>
<td>98%</td>
<td>CENTRAI Drug House (B) LTD.</td>
</tr>
<tr>
<td>n-hexane</td>
<td>65-70°C</td>
<td>LOBA CHEMIE</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>37%</td>
<td>SHAMPLAB Laboratory Chemicals</td>
</tr>
<tr>
<td>Acetic anhydride</td>
<td>98%</td>
<td>SURE CHEM Product</td>
</tr>
<tr>
<td>α-naphthol</td>
<td>99%</td>
<td>Sd fine-chem Limited</td>
</tr>
<tr>
<td>Ammonium hydroxide</td>
<td>25%</td>
<td>SHAMPLAB Laboratory Chemicals</td>
</tr>
<tr>
<td>Chemical</td>
<td>Purity</td>
<td>Supplier</td>
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<td>----------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>96%</td>
<td>N/A</td>
</tr>
<tr>
<td>Iodine solution</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Bismuth nitrate</td>
<td>N/A</td>
<td>PSPARK SCIENTIFIC LIMITED</td>
</tr>
<tr>
<td>Potassium Iodide</td>
<td>99%</td>
<td>LAB TECH CHEMICALS</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>99%</td>
<td>LOBA CHEMIE</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>95-98%</td>
<td>SHAMPLAB Laboratory Chemicals</td>
</tr>
<tr>
<td>Sodium potassium tartarate</td>
<td>99.5%</td>
<td>Sd fine-chem Limited</td>
</tr>
<tr>
<td>Picric acid</td>
<td>99.5%</td>
<td>BDH Laboratory Supplies</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>99.5%</td>
<td>Sd fine-chem Limited</td>
</tr>
<tr>
<td>Iodine</td>
<td>99.5%</td>
<td>Sd fine-chem Limited</td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>85%</td>
<td>Sd fine-chem Limited</td>
</tr>
</tbody>
</table>

2.1.4 Preparations of Solutions (Reagents)

- **Ethanol 96%**
  
  To 96 ml of absolute ethanol 4 ml of distilled water were added.
- **Sodium hydroxide 0.1 M solution**
  4 g of analytical grade reagent sodium hydroxide were dissolved in distilled water and was completed up to 1000 ml in volumetric flask.

- **Phenolphthalein 0.1%**
  0.1 g of phenolphthalein was dissolved in 80 ml of alcohol and was diluted to 100 ml with distilled water.

- **Hydrochloric acid 0.05 M**
  4 ml of conc. HCl were pipetted and sufficient amount of distilled water was added to the mark of 1000 ml volumetric flask.

- **Dragendorff’s reagent**
  Solution A: 0.17g of Bismuth nitrate was weighted and 2ml, 8ml of acetic acid and H₂O were added respectively.
  Solution B: 4 g of KI were weighted and 10ml and 20 ml of acetic acid and H₂O were added respectively.
  Solution A and B were mixed and diluted to 100ml.

- **Fehling’s reagent**
  Fehling’s A: 35 g of CuSO₄ were dissolved in 500 ml distilled water. 3 ml of concentrated sulphuric acid were added to it.
  Fehling’s B: 17.3 g of sodium potassium tartarate (Rochelle salt) and 60 g of sodium hydroxide were dissolved in 500 ml distilled water.

- **Hager’s reagent**
  1g of picric acid was dissolved in 100ml of distilled water.
Mayer’s reagent

1.358g of HgCl₂ was dissolved in 60ml of distilled water and poured into a solution of 5g of KI in 10ml of H₂O; sufficient distilled water was added to make 100 ml.

Wagner’s reagent

2g of iodine and 6g of KI was dissolved in 100ml of distilled water.

2.2 Methods

2.2.1 Extraction of ginger oil

Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor for several times. This process involves extraction followed by evaporation of the solvent. The vapours of the solvent are taken to a condenser and the condensed liquid is returned to the plant for continuous extraction.

