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Chemical Characterization of Coriandrum sativum Extracts and Their Applications

A thesis submitted in fulfillment of the requirements for the Degree of Ph.D. in Organic Chemistry

Submitted by:

Abd Almohimen Gabr Alla Mohammed Alhassan

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Supervisor:

Prof:. Faroug Bakheit Mohamed Ahmed Elsonni

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قال تعالى:

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انْظُرُوا إِلَىٰ ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَٰلِكُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ ﴿٩٩﴾)

صدق الله العظيم

سورة الانعام

DEDICATION

By the grace of God Almighty, this research has been completed.

I dedicate this work to the soul of my mother, who taught me that the secret to success is patience.

To my parents, my role models in life, and the ones to whom I owe the ultimate success.

To my sister and brothers, who spared no effort in supporting me.

To my wife, who provided me with every comfort and patiently endured to complete this research.

To my children, Muhammad, Al-Baraa, Hour and Omer.

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Abstract

Coriandrum sativum L., an annual herbaceous plant of the Apiaceae family, is native to the Mediterranean and Nile basins and is widely recognized as one of the most valuable medicinal and aromatic spices. It's essential oils, though present in minor quantities across its various organs—including leaves, stems, flowers, and seeds—are the primary source of its distinctive organoleptic and bioactive properties, underpinning its extensive applications in the food, pharmaceutical, and perfumery industries. The present study was undertaken to systematically evaluate the phytochemical composition, antioxidant potential, and antimicrobial efficacy of C. sativum extracts prepared using six solvents of varying polarity: chloroform, ethyl acetate, petroleum ether, hexane, ethanol, and water. Extraction was performed via steam distillation under both cold and hot conditions, with and without subsequent centrifugation. Qualitative phytochemical screening revealed a diverse array of secondary metabolites, including alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins, and saponins. The presence and relative abundance of these compounds were found to be markedly influenced by solvent polarity, extraction temperature, and the centrifugation purification step. Antioxidant activity was quantitatively assessed over a five-week storage period by monitoring the peroxide value in both fresh and used sunflower oil. The results demonstrated that the extracts, particularly those obtained via hot extraction combined with centrifugation, exhibited significant antioxidative effects, not only in retarding lipid oxidation but also in reducing pre-existing peroxide levels in oxidized oil systems. Gas chromatography-mass spectrometry (GC-MS) analysis of the ethanolic, hexane, and aqueous-distilled extracts identified a range of bioactive constituents, including terpenoids, fatty acid esters, and phenolic compounds, corroborating the observed bioactivities. Antimicrobial screening indicated that the water-distilled essential oil possessed the most potent and broad-spectrum activity

against a panel of pathogenic bacteria and fungi, surpassing the efficacy of the majority of organic solvent-based extracts. Collectively, these findings conclusively establish that optimized extraction protocols specifically, hot extraction followed by centrifugation yield highly purified and potent coriander extracts with considerable promise as natural antioxidants for food preservation and as effective antimicrobial agents .

الملخص

يُعدُّ نبات الكزبرة (Coriandrum sativum L.) من النباتات العُشبية الحولية التابعة للفصيلة الخيمية (Apiaceae)، و موطنه الأصلي حوض البحر الأبيض المتوسط وحوض النيل، ويُعتبر من أكثر التوابل والنباتات الطبية قيمةً وفائدةً. وعلى الرغم من أن الزيوت العطرية الموجودة في أجزائه المختلفة - بما فيها الأوراق والسيقان والأزهار والثمار (البذور) - لا تشكِّل سوى نسبة ضئيلة من تركيبه الكيميائي، إلا أنها تمثل المصدر الرئيسي لخواصه الحسية والبيولوجية المميزة، التي تُكسبه قيمته التطبيقية الواسعة في صناعات الأغذية والأدوية والعطور. هدفت هذه الدراسة إلى التقييم المنهجي للتركيب الكيميائي النباتي، والنشاط المضاد للأكسدة، والفعالية المضادة للميكروبات لمستخلصات نبات الكزبرة المحضَّرة باستخدام ستة مذيبات مختلفة القطبية، وهي: الكلوروفورم، وخلات الإيثيل، والإيثر البترولي، والهكسان، والإيثانول، والماء. وقد تم استخلاص الزيوت بطريقة التقطير البخاري وتحت ظروف الاستخلاص البارد والساخن، وذلك قبل وبعد عملية الطرد المركزي. أظهر الفحص الكيميائي النباتي النوعي وجود مجموعة غنية ومتنوعة من المستقلبات الثانوية، شملت القلويدات، والفلافونويدات، والتربينويدات، والستيرويدات، والجليكوسيدات، والتانينات، والصابونينات. وقد تبيَّن أن وجود هذه المركبات وتركيزاتها النسبية يتأثران بشكل ملحوظ بقطبية المذيب، ودرجة حرارة الاستخلاص، وكذلك بخطوة التنقية بالطرد المركزي. تم تقييم النشاط المضاد للأكسدة كمياً عن طريق رصد رقم البيروكسيد في زيت دوار الشمس الطازج والمستعمل على مدى خمسة أسابيع من التخزين. وأظهرت النتائج أن المستخلصات، ولا سيما تلك التي تم الحصول عليها بالاستخلاص الساخن المُتبوع بالطرد المركزي، تمتلك فعالية مضادة للأكسدة واضحة، ليس فقط في تثبيط الأكسدة، بل أيضاً في خفض مستويات البيروكسيدات المُسبقة التكوُّن في أنظمة الزيوت المؤكسدة. كما كشف تحليل كروماتوغرافيا الغاز المقرونة بمطياف الكتلة (GC-MS) للمستخلصات المقطَّرة بالإيثانول والهكسان والماء عن احتوائها على مجموعة من المركبات النشطة بيولوجياً، مثل التربينويدات، وإسترات الأحماض الدهنية، والمركبات الفينولية، مما يؤكد النشاطات البيولوجية المرصودة. أشارت اختبارات النشاط المضاد للميكروبات إلى أن الزيت العطري المستخلص بالتقطير المائي كان الأكثر فعالية والأوسع طيفاً في تثبيط مجموعة من البكتيريا والفطريات الممرضة، متفوقاً بذلك على معظم المستخلصات العضوية. وختاماً، تؤكد هذه النتائج بشكل قاطع أن تحسين عمليات الاستخلاص - وبخاصة الاستخلاص الساخن المُقرون بالطرد

المركزي - يُنتج مستخلصات كزبرة عالية النقاء والفعالية، تحمل إمكانيات واعدة كمواد مضادة للأكسدة طبيعية لحفظ الأغذية، وكعوامل مضادة للميكروبات .

LIST OF ABBREVIATIONS

AOAC :Association of Official Analytical Chemists
PV : Peroxide value
FFA : Free fatty acid
BPC:British Pharmacopoeia commission
EOA :Essential oil association
FAO : Food and Agricultural organization
FFA : Free fatty acid
GC-MS:Gas chromatography-mass spectrometry
ISO :International standards organization
NRA : National renderers association
SV :Saponification value
AOACS:Association of Official Analytical Chemists Standard
TBA :Thiobarbituric acid value
UV :Ultraviolet
IR :Infrared Analysis
NMR:Nuclear Magnetic Resonance Spectroscopy
TLC :Thin-layer Chromatography
HPLC:High performance liquidChromatography

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CHAPTER ONE
General framework

1.1. Introduction

Coriander (*Coriandrum sativum*) belonging to the family Umbelliferae/ Apiaceae is a glabrous aromatic, herbaceous annual plant , which has a long history as a culinary herb being the source of aroma compounds and essential oils with biologically active components possessing antibacterial, antifungal and antioxidant activities. C. sativum is useful in food preparation (as a flavouring agent and adjuvant) and preservation as well in preventing food borne diseases and food spoilage .

Coriandrum sativum L. (coriander) is a widely used culinary and medicinal herb. Its seeds and leaves are rich sources of bioactive compounds, including flavonoids, terpenoids, phenolic acids, and essential oils, which are responsible for its antioxidant, antimicrobial, and various pharmacological properties. The efficiency of extracting these compounds is highly dependent on the solvent's polarity, as different compounds have different solubilities. *Coriandrum sativum* is an aromatic and medicinal plant rich in phenolic compounds, flavonoids, and essential oils. These bioactive compounds possess antioxidant and antimicrobial properties, making coriander a valuable source of natural bioactive ingredients .

However, the efficiency of extracting these compounds varies depending on the solvent polarity. Polar solvents tend to extract more hydrophilic compounds, whereas non-polar solvents favor lipophilic ones. Moreover, the centrifuge technique can play an important role in separating and identifying secondary metabolites by fractionating compounds according to their density.

This study aims to systematically compare the extraction efficiency of polar and non-polar solvents, identify the most effective one through phytochemical screening, and evaluate the biological activities of the optimal extract. This includes assessing its potential as a natural antioxidant for edible oils and its efficacy against common bacteria and fungi.

1.2. Research Problem

There is a lack of comprehensive comparative studies evaluating the efficiency of both polar and non-polar solvents in extracting the active constituents of coriander, as well as assessing the chemical and biological properties of the resulting extracts.

1.3. Research Justifications

1.3.1. Natural Alternatives to Synthetic Additives

Increasing concerns about the harmful effects of synthetic antioxidants and preservatives in food have encouraged the search for natural and safe alternatives. *Coriandrum sativum* is a promising source of such natural compounds.

1.3.2. Variation in Solvent Efficiency

The polarity of solvents greatly influences the types and yields of phytochemicals extracted. Comparing polar and non-polar solvents provides essential information about optimal extraction conditions.

1.3.3. Biological Significance

The antioxidant and antimicrobial activities of coriander extracts can contribute to improving food quality, extending shelf life, and potentially providing health benefits.

1.3.4. Scientific Contribution

This study provides comparative data on the extraction efficiency and biological activity of coriander using different solvents, enriching scientific knowledge in applied chemistry.

1.3.5. Technological Justification (Centrifuge Use)

The use of the centrifuge enhances the separation and detection of secondary metabolites, providing a more accurate chemical profile of the extracts.

1.4. Significance of the Study

1.4.1. Scientific Significance

This study contributes to the growing field of natural product research by identifying the most effective solvent for extracting bioactive compounds from *Coriandrum sativum*. The findings will enhance understanding of the relationship between solvent polarity and phytochemical yield.

1.4.2. Practical Significance

The results may help in developing natural antioxidants and antimicrobials that can be used safely in the food, cosmetic, and pharmaceutical industries, reducing dependence on synthetic additives.

1.4.3. Health and environmental importance

Promoting the use of natural extracts from coriander supports sustainable and eco-friendly approaches while minimizing health risks associated with synthetic compounds.

1.4.4. Technological and analytical significance

Incorporating centrifuge technology as a supporting tool for identifying secondary metabolites highlights its importance in improving precision and efficiency in phytochemical studies.

1.4.5. Educational and Research Value

The research provides valuable training in extraction techniques, phytochemical screening, and bioassays, which can benefit students and researchers in chemistry, biochemistry, and food science disciplines.

1.5. Research Hypothesis

- The polarity of the extraction solvent will significantly influence the yield and phytochemical profile of the coriander extracts.
- Centrifugation will be a crucial step in clarifying the extracts and aiding in the preliminary separation of components for analysis.
- Extraction efficiency varies depending on solvent polarity.
- Polar solvents extract higher levels of phenolic and flavonoid compounds.
- The extracts will show significant antioxidant and antimicrobial activity.
- The centrifuge assists in improving the detection and separation of secondary metabolites

1.5. Aim and Objectives

1.5.1. Genaral Objective

To determine the optimal solvent for extracting bioactive compounds from coriander and to comprehensively evaluate the chemical composition and biological efficacy of the resulting extract.

1.5.2. Specific Objectives this study aims to

- extract bioactive compounds from coriander seeds using three solvents of varying polarity: Ethanol (polar), Chloroform (non-polar), and Ethyl Acetate (intermediate polarity).
- determine the extraction yield of each solvent and select the best one based on yield and preliminary phytochemical profile.
- perform a detailed qualitative and quantitative phytochemical analysis of the selected optimal extract.
- evaluate the *in vitro* antioxidant activity of the extract by measuring its ability to inhibit peroxide formation in edible oils.
- assess the antimicrobial activity of the extract against selected Gram-positive and Gram-negative bacteria and common fungi/yeast.

- To employ centrifugation as a key and a supportive analytical tool to separate, clarification, fractionation and identify secondary metabolites from the extracts.

1.6. Research Delimitations

1.6.1. Subject Delimitation

This study is limited to the extraction and analysis of bioactive compounds from *Coriandrum sativum* (coriander) using three specific solvents; ethanol, chloroform, and ethyl acetate. Other solvents or extraction techniques are not included.

1.6.2. Spatial Delimitation (Location)

The experimental work will be carried out in the laboratory facilities of the Department of Chemistry and Biochemistry (or related department), where necessary equipment such as the centrifuge and spectrophotometers are available.

1.6.3. Temporal Delimitation (Time Frame)

The study will be conducted within a defined period sufficient to complete the extraction, chemical analyses, and biological tests three years.

1.6.4. Material Delimitation

The research focuses solely on coriander plant seeds obtained from local sources, without comparison to other plant species.

1.6.5. Analytical Delimitation

The biological evaluation is limited to antioxidant activity (peroxide value test) and antimicrobial activity against selected bacteria and fungi. Other bioassays or cytotoxic studies, are excluded.

1.7. Expected outcomes

- ✓ Identification of the most efficient solvent for extracting bioactive compounds from coriander.
- ✓ A comprehensive phytochemical profile (qualitative and quantitative) of the optimal coriander extract.
- ✓ Scientific data demonstrating the efficacy of coriander extract as a natural antioxidant for stabilizing edible oils against oxidative rancidity.
- ✓ Quantitative evidence of the extract's antimicrobial potential against a panel of food-borne pathogens and spoilage microorganisms.
- ✓ A list of key volatile and non-volatile compounds identified via GC-MS, correlating them to the observed bioactivities.

CHAPTER TOW
Theoretical framework

2.1. *Coriandrum sativum* (Coriander)

Coriander is an annual apiaceae herb, which grows in mediterranean countries, and is widely used in food and pharmaceutical industries. In traditional medicine, seeds are used in the treatment of gastrointestinal problems, rheumatism and pain joints. Recent studies have also demonstrated a hypoglycemic action and effects on carbohydrate metabolism. It has also been reported the antimicrobial effect of coriander leaves and seeds against several microorganisms .Furthermore in food industry, leaves and seeds are employed as condiment, being used to flavour various commercial foods, as liqueurs, teas, meat products and pickles .

Phytochemistry, or plant chemistry, has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function.

In all these operations, methods are needed for separation, purification and identification of the many different constituents present in plants. Thus, advances in our understanding of photochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. One of the challenges of photochemistry is to carry out all the above operations on vanishingly small amounts of material. Frequently, the solution of a biological problem in, say, plant growth regulation, in the biochemistry of plant-animal interactions, or in understanding the origin of fossil plants depends on identifying a range of complex chemical structures which may only be available for study in microgram amounts .

Edible oil is oxidized during processing and storage via autoxidation and photosensitized oxidation, in which triplet oxygen ($^3O_2$) and singlet oxygen ($^1O_2$) react with the oil, respectively. Lipid hydroperoxides formed by $^3O_2$ are conjugated dienes, whereas $^1O_2$ produces both conjugated and non-conjugated dienes Autoxidation of oil is accelerated by the presence of free fatty acids, mono- and diacylglycerols, metals such as iron, and thermally oxidized compounds.(Raal*, et al* 2004).

By definition, the oxidative stability of oil is a measure of the length of time taken for oxidative deterioration to commence. On a general level, the rates of reactions in auto oxidation schemes are dependent on the hydrocarbon structure, heteroatom concentration heteroatom speciation, oxygen concentration, and temperature.

If untreated, oils from vegetable origin oxidize during use and polymerize to a plastic like consistency .

Even when they are not subjected to the intense conditions of industrial applications, fats and oils are liable to rancidity. This happens more so in fats that contain unsaturated fatty acid radicals (Sharma, *et al*., 2007).

To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase). Produced internally or the dietary antioxidants: vitamin A, vitamin C, and vitamin E. Antioxidant dietary supplements do not improve health nor are they effective in preventing diseases as shown by randomized clinical trials including supplements of beta-carotene, vitamin A, and vitamin E singly or in different combinations having no effect on mortality rate. Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers .(Sriti,J.,*et al* 2011).

However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors. Consequently, packaging of fresh fruits and vegetables contains an 8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions , The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects .Some studies have demonstrated that the antioxidant potential of an essential oil depends on its composition. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties. Regarding the antioxidant properties, thymol and carvacrol are potentially active phytochemicals. (Hassan, *et al*.,. 2014).

The activity of these natural products is directly related to their phenolic compounds having redox properties and thus plays an important role in neutralizing harmful free radicals. The antioxidant activity of essential oils is also due to certain alcohol, esters, ketones, aldehyde, and monoterpenes.

A natural product is a natural compound or substance produced by a living organism that is, found in nature In the broadest sense, natural products include any substance produced by life (Anita,D., *et al*., 2014).

Natural products can also be prepared by chemical synthesis (both semi-synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients .Within the field of organic chemistry, the definition of natural products is usually restricted to organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites. Secondary metabolites (or specialized metabolites) are not essential for survival, but nevertheless provide organisms that produce them an evolutionary advantage Many secondary metabolites are cytotoxic and have been selected and optimized through evolution for use as "chemical warfare" agents against prey, predators, and competing organisms Secondary or specialized metabolites are often unique to species, which is contrasted to primary metabolites which have broad use across kingdoms. Secondary metabolites are marked by chemical complexity which is why they are of such interest to chemists (Sharma, *et al*., 2007).

Natural sources may lead to basic research on potential bioactive components for commercial development as lead compounds in drug discovery. Although natural products have inspired numerous drugs, drug development from natural sources has received declining attention in the 21st century by pharmaceutical companies, partly due to unreliable access and supply, intellectual property, cost, and profit concerns, seasonal or environmental variability of composition, and loss of sources due to rising extinction rates. Natural products are often divided into two major classes, the primary and secondary metabolites. Primary metabolites have an intrinsic function that is essential to the survival of the organism that produces them. Secondary metabolites in contrast have an extrinsic function that mainly affects other organisms.

Secondary metabolites are not essential to survival but do increase the competitiveness of the organism within its environment. Because of their ability to modulate biochemical and signal transduction pathways, some secondary metabolites have useful medicinal properties Natural products especially within the field of organic chemistry are often defined as primary and secondary metabolites. A more restrictive definition limiting natural products to secondary metabolites is commonly used within the fields of medicinal chemistry and pharmacognosy (Teshale, *et al*., 2013).

2.1.1. Plants as a source of antioxidants

Antioxidants can be defined as bioactive compounds that inhibit or delay the oxidation of molecules. Antioxidants are categorized as natural or synthetic antioxidants. Some synthetic antioxidants commonly used are: BHT, BHA, propyl gallate, and tertbutylhydroquinine. Many scientists have concerns about safety because synthetic antioxidants have recently been shown to cause health problems such as liver damage, due to their toxicity and carcinogenicity. Therefore, the development of safer antioxidants from natural sources has increased, and plants have been used as a good source of traditional medicines to treat different diseases Many of these medicinal plants are indeed good sources of phytochemicals that possess antioxidant activities. Some typical examples of common ingredients that have been used in ethnic foods are tamarind, cardamom, lemon grass, and galangal basil. These spices or herbs have been shown to contain antioxidants. Deterioration of food due to either bacterial or fungal infection has always been a major concern, causing huge losses to food industries and societies throughout the world . (Wangensteen, *et al*.2004)

Moreover, the spread of food pathogens has become a major public health concern. With an increasing awareness of the negative effects of synthetic preservatives, there has been increased demand for the use of nontoxic, natural preservatives, many of which are likely to have either antioxidant or antimicrobial activities Herbs have always been used for flavor and fragrance in the food industry, and some of them have been found to exhibit antimicrobial properties Therefore, the call for screening and using plant materials for their antioxidant and antimicrobial properties has increased, The major compounds present in coriander are Aliphatic - hydrocarbons, aldehydes and alcohols; Monoterpene - oxides, hydrocarbons, esters and alcohols; Carbonyls, Phenols, Sesquiterpenes and a few miscellaneous compounds like acetic acid and α-pdimethyl styrene (Harborne, *et al*., 1998).

The content and composition of essential oil varies with various climatic conditions. The different phytonutrients present in coriander provide various health benefits including aflatoxin protective, antibacterial, antimutagenic, antispasmodic, carminative, cytotoxic, fungicidal, hypoglycemic, hypolipidemic, insecticidal, lipolytic, stimulant, and stomachic. (Yeung, ,*et al*.2011)

The coriander oil acts as a crucial ingredient in curry mixes and as ground or volatile isolate used in flavouring beverages, bakery products, sweets and tobacco goods

Several studies have been done on the composition of coriander essential oil. reported that coriander seed oil contains more than 52 compounds out of which α-pinene (4.09 %), geranyl acetate (8.12 %) and linalool (75.30 %) are the major ones. The 20 compounds identified in the essential oil of coriander seed by (Bandoni, *et al*., 1998) covered 96.6–99.7 % of total oil content. The major compounds identified included α-pinene, camphor, geraniol, geranyl acetate, linalool and γ-terpinene. The free radicals after oxidation by metabolic process cause significant damage to cells. The antioxidants prevent the damage and thus help to maintain healthy condition of the cells. The secondary metabolites like polyphenols present in spices prove advantageous physiologically by their antioxidant action. These polyphenols exhibit various health-promoting activities such as antioxidant, antihepatotoxic, antimicrobial, anti-inflammatory and antitumor. Several experiments have been carried out to ascertain the antioxidant potential of coriander (Sriti,J., *et al*., 2011).

demonstrated that 2.7 mg of total phenolics present in the aqueous extract of coriander showed substantial antioxidant activity.showed that the aqueous extract of selected coriander leaves acts as a natural reducing agent with antioxidant activity. Thus, coriander can serve as a natural source of food preservative and protect human health.

The importance of classical plant systematics as a tool for the conservation of biodiversity is becoming evident . Yet this aspect of cultivated plants and their diversity on the infra-specific level is often neglected. Genebanks conserve much of the diversity of cultivated plants, and were formerly established to assist modern plant breeding and crop research. Today, genebanks must also confront the continuing gene erosion occurring in cultivated plants and in their wild relatives and weeds. But if the task facing genebanks has changed, the classical methods for studying the biodiversity of cultivated plants as presented by N.I. are still fundamental to their description, to understanding their use, and, as stated above, to the protection of biodiversity. (Vertuani S, *et al*., 2004).

This publication describes the genetic resources of coriander (*Coriandrum sativum* L.) and discusses various aspects of the origin, history, use, breeding and agronomy of a species whose entire potential has not yet been fully recognized. Many interesting publications on the breeding and genetic resources of coriander that were published in the former Soviet Union have gone unnoticed in Western countries, a fact to which particular attention is drawn here.Infra-specific classification is part of a holistic approach to biodiversity.

2.1.2. Taxonomy of *Coriandrum sativum*

The ḡenus Coriandrum includes the cultivated plant C. sativum and the wild species C. tordylium. The latter is described for southeastern Anatolia and northern Lebanon Herbarium specimens of C. tordylium from Edinburgh (Scotland) and Turku (Finland) demonstrate that this annual species is very similar to the cultivated C. sativum. The wild species might be interest ing for coriander breeding. The closest genus to Coriandrum is Bifora. This genus includes the species B. Americana, native to the southeastern areas of North America It also includes two other species which originate in the Mediterranean area: B. radians and B. testiculata.

The latter species occurs as a weed in winter crops in southern Europe, the Mediterranean area and the Near East . Bifora radian is morphologically similar to coriander, but the fruits have a different shape and do not contain essential oils. On the other hand, the fatty oil content of the fruits is very high in both species, with 41.5% of the dry matter in B. testiculata and 49.5% in B. radians . (Eisenmenger., *et al*., (2006).

Fruits of C. sativum contain, depending on the genotype, up to 27.7% of fatty. Plant breeders have tried to use B. radians as a genetic resource in coriander breeding, but even using embryo-rescue techniques. The six other genera belonging to the tribe Coriandreae comprise wild plants from Central Asia. They are perennials and, judging from the herbarium specimens at the Botanical Institute in St. Petersburg, their morphology is quite different from that of coriander. .