500 grams of powdered ginger roots was packed in the extractor part (100 grams each time) and placed in central compartment of soxhlet assembly and the rest pieces of the apparatus were connected, a volume of about 1L of n-hexane was used for extraction, and anti-bumping granules were added to the flask to avoid bumping of the solvent, the thermostat of the heat mantle was adjusted to the boiling point of the solvent 68.95°C and the process took about 8 hours, after completed extraction the lower vessel was removed, solvent recovered and the extract was concentrated and the percentage yield was calculated, the solvents were recovered by using rotary evaporator.
2.2.2 Phytochemical Screening of the Extract

2.2.2.1 Test for alkaloids
About 30 ml aliquot of the prepared extract were evaporated to dryness in an evaporating dish on a water bath. About 5 ml of 2N HCl were added to the dried samples and stirred while heating on the water bath for 10 minutes, cooled, filtered and divided equally into three test tubes:
- To the first portion few drops of Mayer’s reagent were added, while buff precipitate was taken as presumptive evidence for the presence of alkaloids.
- To the second portion few drops of Dragendorff’s reagent were added, yellowish-orange precipitate was taken as an evidence for the presence of alkaloids.
- The third portion was treated with few drops of Wagner’s reagent and turbidity or precipitate was considered as evidence for the presence of alkaloids.

2.2.2.2 Test for saponins
2 ml of the extract were placed in a test tube and 2ml of distilled water were added, and the tube was corked and shaken vigorously for about 30 seconds, then allowed to stand for 15 min, and the presence of the forth was observed.

2.2.2.3 Test for flavonoids
About 40 ml aliquot of the prepared extract was evaporated to dryness on a water bath, cooled and residue was defatted several times with petroleum ether. The defatted residue was dissolved in 15 ml of 96% ethanol and filtered. The filtrate was used for the following tests:
- To 3 ml of the filtrate in a test tube, 1 ml of 1% aluminum chloride solution in methanol was added. Formation of a yellow color indicated the presence of flavonoids.
- To 3 ml of the filtrate in a test tube, 1 ml of potassium hydroxide solution was added. A dark yellow color indicated the presence of flavonoid compounds.
- To 3 ml of the filtrate, 0.5 ml of concentrated HCl and a few magnesium turnings (0.5 g) were added. Production of a definite color change to pink or red was taken as presumptive evidence that flavonoid compounds were present in the plant sample.

2.2.2.4 Test for tannins
About 25 ml of the prepared extract were evaporated to dryness on a water bath. The residue was extracted several times with n-hexane and filtered. The insoluble residue was stirred with 10 ml of hot saline solution. The mixture was cooled and filtered. 5 ml of this solution were treated with few drops of the Gelatin-salt reagents. Formation of an immediate precipitate was taken as evidence for the presence of tannins.
Positive tests are confirmed by the addition of few drops of 1% FeCl₃, test reagent to another portion of the solution and should result in a characteristic blue, blue-black, green or blue-green color.

2.2.2.5 Test for unsaturated sterols and/or triterpenes
The dried powdered plant (1 g) was extracted with few ml of ethanol, filtered and evaporated to dryness, the residue was dissolved in 10 ml of chloroform and the solution was filtered. The chloroform filtrate was tested as follow:

**Liebermann-Burchard's test**
To 2 ml of the chloroform filtrate, 1 ml of acetic anhydride was added and 2 ml of concentrated sulphuric acid along the side of the tube. A reddish violet colour at the junction of the two layers indicated the presence of unsaturated sterols and/or triterpenes.
2.2.2.6 Test for coumarin

About 2ml of hot water were added to 5ml of the evaporated extract:
1st. tube: the previous solution + NH₄OH
2nd. tube: the previous solution
3rd. tube: distilled water
They were detected under UV lamp.

2.2.2.7 Test for carbohydrates and/or glycosides

The dried powdered plant (5 g) were boiled with 100 ml of distilled water; the aqueous solution was filtered through a filter paper and the liquid obtained was tested as follow:

Molish's test:

A. An aliquot of the filtrate (2 ml) was mixed with 0.2 ml of ethanolic α-naphthol (20%) followed by 2 ml of sulphuric acid (98%) poured carefully on the side of the test tube to form two layers. A violet zone at the junction of the two layers indicated the presence of carbohydrates.

B. The filtrate (5 ml) was heated with 5 ml Fehling's solution, and a red precipitate indicated the presence of reducing sugars.

2.2.2.8 Test for anthracene glycosides

About 2ml of 25% NH₄OH was added to 3ml of the extract, a pink color was observed.