Thus it seems doubtful that they can be crossed successfully with the cultivar. None of the Central Asian species is available in any botanical garden or germplasm collection as a living sample, and it is difficult to judge whether they can be used for breeding purposes. The chromosome numbers are similar in most of the species.

The relatively small tribe Coriandreae belongs to the subfamily Apioideae, which includes most of the genera of the Umbelliferae and originates in the temperate

geographical areas of Europe and Asia The taxonomy of the other cultivated plants of the Umbelliferae is carefully presented by

2.1.3. Common names of *Coriandrum sativum*

The name of coriander is differ according to the regions and countriesArab (kuzbara, kuzbura), England (coriander, collender), Chinese (parsley), Ethiopian (dembilal), French (coriander), German (Koriander), Greek (koriannon), Hindi (dhania, dhanya), Italian (coriandolo), Japanese (koendoro), Russian (koriandr), Spanish (coriandro) and Turkish (kisnis).

2.1.4. Geographical variation of coriander plant

The fatty oil content had a wide range of variation, and extremely high values of up to 27.7% were found in the leafy Syrian types. In general, the leafy types of coriander from the Caucasus had high fatty oil content. The accessions of Mediterranean, African, Near Eastern and Indian origin, on the other hand, showed them- selves not to be a homogeneous group with respect to this character. The composition of the fatty acids is quite constant in all accessions. Petroselinic acid is always the principal component, but the accessions with large fruits tend to have more petroselinic acid in their fatty oil than the others.

2.1.4. Areas of production and consumption

World production of coriander fruits is difficult to estimate, since official statistics seldom contain figures relating to this crop. A considerable quantity of coriander is grown in home gardens or on a small scale, and is not recorded in any statistics. Taking the different observations on this subject into account, the world-wide production of coriander may be estimated at approximately 550 000 ha annually. The yearly production of coriander fruits may be estimated at about 600 000. The main producers of coriander fruits are the Ukraine, Russia, India, Morocco, Argentina, Mexico and Romania. Other countries that produce at least some coriander for use of the fruits, by geographical region, are:

- The Near East (Iran, Israel, Kuwait, Lebanon, Syria and Turkey).
- The Middle East (Bhutan, Kasachstan, Kirgysia, Pakistan and Tadjikistan)
- The Far East (Burma, China and Thailand)
- The Americas (Argentina, Chile, Costa Rica, Guatemala, Paraguay, USA and Canada)
- Europe (Bulgaria, Czechoslovakia, England, France, Hungary, Italy, the Nether-lands, Poland and Yugoslavia).

As the list shows, coriander is grown in almost all agriculturally viable areas, with the exception of the tropics and the Polar Regions. The tropical climate is unfavourable for ripening of the fruits, and coriander is only cultivated for the use of the fruits in mountainous areas of the tropics. The plant is sometimes cultivated for use as a vegetable in tropical areas, e.g. in Cuba. The list provided is based on export figures presented by (Aberoum, *et al*,2008).

The volatility of the market for this fruit is shown by these widely varying production figures. The main exporters of coriander are the Ukraine, Russia, India and Morocco, and the main importers are the USA, Sri Lanka and Japan. Other countries importing coriander are Malaysia, Chile, Bolivia and some countries in the Middle East.

2.1.5. Growing and description of coriander

Coriander enjoys a sunny position to grow in but appreciates a little shade during the hottest part of the day. Coriander has a tendency to run to seed if stressed; this is where it flowers prematurely and develops seeds instead of growing lush foliage. This is fine if you are growing the plant for its seeds, but not if you are growing it for its. It is an upright and short-lived herbaceous plant usually growing 1-2 m.

I. Leaves

Coriander is best grown from seed directly into the soil. Also grow in modules and have had good results, take care not to touch the roots when planting. Prepare the soil thoroughly by digging it over, removing any weeds and incorporating organic matter such as well-rotted manure or compost. Rake the soil so it's level and sow seeds 4cm part in drills 1cm deep. If you are growing coriander for its seeds, grow the plants in full sun. This will cause them to develop seeds quicker as the hot stressful conditions will trigger flower production.

Coriander leaves are small herb having many branches and sub-branches New leaves are oval but aerial leaves are elongated

Flowers are white, having slightly brinjal like shades while fruit are round in shape

Macroscopic characteristic of fruit is globular, mericarps usually united by their margins forming a cremocarp about 2-4 mm in diameter, uniformly brownish-yellow or brown, glabrous, sometimes crowned by then remains of sepals and styles, primary ridges 10, wavy and slightly inconspicuous secondary ridges 8and straight with aromatic odor (Figueiredo, *et al*., 2004),

II. Flowers

Umbels of small whitish flowers that are followed by pairs of brownish-yellow, deeply furrowed "seeds". The white flowers are borne inlarge numbers in dense flat-topped clusters at the tips of the branches (in terminal compound umbels). Individual flowers are small (2-4 mm across), have five incurved petals and five stamens, and are borne on stalks (pedicels) up to 5 cm long. Many (about 15) of these stalks (pedicels) radiate from the same point and form a small cluster of flowers (an umbel), with several (6-20) of these smaller clusters (often called rays) being grouped together into a much larger cluster (a compound umbel) that is subtended by several small leafy bracts (about 5 mm long). Flowering occurs mostly during spring and summer.

III. Fruit

The fruit turns from green to greyish-brown in color as it matures and resembles a capsule. It actually consists of two one-seeded structures (mericarps) that readily split apart when the fruit is fully mature. Each of these (seeds), 2-4 mm long, hairless (glabrous), but has five prominent yellowish-colored ribs. There are many varieties of *Coriandrumsativum* which differ in the fruit size and oil yield .

IV. Seeds

Coriander seeds are frequently used in the making of comfits and other kinds of confectionery, as a flavoring in breads, and as an ingredient in curry powder and other condiments. The seeds, have a pleasing aromatic smell which improves with time.It was used in medicines to disguise the unpleasant flavors of some drugs. The seeds are also used for flavoring several types of liquors,growing cycle: hardy annual herb height: grows to 24 to 30 inches tall (Nadeem., *et al*., 2013),

2.1.6. Physicochemical Composition

Total ash: not more than 6 per cent, acid insoluble ash: not more than 1.5 per cent, water -soluble extractive: notmore than 19 per cent, alcohol soluble extractive: not more than 10 per cent and volatile oil: not less than 0.3% v/w .

2.1.7. Chemistry composition of Coriander

Coriander fruit has been reported as containing both essential oil (rich in linalool) and vegetal oil (rich in petroselinic acid) Content and Active Ingredients. The active ingredients of coriander are: volatile oil (0.4 to 1.7%): main component D (+) linalool (Coriandrol 60-70%).Also includes borneol, p-cymene, alpha-pinene, camphor, geraniol, limonene.Fatty oil (13-21%): acid, oleic and linoleic.A range of aldehyde compounds

are largely responsible for the aroma of coriander leaves. The largest proportions of these are those aldehydes with 6-10 carbon atoms, particularly decyl (10) and nonyl (9) aldehydes. (Zawislak G., 2011).

Other major constituents of the leaves are 2-decenoic acid, decanoic acid (also known as capric acid) and tetradecenoicacid.The chemical composition of coriander seeds is slightly different,with the alcohol linalool being the major constituent.Coriander leaves contain high levels of organic compounds called aldehydes.The same aldehydes, or similar, are often commonly found in soaps and lotions. However, perception of this facet of coriander's taste isn't purely chemical. Scientists have discovered that dislike of the taste of coriander may also be influenced, to an extent, by genetic factors. Studies have also suggested that crushing coriander leaves may lead to faster breakdown of aldehydes, diminishing the soapy taste.

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2.1.8. Chemical Constituents

protein 21.93 and 12.37 g, total lipid (fat) 4.78 and 17.77 g, carbohydrate 52.10 and 54.99 g,fiber 10.40 and 41.9 g, calcium 1246 and 709 mg, iron 42.46 and 16.32 mg, phosphorus 481 and 409 mg, magnesium 694 and 330 mg, potassium 4466 and 1267mg, sodium 211 and 35 mg, zinc 4.72 and 4.70 mg, vitamin C 566.7 and 21 mg, thiamin 1.252 and 0.239 mg, riboflavin 1.500 and 0.290 mg, niacin 10.707 and2.130 mg, vitamin B-120.00 and 0.00µg, vitamin A, RAE 293 and 0.00 µg, vitamin A, IU 5850 IU and 0 IUand vitamin D (D2 + D3) 0.00 and 0.00 µg, respectively.

The phytochemical screening of plant showed the presence of essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols and glycosides . The most important constituents of coriander fruits were the essential oil and fatty oil. The essential oil content of dried coriander fruits varies between 0.03 and 2.6%, while the fatty oil content varies between 9.9 and 27.7% (Coskuner, *et al*., 2007).

The variations in the oil constituents of *Coriandrum sativum*leaves and seeds could be attributed to the variations in the cultivar and not due to geographic divergence and ecological conditions.

However, the compounds isolated from coriander essential oil were included: monoterpene hydrocarbons (p-cymene, camphene, Δ-3-carene, limonene (dipentene), myrcene, cis- and trans-ocimene,α-phellandrene, β-phellandrene, α- pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, terpinolene, α-thujene); monoterpene oxides and carbonyls (camphor, 1,8- cineole, linalol oxide, carvone, geranial); monoterpene alcohols (borneol, citronellol, geraniol, linalool, nerol, α-terpineol, 4-terpinenol); monoterpene esters (bornyl acetate, geranyl acetate, linalyl acetate, α-terpinyl acetate); sesquiterpenes (β-caryophyllene, caryophellene oxide, elemol, nerolidol); phenols (anethole, myristicin, thymol); aliphatic hydrocarbons (heptadecane, octadecane); aliphatic alcohols (decanol, dodecanol); aliphatic aldehydes (octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, tetradecanal, 3 - octenal, 2-decenal, 5-decenal, 8-methyl-2-nonenal, 8- methyl-5-nonenal, 6-undecenal, 2-dodecenal, 7- dodecenal, 2-tridecenal, 8- tridecenal, 9-tetradecenal, 10-pentadecenal,3,6-undecadienal, 5,8-tridecadienal) and Miscellaneous compounds: acetic acid, α-pdimethyl styrene .

The analysis of the essential oil conducted by gas chromatography-mass spectroscopy, revealed 33 components, representing 99.99% of the total oil from the seeds of coriander. The major components were linalool (55.09%), α-pinene (7.49%), 2,6-octadien-1-ol, 3,7-dimethyl-, acetate, (E)- (5.70%), geraniol (4.83%), 3-cyclohexene-1-methanol, α,α,4-trimethyl- (4.72%), hexadecanoic acid (2.65%), tetradecanoic acid (2.49%), 2-α-pinene (2.39%), citronellyl acetate (1.77%), and undecanal (1.29%) (Ciocarlan and Zarbock, 2015). Sudanese *Coriandrum sativum* oils contained seventy eight compounds with sabinene (17.63%), camphor (15.5%), cis-beta-ocimene (10.11%), trans-beta-ocimene (5.64%), alpha pinene (4.69%) and norboreneolacetate (4.09%) as the main constituents .

Chromatographic analysis yielded 93.0% neutral lipids, 4.14% glycolipids, and 1.57% phospholipids. Six triacylglycerol molecularspecies were detected but one component (C54:3) corresponding to tripetroselinin, and/or dipetroselinoyloleoyl glycerol comprised more than 50% of the totaltriacylglycerols. Sterol content was estimated to be at ahigh level (5186 µg /g oil). Stigmasterol, β -sitosterol, 5-avenasterol, and campesterol were found to be the sterol markers. The major phospholipid subclasses were phosphatidylcholine followed by phosphatidyl ethanolamine, phosphatidy-linositol and phosphatidylserine .

The leaves and stems of Korean *coriandrum sativum* were extracted and the essential oil composition were studied. Thirty-nine components representing 99.62% of the total oil were identified from the leaves. The major components were cyclododecanol (23.11%), tetradecanal (17.86%), 2-dodecenal (9.93%), 1-decanol (7.24%), 13-tetradecenal (6.85%), 1-dodecanol (6.54%), dodecanal (5.16%), 1-undecanol (2.28%), and decanal (2.33%). On the other hand, thirty-eight components representing 98.46% of the total oil were identified from the stems of the coriander (Chung, *et al*., 012). The leaf oil of *Coriandrum sativum* from Bangladesh contained 44 compounds mostly of aromatic acids, the major were 2-decenoic acid (30.82%), E-11-tetradecenoic acid (13.4%), capric acid (12.7%), undecyl alcohol (6.4%) and tridecanoic acid (5.5%). Other major constituents in the leaf oil were undecanoic acid (2.13%), 2-dodecanal (1.32%), 2-undecenal (3.87%), cyclododecane (2.45%), decamethylene glycol (1.15%), decanal (1.35%) and dodecanoic acid (2.63%). The seed oil contains 53 compounds of which the major compounds are linalool (37.65%), geranyl acetate (17.57%) and γ-terpinene (14.42%).

Other major compounds in the fruit oil are β- pinene (1.82%), m-cymene (1.27%), citronellal (1.96%), citronellol (1.31%), citral (1.36%), geraniol (1.87%), citronellyl acetate (1.36%), α-cedrene (3.87%), and α- farnesene (1.22%) and β-sesquiphell-andrene(1.56%). The seed oil contained 53 compounds; the major compounds were linalool (37.7%), geranyl acetate (17.6%) and γ-terpinene (14.4%).

However, the components isolated from seeds oil and their percentage were: γ-Terpinene 14.42, Camphene 0.14, E-Verbenol 0.27, Sabinene 0.23, β-Pinene 1.82, 2- Oxabicyclo[2.2.2]0ctan-6-l,1,2,3-trimethyl 0.02, β-Myrcene 0.55, Cyclooctanol 0.02, α-Thujene 0.04, m Cymene 1.27, Limonene 0.40, E-Ocimene 0.05, Z-Ocimene 0.04, Lilac alcohol 0.11, α-Terpinene 0.04, Z- Verbenol 0.11, Linalool 37.65, Isothujol 0.04, α-Campholenal 0.22, Citronellal 1.96, Umbellulone 0.11, Borneol 0.32, 4-Terpineol 0.06, Terpinyl acetate 0.31, Decanal 0.14, Z-verbenone 0.10, Citronellol 1.31, Citral 1.36, Geraniol 1.87, Eugenol 0.90, Carveol 0.15, Undecanal 0.58, Methyl geranate0.17, Myrtenyl acetate 0.43, Citronellyl acetate 1.36, Geranyl acetate 17.57, Z-myrtenyl acetate 0.10, β-Elemene 0.06, Dodecenal 0.15, Caryophyllene 0.33, β-Farnesene 0.07, 2-Dodecenal 0.18, Curcumene 0.98, α-Cubebene 0.13, α-Cedrene 3.87, α-Farnesene 1.22, β-Bisabolene 0.80, β-Sesquiphellandrene 1.56, E-Nerolidol 0.13, Artumerone 0.04, 8-Hexadecenal, 14-methyl-, (Z) 0.22, α-Bisabolol 0.15 and n-Hexadecanoic acid 0.08 (Derbesy, *et al*. 1993)

Many isocoumarins were isolated from the aerial parts of Coriandrum sativum, including coriandrone A, coriandrone B, isocoumarins, coriandrin and dihydro coriandrin . Caffeic acid, protocatechinic acid, and glycitin were characterized as the major polyphenolics of coriander aerial parts (Rajeshwari and Andallu); found that the ethanolic extract of coriander seeds contained many flavonoids including caffeic acid, chlorogenic, quercetin and rutin However, the total polyphenolic content of the seeds was found to be 12.2 gallic acid equivalents (GAE)/g while total flavanoid content was found to be 12.6 quercetin equivalents /g. The amount of flavonoids in 70% ethanol extract was found to be 44.5 µg and that of the total phenols was 133.74µggallic acid equivalents per mg of the hydro-alcohol extract of *Coriandrumsativum* leaves (Paolini J, (Costa J, et al., 2005).

2.1.9. Uses of *Coriandrum sativum*

There exist very different uses of coriander and these are based on different parts of the plant. The traditional uses of the plant, which are based on the primary products, i.e. the fruits and the green herb, are twofold: medicinal and culinary. During industrialization, the specific chemical compounds of coriander were recognized and identified, and these became important as raw materials for industrial use and further processing. The essential and fatty oils of the fruits are both used in industry, either separately or combined. After extraction of the essential oil, the fatty oil is obtained from the extraction residues either by pressing or by extraction.

A further benefit of coriander derives from the reproductive biology of this plant. Coriander produces a considerable quantity of nectar and thereby attracts many different insects for pollination, an external effect which is of both ecological and economic value. Coriander is also a good melliferous plant, and Luk'janov and Reznikov (1976) state that one hectare of coriander allows honey bees to collect about 500 kg of honey.

2.1.9.1. Medical uses

Coriander has been used in medicine for thousands of years . The first medicinal uses of the plant were reported by the ancient Egyptians. General references to coriander's medical uses are also found in classical Greek and Latin literature .

The coriander fruits are believed to aid digestion. Many other fruits of umbelliferous plants have been used in medicine since antiquity as they also affect the digestive system and some act as an aphrodisiac. Some of these, such as hemlock (Conium maculatum L.), are poisonous .

Coriander is also used externally to treat ulcers and rheumatism; these and several other medicinal uses are recorded by Hegi (1926). Losch (1903) describes how the fruits need

to be soaked in wine or in vinegar overnight before being re-dried, in order to remove chemical compounds contained in the fresh fruits, which cause dizziness. These are mentioned in the older references (Linnaeus 1780; Reichenbach 1833; Losch 1903; Hegi 1926). Fruits thus treated were used for medicinal purposes, and also to treat halitosis. Today, the plant is still sometimes used for these purposes in folk medicine. The medical uses of coriander in the modern era .

They are used chiefly, however, to conceal the taste or smell of other ingredients in pharmaceutical preparations (this use is also reported by Jansen , and to correct the gripping qualities of rhubarb and senna. The seeds are chewed as a remedy for halitosis.

The drug which is known as coriandri fructus or fructus coriandri is hardly used in current orthodox medicine. It is still on the German and Austrian official lists of pharmaceutical plant drugs, however. The antibacterial effects of the essential oil of coriander are mentioned by (Pino, *et al*. 1996).

2.1.9.2. Use of the fruits as a spice

The ripe fruits of coriander have a pleasant flavour owing to the particular composition of the essential oil .The use of coriander as a spice has also been reported since ancient times. The fruits are used in the preparation of fish and meat, but also for baking. The famous Russian rye bread 'Borodinskij chleb' is spiced with coriander .In India, coriander is very popular as a spice and is also cheap. In Ethiopia, coriander is widely used along with other spices to add flavour to 'berbere' which is a spiced, hot red-pepper powder used for numerous meat and vegetarian dishes Today, most coriander is consumed in the form of curry powder, of which it forms 25-40% In India, the fruits are also extensively employed as a condiment in the preparation of pickling spices, sausages and seasonings, and for flavouring pastry, cookies, buns and cakes, and tobacco products. The entire young plant is used to prepare chutneys and sauces. Coriander is used also to flavour several alcoholic beverages, (Oyedeji, F. *et al*., 2006).

2.1.9.3. Use of the green herb as a vegetable

Another primary product is the fresh green herb of coriander, used because of its specific flavour, which is completely different from that of the ripe fruits. In the Caucasus, Iran, Iraq, Mexico and in South America, with the exception of Argentina, coriander is mainly used in this form. The green herb is also consumed on a large scale in India, China, Thailand, Malaysia, Indonesia, and the American Midwest and in the Near East

The Georgian name 'kinza', also used in Russian, refers to the green plant, and the English term 'Chinese parsley' also describes the herb. The same is true for the Spanish

name ‘cilantro’, which denotes the plant, while the fruits are called ‘simiente de cilantro’.

The name ‘cilantro’ is frequently used in American English to refer to the green herb or the dried leaves , probably because the use of the green plant was introduced in the USA by Spanish-speaking Mexicans. The French name ‘persil arabe’ also means the green herb. Whether the ancient Egyptians also used the green herb cannot be determined from the available literature, and the later Greek and Latin authors only mention the use of the fruits.

The green plant is a most important ingredient in cooking. Here, the local varieties are characterized by high production of green matter . Although at present the use of the green herb is much more lim- ited in the industrialized countries, its popularity seems likely to increase, as the market for such ‘ethnic’ foods is growing.

The characteristic smell of the green plant is caused by the aldehydic contents of the essential oil. During ripening, these decrease, and after ripening and drying, they are no longer found in the fruits (Lörincz and Tyihák 1965). Lastly, the green herb’s high vitamin C (ascor- bic acid, up to 160 mg / 100 g), vitamin A (carotin, up to 12 mg / 100 g) and vitamin B2 content (up to 60 mg / 100 g) should be noted .

2.1.9.4. Use of the essential oil of the fruits

The first factory for the steam distillation of the essential oil of coriander was built in Russia in 1885 in the Vorone district In this area, the introduction of coriander as a field crop began as early as 1830, and it remains the principal producer of coriander for this purpose. The oil is obtained by steam distillation of the crushed fruits and a continuous and completely automated processing tech- nique has been developed.

Recently, the essential oil has also been processed by liquid carbon dioxide extraction. The extracted essential oil is used in the flavouring of a number of food products and in soap manufacture. It is principally used as a flavouring agent in the liquor, cocoa and chocolate industries.

Like the fruits, it is also employed in medicine as a carminative or as a flavouring agent. It has the advantage of being more stable and of retaining its agreeable odour longer than any other oil of its class. Decylaldehyde (yield 0.1% of the weight of coriander oil), obtained by treating the oil with bisulphite, is reported to be useful for perfumery purposes. The commercial oil is extensively adulterated with sweet orange oil, cedar-wood oil, turpentine and anethole or aniseed oil.

The main component, linalool, is used as a base for further tech- nical processing. Today, oleochemically synthesized linalool is usually used in the non-food sec- tor, as it

is cheaper at present. The demand for essential oils is rising in Western countries, and the full potential of this use of coriander has not yet been recognized .

After extraction of the essential oil, the residues are used as ruminant feed, since their composition is nearly the same as that of the whole fruits, and therefore still contains digestible fat and protein .Because of the high crude fibre content, coriander oil cake can only be fed to ruminants, but the nutritional value of this feed is limited

(Frank,C. *et al*., 1995),

2.1.9.5. Using of the fatty oil of the fruits

The fatty oil is obtained either by pressing or by extraction of the fruits. This oil has special qualities that make it suitable for use as a lubricant in some technical processes with special demands .

The main component of the fatty seed oil, petroselinic acid (C 18:1(6c)) is an isomer of the usual oleic acid (C18:1(9c)). In petroselinic acid, the single double bond has a different position; the biosynthesis is described by Meierzu (Dambroth and Bramm 1991). The high petroselinic acid content gives the oil peculiar physicochemical properties that make it potentially suitable for use in surfactants, polymers or as oleochemical raw material .

Petroselinic acid is a characteristic component of the fatty oil of umbelliferous plants, and the fruits of several medicinal and aromatic plants have been screened for this fatty acid. In temperate climates, coriander is the plant with the greatest potential for petroselinic acid production. In the past, the ozonolysis of petroselinic acid at the double bond to yield lauric (C12:0) and adipic acid was proposed .

The use of coriander dated back to around 1550 BC, and it was one of the oldest spice crops in the world. Medicinally, it was used as stimulant, aromatic and carminative. The powdered fruit, fluid extract and oil are chiefly used medicinally as flavouring to disguise the taste of active purgatives and correct their griping tendencies. The whole or ground seed (fruit) was an ingredient of pickling spices, also used to flavor various commercial foods, particularly, to prepare some instant soups and dishes, in many cakes, breads and other pastries, alcoholic beverages, frozen dairy desserts, candy, and puddings. The fruit essential oil was a common ingredient in creams, detergents, surfactants, emulsifiers, lotions, and perfumes (Dorado, *et al*., (2005).