2.2.3 Determination of density

A clean, dry and empty density bottle was weighted by a sensitive balance w₀, and then it was filled with distilled water and was weighted again w₂, and then it was washed by alcohol and petroleum ether respectively until it was completely dried, and then it was filled with ginger oil and weighted again w₁.
Density \( \frac{w_1 - w_0}{w_2 - w_0} \)

Where:

\( w_0 \equiv \) the weight of the empty density bottle.

\( w_1 \equiv \) the weight of the density bottle and the oil.

\( w_2 \equiv \) the weight of the density bottle and the water.

### 2.2.4 Determination of optical rotation

Determination of optical rotation is carried in a polarimeter and is useful for detection of adulteration and identification of the variety of the sample. The zero of the polarimeter and the angle of rotation of polarized light at the wavelength of the D-line of sodium (\( \lambda = 589.3 \) nm) at \( 20 \pm 0.5 \) °C was determined. Measurements may be carried out at other temperatures only where the monograph indicates the temperature correction to be made to the measured optical rotation. Determine the zero of the apparatus with the tube closed; for liquids the zero is determined with the tube empty and for solids filled with the prescribed solvent. The specific optical rotation was calculated using the following formulae.

\[
\left[ \alpha \right]_D^{20} = \frac{a \times 100}{\ell \times c}
\]

\( t = 20 \)°C

\( D = \) sodium D line

\( a = \) observed angle of rotation

\( l = \) length of the polarimeter tube in dm.
c = concentration in g/100ml.

2.2.5 Determination of refractive index
The light source was turned on, the double-prism of the refractometer was opened, and both glass surfaces were cleaned with a filter paper and the double-prism was closed. A pipette was used to fill the space between the two prisms with the ginger oil; the refractometer scale knob was turned to get a clear interface between the illuminated and dark regions. The micrometric screw was used for the additional refinement of the scale, until the clear interface appeared. The integer value from the rough scale and decimal number from the refined scale was read out.

2.2.6 GC-MS method validation:
Gas chromatographic analyses were carried by Shimadzu GC-MS model GC-2010. Methyl silicone column was used (50m x 0.2mm, 0.17μm) for the analyses. The conditions were as follows: temperature programming from 80°C-200°C, rate at 5°C /min, hold at 80°C for 1 min, hold at 200°C for 20 min, FID temperature 300°C, injection temperature 250°C, carrier gas : nitrogen at a flow rate of 1mL/min, split ratio of 1:5. Quantitative analysis data were retained from electronic integration of area percentage without the use of response factors.

2.2.6.1 GC-MS analyses
GC-MS analyses were carried out in a Shimadzu GC-MS model GC-2010 equipped with Mass spectrophotometer GC-MS QP 5050 A. A 30 M capillary silicon column was used for the analysis. Temperature programming conditions were as follows, 80°-200° C, rate at the rate of 5° C per min, hold at 80° C for 1 min, hold at 200° C for 25 min, column start temperature 80° C, injection temperature 250° C, interface temperature 270° C, carrier gas helium, flow rate of 1 ml/min, split ratio 1:50. The percentage composition of the oil was calculated
automatically from the FID peak areas without any correction. The retention indices of compounds were determined relative to the retention times of a series of n-alkanes with linear interpolation. Identification of the oil components was done by comparison of their mass spectra with the Wiley GC-MS library as well as by comparing them with those reported in literature. The identification of each compound was confirmed by comparison of its retention index either with those of authentic compounds or from literature.

**2.2.7 Determination of acid value**

10 g of ginger oil were dissolved in 50 ml of mixture of ethanol 96% and petroleum ether previously neutralized with 0.1 M sodium hydroxide and 2 drops of phenolphthalein solution were added as indicator and then was titrated with 0.1 M potassium hydroxide until the pink color persisted for at 15 s.

\[
\text{Acid value } IA = \frac{4.0 \ n}{m}
\]

Where:

- \( n \) is the mls of titrant
- \( m \) is the g of oil

**2.2.8 Determination of saponification value**

40 g of potassium hydroxide were dissolved in 20 ml of water and sufficient ethanol 96% was added to produce 1000 ml. Allowed to stand overnight and the clear liquid was poured off.