However, seeds were applied locally to alleviate swelling and pains. Paste of green coriander was used for headache. Externally, powdered green coriander was used to alleviate burning sensation and pain in diseases like inflammation caused by erysipelas and lymphadenopathy. Decoction of green coriander was used in stomatitis. Nasal

drops of green coriander act as a haemostat and thus stop bleeding in epistaxis. Juice or decoction of green coriander was used in conjunctivitis. The seeds were included in many prescriptions as carminative and for the treatment of fever, diarrhoea, vomiting and indigestion. Coriander was used internally as tonics. It was also used for syncope and memory loss. Fresh juice of leaves was used as gargle in sore throat and stomatitis. Paste of leaves was locally applied for swellings and boils and was applied over forehead and temples for headache (Choe . *et al*.2006).

2.2. Essential oils

Essential oils are also known as volatile oils, ethereal oils, aetheroleum, or simply as the oil of the plant from which they were extracted, such as oil of clove. Essential oil is a concentrated hydrophobic liquid volatile containing(easily evaporated at normal temperatures) chemical compounds from plants. An essential oil is essential in the sense that it contains the essence of the plant's fragrance the characteristic fragrance of the plant from which it is derived. The term "essential" used here does not mean indispensable or usable by the human body, as with the terms essential amino acid or essential fatty acid, which are so called because they are nutritionally required by a living organism.

Essential oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, sfumatura, absolute oil extraction, resin tapping, wax embedding, and cold pressing. They are used in perfumes, cosmetics, soaps, air fresheners and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products. Essential oils are often used for aromatherapy, a form of alternative medicine in which healing effects is ascribed to aromatic compounds. Aromatherapy may be useful to induce relaxation, but there is not sufficient evidence that essential oils can effectively treat any condition

Improper use of essential oils may cause harm including allergic reactions, inflammation and skin irritation. Children may be particularly susceptible to the toxic effects of improper use. Essential oils can be poisonous if ingested or absorbed through the skin (Keskin, *et al*., 2011)

2.2.1. Extraction of essential oil

Oils contained within plant cells are liberated through heat and pressure from various parts of the plant matter; for example, the leaves, flowers, fruit, grass, roots, wood, bark, gums and blossom. The extraction of essential oils from plant material can be achieved by various methods, of which hydro-distillation, steam and steam/water distillation are the most common method of extraction .Other methods include solvent extraction, aqueous infusion, cold or hot pressing, effleurage, supercritical fluid extraction and phytonic process. This later process has been newly developed; it uses refrigerant hydrofluorocarbons solvents at low temperatures (below room temperature), resulting in good quality of the extracted oils .

2.2.1.1. Plant Material

Fresh plant tissues should be used for phytochemical analysis and the material should be plunged into boiling alcohol, plants may be dried before extraction. If this is done, it is essential that the drying operation is carried out under controlled conditions to avoid too many chemical changes occurring. It should be dried as quickly as possible, without using high temperatures, preferably in a good air draft. Once thoroughly dried, plants can be stored before analysis. Indeed, tests for flavonoids, alkaloids, quinones and terpenoids have been successfully carried out on herbarium plant tissue.The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance that is being isolated. In general, it is desirable to 'kill' the plant tissue, i.e. prevent enzymatic oxidation or hydrolysis occurring, and plunging fresh leaf or flower tissue, suitably cut up where necessary, into boiling ethanol is a good way of achieving this end (Li HB,et al., (2008).).

2.2.1.2. Solvent Selection

The classical chemical procedure for obtaining organic constituents from dried plant tissue (dried seeds, root, leaf) is to continuously extract powdered material in a soxhlet apparatus with a range of solvents, starting in turn with ether, petroleum and chloroform (to separate lipids and terpenoids) and then using alcohol and ethyl acetate (for more polar compounds). This method is useful when working on the gram scale.

Ideal solvents for fat extraction should have a high solvent power for lipids and low or no solvent power for proteins, amino acids, and carbohydrates. They should evaporate readily and leave no residue, have a relatively low boiling point, and be nonflammable

and nontoxic in both liquid and vapor states Alcohol, in any case, is a good all-purpose solvent for preliminary extraction .

Most flowers contain too little volatile oil to undergo expression, but their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils .

Extracts from hexane and other hydrophobic solvents are called concretes, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil-soluble) plant material. Although highly fragrant, concretes contain large quantities of non-fragrant waxes and resins. Often, another solvent, such as ethyl alcohol, is used to extract the fragrant oil from the concrete. The alcohol solution is chilled to 18 °C (0 °F) for more than 48 hours which causes the waxes and lipids to precipitate out. The precipitates are then filtered out and the ethanol is removed from the remaining solution by evaporation, vacuum purge, or both, leaving behind the absolute. The ideal solvent should penetrate sample particles readily, be in single component form to avoid fractionation, and be inexpensive and no hygroscopic .

It is difficult to find an ideal fat solvent to meet all of these requirements. Ethyl ether and petroleum ether are the most commonly used solvents, but n-hexane are used to extract oil from soybeans. Ethyl ether has a boiling point of 34.6 isabetter solvent for fat than petroleum ether. It is generally expensive compared to other solvents, has a greater danger of explosion and fire hazards, is hygroscopic, and forms peroxides. Petroleum ether is the low boiling point fraction of petroleum and is composed mainly of pentane and hexane. It has a boiling point of 35–38 C and is more hydrophobic than ethyl ether. It is selective for more hydrophobic lipids, cheaper, less hygroscopic and less flammable than ethyl ether. The detailed properties of petroleum ether for fat extraction are described in AOAC method 945.16 . Instead, a solvent such as n-hexane or supercritical carbon dioxide is used to extract the oils. Extracts from n-hexane and other hydrophobic solvents are called concretes, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil-soluble) plant material (Simon, 1987). Although highly fragrant, concretes contain large quantities of non-fragrant waxes and resins. Often, another solvent, such as ethyl alcohol, which is more polar in nature, is used to extract the fragrant oil from the concrete. The alcohol solution is chilled to -18 °C (0 °F) for more than 48 hours which causes the waxes and lipids to precipitate out. The precipitates are then filtered and the ethanol is removed from the remaining solution by evaporation, or

both, leaving the absolute. Supercritical carbon dioxide is used as a solvent in supercritical fluid extraction (Kravchenko, A. I. 2011).

2.2.1.3. Method of extraction

I. Single solvent extraction

It is well known that mono- and sesquiterpenes are much less soluble in alcohol than the oxygenated constituents; with the solubility of these latter compounds increased in the following order: ether and oxides < esters < ketones < aldehydes < alcohols. As a result, alcohol washing can be used to remove the oxygenated compounds from the oil. The process of removing the alcohol is, however, more difficult requiring a series of brine washings followed by a careful fractional distillation to remove all of the alcohol (Kumar and Tripathi, 2011).

The main advantage of this method is that the resulting oil is free of both mono- and sesquiterpene.Also, the use of alcohol to deterpenify oils such as citrus oils, where aldehydes are the important aroma components, is not recommended. This is because of the ease of acetal formation resulting in a change in odour and composition of the terpeneless oil (Fecka and Raj (2007).

II. Two-Phase Solvent System

In 1937, Dijck and Ruys described the removal of terpenes from an essential oil by the use of a binary two-phase solvent system.The apparatus is constructed so that the tube is on a slight incline, thereby allowing the alcohol to flow in a countercurrent way with gravity while pentane is introduced under pressure .

During extraction, the hydrocarbons of the oil and the oxygenated compounds dissolve in the alcohol. Each fraction must then be concentrated to remove the solvent. This is done by fractional distillation. The oils produced this way are free from monoterpene and sesquiterpene hydrocarbons. In their original report, Dijck and Ruys showed that this technique could be used to successfully deterpenify lemon, orange, and ginger oil (two oils rich in monoterpene hydrocarbons, and one rich in sesquiterpene hydrocarbons (Boucard and Serth, 1991).Each extract was then placed in a separator funnel and the aqueous alcohol plus oxygenated orange oil constituents were separated from the insoluble mono- and sesquiterpene .

2.2.1.4. Distillation of oils

Distillation is the process of separating the component or substances from a liquid mixture by selective evaporation and condensation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that

increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of the mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction .

I. Applications of distillation

Early forms of distillation were batch processes using one vaporization and one condensation. Purity was improved by further distillation of the condensate. Greater volumes were processed by simply repeating the distillation. Chemists were reported to carry out as many as 500 to 600 distillations in order to obtain a pure compound.The application of distillation can roughly be divided in four groups: laboratory scale, industrial distillation, distillation of herbs for perfumery and medicinals (herbal distillate), and food processing .

The latter two are distinctively different from the former two in that in the processing of beverages and herbs, the distillation is not used as a true purification method but more to transfer all volatiles from the source materials to the distillate.The main difference between laboratory scale distillation and industrial distillation is that laboratory scale distillation is often performed batch-wise, whereas industrial distillation often occurs continuously (Hassan, *et al*., 2014).

II. Batch distillation

Heating an ideal mixture of two volatile substances A and B (with A having the higher volatility, or lower boiling point) in a batch distillation setup (such as in an apparatus depicted in the opening figure) until the mixture is boiling results in a vapor above the liquid which contains a mixture of A and B. The ratio between A and B in the vapor will be different from the ratio in the liquid: the ratio in the liquid will be determined by how the original mixture was prepared, while the ratio in the vapor will be enriched in the more volatile compound (Kravchenko, 2014).

The vapor goes through the condenser and is removed from the system. This in turn means that the ratio of compounds in the remaining liquid is now different from the initial ratio (i.e., more enriched in B than the starting liquid).The result is that the ratio in the liquid mixture is changing, becoming richer in component B. This causes the boiling point of the mixture to rise, which in turn results in a rise in the temperature in the vapor, which results in a changing ratio of A : B in the gas phase (as distillation continues, there is an increasing proportion of B in the gas phase). This results in a slowly changing ratio A:B in the distillate.If the difference in vapor pressure between the two components A and B is large (generally expressed as the difference in boiling points), the mixture in the

beginning of the distillation is highly enriched in component A, and when component A has distilled off, the boiling liquid is enriched in component B (Hassan *et al*., 2014)

III. Continuous distillation

Continuous distillation is an ongoing distillation in which a liquid mixture is continuously (without interruption) fed into the process and separated fractions are removed continuously as output streams occur over time during the operation. Continuous distillation produces a minimum of two output fractions, including at least one volatile distillate fraction, which has boiled and been separately captured as a vapor, and then condensed to a liquid. There is always a bottoms (or residue) fraction, which is the least volatile residue that has not been separately captured as a condensed vapor (Holmyard, *et al*., 1990).

Continuous distillation can be run at a steady state for an arbitrary amount of time. For any source material of specific composition, the main variables that affect the purity of products in continuous distillation are the reflux ratio and the number of theoretical equilibrium stages, in practice determined by the number of trays or the height of packing. Reflux is a flow from the condenser back to the column, which generates a recycle that allows a better separation with a given number of trays. Equilibrium stages are ideal steps where compositions achieve vapor–liquid equilibrium, repeating the separation process and allowing better separation given a reflux ratio. A column with a high reflux ratio may have fewer stages, but it refluxes a large amount of liquid, giving a wide column with a large holdup. Conversely, a column with a low reflux ratio must have a large number of stages, thus requiring a taller column (Kravchenko, *et al*., 2011).

2.2.1.5. Types of distillation

I. Industrial Distillation

Large scale industrial distillation applications include both batch and continuous fractional, vacuum, azeotropic, extractive, and steam distillation. The most widely used industrial applications of continuous, steady-state fractional distillation are in petroleum refineries, petrochemical and chemical plants and natural gas processing plants .

II. Laboratory scale distillation

Laboratory scale distillations are almost exclusively run as batch distillations. The device used in distillation, sometimes referred to as a still, consists at a minimum of a re-boiler or pot in which the source material is heated, a condenser in which the heated vapor is cooled back to the liquid state, and a receiver in which the concentrated or purified liquid, called the distillate, is collected.

III. Simple Distillation

In simple distillation, the vapor is immediately channeled into a condenser. Consequently, the distillate is not pure but rather its composition is identical to the composition of the vapors at the given temperature and pressure. That concentration follows Raoult's law. As a result, simple distillation is effective only when the liquid boiling points differ greatly (rule of thumb is 25 °C) or when separating liquids from non-volatile solids or oils. For these cases, the vapor pressures of the components are usually different enough that the distillate may be sufficiently pure for its intended purpose (Harmandar et al.,2009)

IV. Steam distillation

Steam distillation is a method for distilling compounds which are heat-sensitive. The temperature of the steam is easier to control than the surface of a heating element, and allows a high rate of heat transfer without heating at a very high temperature. This process involves bubbling steam through a heated mixture of the raw material. By Raoult's law, some of the target compound will vaporize (in accordance with its partial pressure). The vapor mixture is cooled and condensed, usually yielding a layer of oil and a layer of water. Steam distillation of various aromatic herbs and flowers can result in two products; an essential oil as well as a watery herbal distillate. The essential oils are often used in perfumery and aromatherapy while the watery distillates have many applications in aromatherapy, food processing and skin care.

V. Short Path Distillation

Distillation technique that involves the distillate travelling a short distance, often only a few centimeters, and is normally done at reduced pressure.A classic example would be a distillation involving the distillate travelling from one glass bulb to another, without the need for a condenser separating the two chambers. This technique is often used for compounds which are unstable at high temperatures or to purify small amounts of compound. The advantage is that the heating temperature can be considerably lower (at reduced pressure) than the boiling point of the liquid at standard pressure, and the distillate only has to travel a short distance before condensing.

VI. Zone distillation

Zone distillation is a distillation process in long container with partial melting of refined matter in moving liquid zone and condensation of vapor in the solid phase at condensate pulling in cold area. The process is worked in theory. When zone heater is moving from

the top to the bottom of the container then solid condensate with irregular impurity distribution is forming. Then most pure part of the condensate may be extracted as product. The process may be iterated many times by moving (without turnover) the received condensate to the bottom part of the container on the place of refined matter. The irregular impurity distribution in the condensate (that is efficiency of purification) increases with number of repetitions of the process (Kravchenko, 2014).

VII. Azeotropic distillation

Interactions between the components of the solution create properties unique to the solution, as most processes entail nonideal mixtures, where Raoult's law does not hold. Such interactions can result in a constant-boiling azeotrope which behaves as if it were a pure compound (i.e., boils at a single temperature instead of a range). At an azeotrope, the solution contains the given component in the same proportion as the vapor, so that evaporation does not change the purity, and distillation does not affect separation traditional.

For example, ethyl alcohol and water form an azeotrope of 95.6% at 78.1 °C. If the azeotrope is not considered sufficiently pure for use, there exist some techniques to break the azeotrope to give a pure distillate. Some techniques achieve this by "jumping" over the azeotropic composition (by adding another component to create a new azeotrope, or by varying the pressure). Others work by chemically or physically removing or sequestering the impurity. For example, to purify ethanol beyond 95%, a drying agent (or desiccant, such as potassium carbonate) can be added to convert the soluble water into insoluble water of crystallization.

VIII. Freeze Distillation

Freeze distillation is an analogous method of purification using freezing instead of evaporation. It is not truly distillation, but a recrystallization where the product is the mother liquor, and does not produce products equivalent to distillation. This process is used in the production of ice beer and ice wine to increase ethanol and sugar content, respectively. It is also used to produce applejack. Unlike distillation, freeze distillation concentrates poisonous congeners rather than removing them; As a result, many countries prohibit such applejack as a health measure. However, reducing methanol with the absorption of 4A molecular sieve is a practical method for production. Also, distillation by evaporation can separate these since they have different boiling points Energy (Forbes, R. J. 1970).

2.2.1.6. Steps of distillation

The distillation process includes five steps as follows (Kumar and Tripathi, 2011):

I. Boiling

The boilling ispoint of a pure liquid is defined as the temperature at which the vapour pressure of the liquid exactly equals the pressure exerted on it by the atmosphere, and is one of its characteristic physical properties.

II. Collection

The gas of the more-volatile substance is carried away so that it can't mix in with the remaining liquid. It may then be condensed and cooled back into a separate liquid.

III. Purification

Distillation would not produce components of extremely high purity by itself. This requires to be performed on the distilled product.

2.2.1.7. Identification techniques of extracts components

I. Phytochemical screening

The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function .

In all these operations, methods are needed for separation, purification and identification of the many different constituents present in plants. Thus, advances in understanding of phytochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. It has been estimated, for example, that there are now over 5500 known plant alkaloids and the pharmacological interest in novel alkaloids that new are being discovered and described.

While phytochemical procedures have today an established all branches of plant science. Phytochemical methods are essential in all chemical and biochemical studies, their application in more strictly biological spheres have only come within the last decade. Even in disciplines remote from the chemical laboratory as systematic, phytogeography, ecology and palaeobotany, phytochemical methods have become important for solving

certain types of problems and will undoubtedly be used with increasing frequency in the future .

II. Chromatographic methods

The separation and purification of plant constituents is mainly carried out using one or other, or a combination, of three chromatographic techniques. The choice of technique depends largely on the solubility properties to be separated:

a. Paper chromatography(PC)

PC is particularly applicable to water soluble plant constituents, namely carbohydrates, amino acids, nucleic acid bases, organic acids and phenolic compounds

b. Thin layer chromatography (TLC)

TLC is the method of choice for separating all lipid soluble components, i.e. lipids, steroids, carotenoids, simple quinones and chlorophylls. The best method of separating and identifying simple 'phenolics' is by TLC. They are normally detected after acid or alkaline hydrolysis of plant tissue (fresh or dried) or of a concentrated aqueous-alcoholic plant extract. For preparative work, TLC is carried out on thick layers of adsorbent and PC on thick sheets of filter paper (Seader, *etal* 1998).

Acid hydrolysis is carried out with 2M HCI for 0.5 hr, the resulting solution being cooled and filtered before extraction. Alkaline hydrolysis is carried out with 2M NaOH at room temperature under nitrogen for 4 hr, acidification being necessary before extraction(Ahmed, *etal*., 2004).In either case, phenols are taken into ether and the ether extract washed, dried and evaporated to dryness. The residue, dissolved in ether, is then on silica gel in acetic acid - chloroform and ethyl acetate-benzene and separately on cellulose M N 300 in benzene-methanol-acetic acid and aqueous acetic acid. Typical Rf values for the common phenols and phenolic acids in these four solvents (Compositae, *etal* 1977).

c. Gas liquid chromatography (GLC)

By contrast, the third technique GLC finds its main application with volatile compounds, fatty acids, monosesquiterpenes, and sulphur compounds. However, the volatility of higher boiling plant constituents can be enhanced by converting them to esters and/or trimethylsilyl ethers so that there are few classes which are completely unsuitable for GLC separation.

d. Gas chromatography – Mass spectroscopy

Gas chromatography is a technique for separating volatile substance by percolating a gas stream over a stationary phase. This depends upon the adsorptive properties of the

column packing to separate sample. 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 .

Library search algorithms are commonly provided with mass spectrometer data systems with the purpose to assist in the identification of unknown compounds (Grob, and Barry, 2004).

High performance liquid chromatography (HPLC)

Even if chromatographic methods, such as HPLC, are used for analysis, the mono- and oligosaccharides must usually be separated from other components of the food before chromatography. Thus, for determination of any mono- (glucose, fructose), di- (sucrose, lactose, maltose), tri- (raf nose), tetra- (stachyose), or other oligomaltodextrins saccharides present, the dried, lipid-free sample is extracted with hot 80% ethanol (final concentration) in the presence of precipitated calcium carbonate to neutralize any acidity Higher oligosaccharides from added malto or fructooligosaccharides also may be extracted. Carbohydrates are soluble in polar solvents. However, much of the composition of a food (other than water) is in the form of polymers, and almost all polysaccharides and proteins are insoluble in ethanol 80% (AOAC, 1999.).

III. Spectrophotometric methods

Food raw materials and products and some ingredients are complex, heterogeneous, biological materials. Thus, it is quite likely that they may contain substances that interfere with measurement of the mono- and oligosaccharides present, especially if spectrophotometric method is used. Interference may arise either from compounds that absorb light of the same wave-length used for the carbohydrate analysis or from insoluble, colloidal material that scatters light, since light scattering will be measured as absorbance. Also, the aldehyde or ketone group of the sugar can react with other components, especially amino groups of proteins, a reaction that simultaneously produces color and destroys the sugar (Kumar *etal*, 2011).

IV. Mass Spectroscopy (MS)

MS, since its relatively recent introduction has revolutionized biochemical research on natural products and has eased the task of the phytochemist in many ways. The value of the technique is that it requires only microgram amounts of material, that it can provide an accurate molecular weight and that may yield a complex fragmentation pattern which is often characteristic. In essence, consists of degrading trace amounts of an organic compound and recording the fragmentation pattern according to mass . The sample

vapour diffuses into the low pressure system of the mass spectrometer where it is ionized with sufficient energy to cause fragmentation of the chemical bonds.

The resulting positively charged ions are accelerated in a magnetic field which disperses and permits relative abundance measurements of ions of given mass-to-charge ratio. The result versus mass constitutes the mass spectral graph, which thus consists of a series of lines of varying intensity at different mass units (Kumar and Tripathi, 2011). In many cases some of the parent compound will survive the vaporization process and will be recorded as a parent ion peak. Very accurate mass measurements (to 0·0001 mass units) can then be performed on this (and any other) particular ions. The accuracy is such to indicate the exact molecular formula of the substance, so that conventional elemental analysis (which normally requires several milligrams of substance) is no longer necessary (Compositae, *etal*, 1977).

V. Infrared Spectroscopy (IR)

IR spectra may be measured on plant substances in an automatic recording IR spectrophotometer either in solution (in chloroform or carbon tetrachloride (1-5 %), as a mull with nujol oil or in the solid state, mixed with potassium bromide. In the latter case, a thin disc is prepared under anhydrous conditions from a powder containing about I mg of material and 10 to 100 mg KBr, using a mould and press. The range of measurement is from 4000 to 667 cm-1 (or 2·5 to 15p.) and the spectrum takes about three minutes to be recorded.

The region in the IR spectrum above 1200 cm-1 shows spectral bands or peaks due to the vibrations of individual bonds or functional groups in the molecule under examination. The region below 1200 cm-1 show bands due to the vibrations of the whole molecule and, because of its complexity, is known as the 'fingerprint' region. Intensities of the various bands are recorded subjectively on a simple scale as being, strong (S), medium (M) or weak (W) . The fact that many functional groups can be identified by their characteristic vibration frequencies makes. Infrared Spectroscopy spectrum is the simplest and often the most reliable method of assigning a compound to its class. IR spectroscopy is most frequently used in phytochemical studies as a 'fingerprinting' device (Kumar and Tripathi, 2011).

2.2.1.8. Chemistry of essential oils

Essential oils consist of a complex mixture of hydrocarbons, alcohols, esters, and carbonyl compounds. Some of these materials are called terpenes. Essential oils, as a group, do not need to have any specific chemical properties in common, beyond conveying characteristic fragrances (Rebey, *et al*., 2014). Extracted oils are complex

mixtures of sometimes hundreds of chemical compounds. Pure essential oils can be essentially classified into two groups .

I. Volatile fraction

Essential oil constituting of 90–95% containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

II. Nonvolatile residue

It comprises 1–10% of the oil containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoid.Most of these compounds can be grouped into a few major classes but there are also many components of essential oils that bear little resemblance to these classes. In the overview of important and characteristic components given below, compounds are classified into four major groups: aliphatic compounds, terpenes and terpene derivatives, benzene derivatives and miscellaneous compounds .

a. Hydrocarbon

Essential oils consist of chemical compounds with hydrogen and carbon building blocks. Basic hydrocarbon found in plants is isoprene.

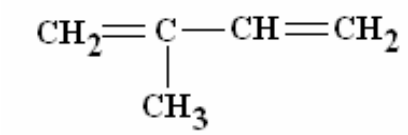


Figure (2-1): Isoprene structure (Baser and Demirci, 2007)

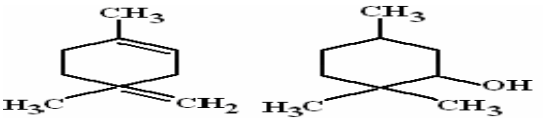
b. Terpenes

The terpenes are the most important. For examples: limonene, pinene, piperene, camphene, etc. Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and Diterpenes, referring back to isoprene units under the hydrocarbon heading, when two isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes .

c. Monoterpenes[$C_{10}H_{16}$]

Monoterpenes are analgesic, bactericidal, expectorant, and Stimulant. Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10). But some of their oxygenated derivatives such as alcohols, Ketones, and carboxylic acids are known as monoterpenoids (Kumar and Tripathi, 2011). Monoterpenes can be cyclic

molecules (Menthol, Monocyclic; Camphor bicyclic; Pinenes (α and β) Pine genera as well.