2 g of the oil were weighed into a 200-ml flask, 25.0 ml of the ethanolic solution of potassium hydroxide were added and boiled under a reflux condenser for 1 hour, and the contents were rotated frequently. While the solution is still hot, the excess
of alkali was titrated with 0.5M hydrochloric acid using 1 ml of phenolphthalein solution as indicator. The operation was repeated without the oil being examined.

\[
\text{Saponification value } I_S = 28.05\frac{v}{w}
\]

Where:

\(v\) is the difference in ml between the titrations.
\(w\) is the weight in g of oil taken.

### 2.2.9 Determination of ester value

\[
Ester \ value \ I_E = I_S - I_A
\]

\(I_S\) is Saponification value
\(I_A\) is Acid value
Chapter three

Results and discussion
Chapter Three

In this chapter the analysis results of the ginger oil obtained were showed in the tables below:

**Table 3.1**: The results of the physical properties of ginger oil

<table>
<thead>
<tr>
<th>Physical property</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance at room temperature</td>
<td>Homogeneous, transparent liquid, lighter than water</td>
</tr>
<tr>
<td>(30°C)</td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>Golden yellow</td>
</tr>
<tr>
<td>Density</td>
<td>0.909</td>
</tr>
<tr>
<td>Optical rotation $[\alpha]_{26}^0$</td>
<td>-35°</td>
</tr>
<tr>
<td>Refractive index $[\eta^i \text{C}]$</td>
<td>1.4860</td>
</tr>
</tbody>
</table>

The physical properties results obtain were found similar to the previous studies when were compared with it. [2,18,19]

**Table 3.2**: The phytochemical screening of the extracts of Ginger (*Zingiber officinale*)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
</tr>
<tr>
<td>Falvonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
</tr>
<tr>
<td>Anthracene</td>
<td>-ve</td>
</tr>
<tr>
<td>Chemical property</td>
<td>Result</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Coumarin</td>
<td>-ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>-ve</td>
</tr>
<tr>
<td>Sterols</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve indicates presence of the chemical group.
-ve indicates absence of the chemical group.

The results of phytochemical screening table 3.2 shows the presence of flavonoids, tannins, saponins, and sterols.

Table 3.3: The results of the chemical properties of ginger oil

<table>
<thead>
<tr>
<th>Chemical property</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponification value</td>
<td>39.5</td>
</tr>
<tr>
<td>Acid value</td>
<td>7.2</td>
</tr>
<tr>
<td>Ester value</td>
<td>32.3</td>
</tr>
</tbody>
</table>

The chemical properties results obtain were found similar to the previous studies when were compared with it. [2,18,19]
The GC/MS analysis of ginger oil (Zingiber officinale Roscoe) shows it contains 21 compounds, the major compounds which found are 1,3-Cyclohexadiene, 5-(1,5-
dimethyl-4-hexenyl)-2-methyl (34.96%), Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) (15.37%), and Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (15.06%).

**Table 3.4: Classification of compounds in the Volatile oils of ginger**

<table>
<thead>
<tr>
<th>Percentage %</th>
<th>Classes of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.3%</td>
<td>Monoterpene hydrocarbons</td>
</tr>
<tr>
<td>24%</td>
<td>Oxygenated monoterpene</td>
</tr>
<tr>
<td>49%</td>
<td>Sesquiterpenes hydrocarbons</td>
</tr>
<tr>
<td>11.6</td>
<td>Oxygenated sesquiterpenes</td>
</tr>
</tbody>
</table>

**Figure 3.2: The major compounds which found by GC-MS**

1, 3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl
Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)

Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl
**Recommendations**

Phytochemical study of the roots extract of *Zingiber officinale* revealed the presence of a variety of constituents in the plant. These constituents contributed to the demonstrated bioactivities of the extracts prepared viz, antimicrobial, antioxidant and anticancer, meanwhile, the plant extracts is completely safe due to its LD50 value.

Based on these results, the following could be recommended:

1. Development of methods for standardization of extracts prepared from the plant and study of their stability and bioavailability.
2. Development of phytopharmaceuticals containing the standardized bioactive ingredients after applying clinical study.
3. Build up new methods about identification and purification of compounds from oils e.g. NMR.
Chapter four

References
Chapter four

References

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