Limonene **Menthol**

Figure (2-2). Structures of some monoterpenes (Tezel, *et al*., 2000)

d. Sesquiterpenes

Sesquiterpenoids contain 15 carbon atoms and these results lower volatilities and higher boiling points than monoterpenoids (Baser and Demiric, 2007). Sesquiterpenes are anti-inflammatory, antiseptic, analgesic and antiallergic. Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic.Diterpenes: Diterpenes are antifungal, expectorant, hormonal balancers, hypotensive. Diterpenes are made up of four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20 skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones Gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. Diterpenes have limited therapeutical importance and used in certain sedatives .

e. Alcohols

Alcohols are antiseptic, antiviral, bactericidal and germicidal. Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low or totally reaction in the body or on the skin. Therefore, they are considered safe to use .

f. Aldehydes

Aldehydes are antifungal, antiseptic, antiviral, bactericidal, disinfectant and sedative. Medicinally, essential oils containing aldehydes are effective in treating Candida and other fungal infections (Baser and Demiric, 2007).

g. Acids

Organic acids in their free state are generally found in very small quantities within essential oils. Plant acids act as components or buffer systems to control acidity (Council of Europe 1996).

h. Esters

Esters are formed through the reaction of alcohols with acids. Essential oils containing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system .

i. Ketones

Ketones are anticatarrhal, cell proliferant, expectorant, and vulnerary. Ketones often found in plants that are used for upper respiratory complaints because they assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually (not always) very toxic .

j. Lactones

Lactones are known to be particularly effective for, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action their ketones (Rhind, J. P. 2012).

2.2.1.9. Uses of essential oils

Essential oils are used for flavorings, perfumes, and aromatherapy. At different periods in history, essential oils have been used medicinally. Current medical applications range from skin treatments to remedies for cancer, and are oftenbased on historical use of these oils. Medicinal claims, however, are now subject to regulation in most countries, and, as a result, have grown vaguer to stay within these regulations . Use of essential oils has revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in carrier oil and used in massage, diffused in the air by heating over a candle flame, or burned as incense (Damasius, *et al*., 2007).

The most effective way to use most essential oils is by external application or inhalation, though some can be very beneficial when taken internally. The use of essential oils include body oils, compresses, cosmetic lotions, baths, hair rinses, inhalation by steam, perfumes and room sprays. Essential oils are very potent some will cause skin irritation or have other harmful effects if not used properly. Unless specifically noted, it is best to dilute all essential oils in a carrier of base oil like Almond, Jojoba or Apricot Kernel before applying to the skin - appropriate dilution is usually only 1 - 10% essential oil in

carrier. For inhalation, a diffuser or oil lamp is effective for releasing essential oils into your environment - a very pleasant way of creating a particular atmosphere (Amorati, *et al*., 1992).

Essential oils are products of the secondary metabolism of plants, and generally are fragrant volatile materials consisting of complex mixtures of mono- and sesqui-terpene hydrocarbons, and oxygenated materials biogenically derived from them hence essential oils are used in flavourings, perfumes, in Aromatherapy, as insect & animal repellents, in pharmaceutical preparations, as agents and in many other ways (Baser *etal*, 2011).

I. Medicinal use of essential oils

Medicinal use of essential oils is seen as in health care community.Despite the lack of proof essential oils provide medicinal value, they retain considerable popular use among of alternative medicine. Studies have shown that certain essential oils may have ability to prevent the transmission of some drug-resistant strains of pathogen, specifically Staphylococcus , Streptococcus and Candida .

Taken by mouth, many essential oils can be dangerous in high concentrations. Typical effects begin with a burning feeling, followed by salivation. In the stomach, the effect is carminative, relaxing the gastric sphincter .

Typical ingredients for such applications include eucalyptus oils, menthol , capsaicin , anise and camphor. Other essential oils work well in these applications, but it is notable that others offer no significant benefit. This illustrates the fact that different essential oils may have drastically different pharmacology. Those do work well for upper respiratory tract and bronchial problems act variously as mild expectorants and decongestants .

Some essential oils, such as those of juniper and agathosma, are valued for their diuretic effects with relatively recent concerns about the overuse of antibacterial agents many essential oils have seen resurgence in off-label use for such properties and are being examined for this use clinically.

Many essential oils affect the skin and mucous membranes in ways that are valuable or harmful. Many essential oils, particularly tea tree oil, may cause a contact dermatitis

They are used in antiseptics and liniments in particular. Turpentine oil and camphor are two typical examples of oils that cause such effects. Menthol and some others produce a feeling of cold followed by a sense of burning. This is caused by its effect on heat-sensing nerve endings. Some essential oils, such as clove oil or eugenol, were popular for many hundred years in dentistry as antiseptics and local anaesthetics. Thymol is well known for its antiseptic effects (Havsteen BH (2002).

II. Antioxidants of essential oils

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells . These oxidative reactions result in a partial loss of minor constituents, primary cause of the health-promoting effects of oil consumption (Visoli and Galli, 1998). Different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells .

The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which added to products to prevent oxidation, and natural chemicals found in foods and body tissue which said to have beneficial health effects. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g. catalase and superoxide dismutase) produced internally or the dietary antioxidants, vitamin A, vitamin C, and vitamin E (Abdoul-Latif *etal*,1998).

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). In general, antioxidantsprotect cell membranes from lipid oxidation. The relative importance and interactions between these different antioxidants is very complex question, with various metabolites and enzyme systems having synergistic. Food antioxidants are compounds that increase the resistance of fats to oxidation and consequent deterioration or rancidity .

Natural antioxidants have also been shown to effectively reduce oxidative rancidity while providing additional sources of nutrients and flavor. It is important to note that antioxidants can’t be expected to stop rancidity.

Their effectiveness lies only in slowing down the rate of oxidation. Natural antioxidants, such as those found in some spices, such as rosemary, sage, and marjoram, have met acceptance for the retardation of rancidity in meat products (Ornano,, *et al*., 2013). There is evidence that antioxidants may be useful in preventing the deleterious consequences of oxidative stress There is an increasing interest in the protective biochemical function of natural antioxidants found in vegetables, fruits and medicinal herbs, and used for the prevention of oxidative damage caused by oxygen free-radical species .

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and

natural chemicals found in foods and body tissue which are said to have beneficial health effects (Stanojevic*etal*, 2016).

Some studies have demonstrated that the antioxidant potential of an essential oil depends on its composition. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties. Regarding the antioxidant properties, thymol and carvacrol are potentially active phytochemicals. The activity of these natural products is directly related to their phenolic compounds having redox properties and thus plays an important role in neutralizing harmful free radicals42. The antioxidant activity of essential oils is also due to certain alcohol, esters, ketones, aldehyde, and monoterpenes. Essential oils with important scavenging capacity of free radicals may play an important role in some diseases prevention, such as, brain dysfunction, cancer, heart disease, and immune system decline. In facts the disease may result from cellular damage caused by free radicals.

The phenolic compound embraces a wide range of plant substances which possess in common an aromatic ring bearing one or more hydroxyl substituents. Phenolic substances tend to be water-soluble, since they most frequently occur combined with sugar as glycosides and they are usually located in the cell vacuole. Among the natural phenolic compounds, of which over a thousand structures are known, the flavonoids form the largest group but simple monocyclic phenols, phenylpropanoids and phenolic quinones all exist in considerable numbers.

The classic procedure for detecting simple phenols is by means of the intense green, purple, blue or black colours many of them give in solution when 1 % aqueous or alcoholic ferric chloride is added. This procedure, modified by using a fresh aqueous mixture of 1 % ferric chloride and 1 % potassium ferric cyanide, is still used as a general means of detecting phenolic compounds on paper chromatograms. However, the majority of phenolic compounds (and especially the flavonoids) can be detected on chromatograms by their colours or fluorescences in UV light, the colours being intensified or changed by fuming the papers with ammonia vapour. The phenolic pigments are visibly coloured and they are thus particularly easily monitored during their isolation and purification. Phenolic compounds are all aromatic, so that they all show intense absorption in the UV region of the spectrum (Ayoughi,*etal* 2011).

III. Bioavailability of essential oils

The term bioavailability, one of the principal pharmacokinetic properties of drugs, is used to describe the fraction of an administered dose of unchanged drug that reaches the systemic circulation and can be used for a specific function and/or stored. By definition,

when a drug is administered intravenously, its bioavailability is 100%. However, when a drug is administer via other routes (such as oral), it has to pass absorption and metabolic barriers, before it reaches the general circulation system, and its bioavailability is prone to decrease (due to gastro-intestinal metabolism, incomplete absorption or first-pass metabolism) (Zare-Shehneh *etal*, 2014).

Bioavailability is measured by pharmacokinetic analysis of blood samples taken from the systemic circulation and reflects the fraction of the drug reaching the systemic circulation. If a compound is poorly absorbed or extensively metabolized beforehand, only a limited fraction of the dose administered will reach the systemic circulation. Thus, in order to achieve a high bioavailability, the compound must be of sufficiently high absorption and of low renal clearance (measurement of the renal or other organ excretion ability). Various factors can affect bioavailability such as biochemical, physiological, physicochemical interactions; habitual mix of the diet; individual characteristics (life-stage and life-style) as well as the genotype (Das, M. 2015).

In the case of essential oils, the comprehension of their bioavailability by studying their absorption, distribution, metabolism and excretion in the human body is necessary. Unfortunately, there exists only limited data on the bioavailability of essential oils, and most studies are based on animal models. All findings confirm that most essential oils are rapidly absorbed after dermal, oral, or pulmonary administration and cross the blood-brain barrier and interact with receptors in the central nervous system, and then affect relevant biological functions such as relaxation, sleep, digestion.

Most essential oil components are metabolized and either eliminated by the kidneys in the form of polar compounds following limited phase I enzyme metabolism by conjugation with glucuronate or sulfate, or exhaled via the lungs as CO_2. For example, after oral administration of (-)-menthol, 35% of the original menthol content was excreted renally as menthol glucuronide .The same happens with thymol, carvacrol, limonene and eugenol. After their oral administration, sulphate and glucuronide forms have been detected in urine and in plasma respectively.The fast metabolism and short half-life of active compounds have led to the belief that there is aminimum risk of accumulation in body tissues.

So far, there is no study that can give us a clear idea and be accurate on the mode of action of the essential oils. Given the complexity of their chemical composition, everything suggests that this mode of action is complex, and it is difficult to identify the molecular pathway of action. It is very likely that each of the constituents of the essential oils has its own mechanism of action

2.2.1.9.1. Antimicrobial activity of essential oil

I. Antibacterial action

Because of the variability of amounts and profiles of the components of essential oils, it is likely that their antimicrobial activity is not due to a single mechanism, but to several sites of action at the cellular level. Then, different modes of action are involved in the antimicrobial activity of essential oils.One of the possibilities for action is the generation of irreversible damage to the membrane of bacterial cells, that induce material losses (cytoplasmic), leakage of ions, loss of energy substrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore toits death (Farhoudi, R. 2013).

Another possibility of action is inhibition of production of amylase and protease which stop the toxin production, electron flow and result in coagulation of the cell content (Pasqua, *et al*., 2007).

II. Antifungal action

Antifungal actions are quite similar to those described for bacteria. However, two additional phenomena inhibiting the action of yeast are worth mentioning: the establishment of a pH gradient across the cytoplasmic membrane and the blocking of energy production of yeasts which involve the disruption of the bacterial membrane (Darughe, *et al*., 2012).

III. Antiviral activity

The complex mixture of essential oils usually shows a higher antiviral activity than individual compounds (due probably to synergism phenomena); with exception of β-caryophyllene which is the most famous antiviral compounds found in many different essential oils from different plant families. Different mechanisms of antiviral activity of different essential oils and their constituents seem to be present. The antiviral activity of the essential oil is principally due to direct virucidal effects (by denaturing viral structural proteins or glycoproteins). Proposed mechanisms suggest that essential oils interfere with the virus envelope by inhibiting specific processes in the viral replication cycle or bymasking viral components, which are necessary for adsorption or entry into host cells, thus, they prevent the cell-to-cell virus diffusion (Ates, etal. 2003)..

2.3. Edible Oils

2.3.1. Characterization of edible oils

Edible oil is a triglyceride extracted from a plant. The term “edible oil " can be narrowly defined as referring only to substances that are liquid at room temperature, or broadly

defined without regard to a substance's state of matter at a given temperature. For this reason edible oil that are solid at room temperature are sometimes called edible fats. Edible oils are composed of triglycerides, as contrasted with waxes which lack glycerol in their structure. Although many plant parts may yield oil, in commercial practice, oil is extracted primarily from seeds. Plant and animal or synthetic fat used for frying, baking, and other types of edible. It is also used in food preparation and flavoring not involving heat, such as salad dressings and bread dips, and in this sense might be more accurately termed edible oil. There are a wide variety of cooking oils from plant sources such as olive oil, palm oil, soybean oil, canola oil, corn oil, peanut oil and other vegetable oils, as well as animal-based oils like butter and lard. Oil can be flavored with aromatic foodstuffs such as herbs, chillies or garlic .

Seed oils are important sources of nutritional oils, industrial raw materials and nutraceuticals. The characteristics of oils from different sources depend mainly on their compositions; no oil from a single source can be suitable for all purposes thus the study of their constituents is important.

Many consumers are looking for variety intheir diets and aware of the health benefits of fresh fruits and vegetables and of special interest are food sources rich in antioxidants (Halliwell, *et al*., 1995)

Oils in the diet are available to the body as fatty acids. Fatty acids, both free and as part of complex lipids, play a number of key roles in metabolism as major metabolic fuel (storage and transport of energy), as essential components of all membranes, and as gene regulators.The total energy intake from oils for a normal healthy adult is approximately 30 energy percent and that in the western diets is about 40 energy percent. High fat diets enhance the incidence of coronary heart disease .

2.3.2. General properties

2.3.2.1. Physical properties

The common physical properties of such oils are that they float on water but are in soluble in it; they are greasy to touch, and have lubricating properties; they are not readily volatile and may be burned without leaving any residue.Physical properties of oil according to colour, refractive index and viscosity play an important role in determining the quality of any oil because they give physical specification for description of the oil (Choe E. And Min D. 2006).

I. Specific Gravity (SG)

Specific gravity of oil is the ratio of the weights of given volume of oil to that of equal volume of distilled water same temperature, all weighting being done in absence of air

and it is expressed without units. Less than SG 1.0 floats on water, greater than SG 1.0 sinks in water, majority of oils “float on water”. In general, specific gravity of spilled oil will increase over time .

This physical property is an important criterion for quality and purity of an essential oil. For determining the specific gravity accuracy to at least the third decimal place is necessary used (Kumar and Tripathi, 2011).

II. Refractive index

Refractive index is defined as a ratio of the sin of the angle of incident to the sin of the angle or refraction when a ray of light of defined wavelength passes from air to the material kept at constant temperature. For measuring refractive index of oil, usually refractometer is used (Kumar and Tripathi, 2011). Usually refractive index reading is taken at 270 0C except for those, which are not liquid at this temperature, in which case a high temperature (300oC) depending on the melting point of the material shall be used.

III. Viscosity

Viscosity is defined as the resistance of liquid to flow. High viscosity implies a high resistance to flow while a low viscosity indicates a low resistance to flow. Viscosity is "thickness" or "internal friction". Thus, water is "thin", having a lower viscosity, while honey is "thick", having a higher viscosity. Put simply, the less viscous the fluid is, the greater its ease of movement (fluidity). One of the most common instruments for measuring kinematic viscosity is the glass capillary viscometer and decreasing temperature increases viscosity .

IV. The color

Color of oil comes from natural coloring matters; carotene, xanthophyll and chlorophyll. Exposing plant leaves to hot air drying may cause quality degradation due to color reactions and decomposition of active ingredients . Color, flavor and texture are key factors in food acceptability. Additionally, color may be used to evaluate composition and chemical changes in foodstuffs, being one of the indicators of product quality .

Color is measured quantitatively using such equipment as spectrophotometers or tristimulus colorimeters. Usually the color is quantified using the units proposed by the Commission International Eclairage (CIE) (Calvo, 2004). Color is quite stable for the oil stored in darkness, as one would expect from visual inspection of the respective absorption spectra. On the other hand, the colour parameters of the samples stored under visible irradiation change continuously and almost monotonously during the storage period (Choe E. And Min D. (2006).

2.3.2.2. Chemicals properties

I. Rancidity

Fats undergo changes during storage which result in the production of taste and odor, which is commonly referred to as rancidity is brought about by the action of air (oxidative rancidity) or by microorganisms (ketonic rancidity) (Weybridge and Surry, (1970). Oxidation a free radical process, the double bonds of an unsaturated fatty acid can undergo cleavage, .There are some factors influencing fat oxidation .

a. Temperature

The rate of fat oxidation is highly depends on temperature. Considerable improvement in storage stability can therefore be gained by lowering the storage temperature76- 87. As an example, it has been found that the storage time for frozen raw, lean meat can be extended approximately by 3 times by lowering the temperature from -15 to -25°C.

b.Oxygen

Oxygen in the air may be displaced by an inert gas such as nitrogen or carbon dioxide to retard oxidative rancidity, or the products may be packed under vacuum. These methods require the use of packaging materials with low oxygen permeability (Kumar and Tripathi, 2011).

c. Type of fat

In general, the softer the fat, the more unsaturated are the fatty acids and the more susceptible they are to oxidation and oxidative rancidity. However, vegetable fats, although unsaturated, are usually more stable than animal fats because they contain natural antioxidants. The most common antioxidant found in vegetable fats is vitamin E.

d. Light

Packages that exclude light can be used to protect the products against fat oxidation.

e. Metals

Metals such as cupper, iron, manganese, and chromium increase rate of fat oxidation. As a result, the preferred storage containers are steel drums, tin, or nonmetallic materials such as plastic.

h. Products from fat oxidation

Traces of oxidized fat in ingredients can accelerate oxidative rancidity in the remainder of the products. Steam treatment under vacuum conditions has been effective in removing products of deterioration (odorous substances) from some oils and

fats.Depending on the type of oil, its age, storage conditions, etc., peroxide value (Mishra,*etal*., 2012)

III. Peroxide value (PV)

PV measures formation by determining the amount of iodine liberated from their reaction with potassium iodide and expressing the result in milliequivalents per kilogram (meq/kg). Hydroperoxides are further oxidized to aldehydes and ketones which are responsible for the changes in odor and flavor of rancid fats . The peroxide number gives information about the number of peroxide compounds in the oil and hence of the age and quality of the edible oil. The lower of peroxide numbers the better and/or newer the oil. The peroxide value test is a commonly requested test used to measure the stability of various food products including pet foods, feeds, and human foods (AOAC, 1999).

The peroxide value is the primary measurement of oils rancidity and it gives us an idea of oils' freshness and storage conditions. It is a commonly used as indicator of the shelf life of a product because an elevated peroxide value will accompany disagreeable odors. It is expected that fresh and well processed oils should show peroxides value less than 12 . If a fat has a peroxide value of < 40 meq/kg and does not smell rancid, it is most likely in the initial stages of oxidation and can readily be used in feed ration. If the peroxide value is > 40 meq/kg and the fat smells rancid, it is likely in its later stages of oxidation (IFRA, 2011).

Oil suitability for most uses depends on its quality and chemical composition. several tests can be used to determine oil purity, such as the determination of triacylglycerols, sterols and other constituents of the unsaponifiable fraction of edible oils are characterized by a wide range of physical and chemical properties, since their composition depend on the type of oil . The edible oils have in their composition fatty acids and triglycerides. Fatty acids are straight chain aliphatic compounds terminated with a COOH group and triglycerides are esters of the propane 1,2,3 triol with three fatty acid residues .

The most important compounds of edible oils as sunflower oil or olive oil are the saturated fatty acids c12:0 (lauric), c16:0 (palmitic), c18:0 (stearic) and the unsaturated fatty acids c18:1 (oleic), c18:2 (linoleic, c18:3 (linolenic).

the two conjugated double bonds in linoleic acid are more susceptible to oxidation than a single double bond in oleic acid. the oils with a rich content of oleic acid is expected to have longer lifetime than oil with a large content of linoleic acid. oxidation slightly reduced content of oleic acid in the olive oil and an increase of saturated constituents

(palmitic and stearic acid). in the sunflower oil, oxidation induced more pronounced compositional changes: contents of linoleic and linolenic acids dropped while at the same time concentration of oleic acid increased.

It is well known that auto-oxidation developed during the storage and heating, is considered as a major cause for degradation of edible oil.

to assess oxidative state of oils can be used the spectroscopic measurements (IR, classical transmission) or gas chromatography (GC) analysis, degradation kinetics of chlorophyll and changes in composition of fatty acids profiles may be selected as parameters to monitor in oils .

2.3.3. Vegetable of edible oil

The essential oils that were used in cooking process are; olive, palm, soybean, canola (rapeseed), pumpkin seed, corn, sunflower, peanut, grape, gsesame, gargan oil. In our country the common use is sunflower oil. A vegetable oil is a triglyceride extracted from a plant such oils have been part of human culture for millennia. The term "vegetable oil" can be narrowly defined as referring only to substances that are liquid at room temperature, or broadly defined without regard to a substance's state of matter at a given temperature. For this reason, vegetable oils that are solid at room temperature are sometimes called vegetable fats. Vegetable oils are composed of triglycerides, as contrasted with waxes which lack glycerin in their structure. Although many plant parts may yield oil, in commercial practice, oil is extracted primarily from seeds (*Habib,etal*, 1986).

2.3.3.1. Production of vegetable oils

To produce vegetable oils, the oil first needs to be removed from the oil-bearing plan components, typically seeds. This can be done via mechanical extraction using an oil mill or chemical extraction using a solvent. The extracted oil can then be purified and if required, refined or chemically altered .

Oils can also be removed via mechanical extraction, termed "crushing" or "pressing." this method is typically used to produce the more traditional oils (e.g., olive coconut etc.), and it is preferred by most health food customers in the united states and in Europe. There are several different types of mechanical extraction: expeller-pressing extraction is common, though the screw press, ram press, and ghani (powered mortar and pestle) are also used. Oil seed presses are commonly used in developing countries, among people for whom other extraction methods would be prohibitively expensive; the ghani is primarily used in India (Eunkyakiat, *etal* 2006).

The processing of vegetable oil in commercial applications is commonly done by chemical extraction, using solvent extracts, which produces higher yields and is quicker and less expensive. The most common solvent is petroleum-derived hexane. This technique is used for most of the "newer" industrial oils such as soybean and corn oils. Supercritical carbon dioxide can be used as a non-toxic alternative to other solvents .

The oil is heated under vacuum to near the smoke point, and water is introduced at the bottom of the oil. The water immediately is converted to steam, which bubbles through the oil, carrying with it any chemicals which are water-soluble. The steam sparging removes impurities that can impart unwanted flavors and odors to the oil.

Differ in two major ways from other oils which are equally saturated. During hydrogenation, it is easier for hydrogen to comeinto contact with the fatty acids on the end of the triglyceride, and less easy for them to come into contact with the center fatty acid. This makes the resulting fat more brittle than tropical oil; soy margarines are less "spreadable". The other difference is that trans-fatty acids (often called trans-fat) are formed in the hydrogenation reactor, and may amount to as much as 40 percent by weight of a partially hydrogenated oil. Hydrogenated oils, especially partially hydrogenated oils with their higher amounts of trans-fatty acids are increasingly thought to be unhealthy (Toshiyuki, 1999) .

Oils may be partially hydrogenated to produce various ingredient oils. Lightly hydrogenated oils have very similar physical characteristics to regular soya oil, but are more resistant to becoming rancid. Hardening vegetable oil is done by raising a blend of vegetable oil and a catalyst in near-vacuum to very high temperatures, and introducing hydrogen. This causes the carbon atoms of the oil to break double-bonds with other carbons, each carbon forming a new single-bond with a hydrogen atom. Adding these hydrogen atoms to the oil makes it more solid, raises the smoke point, and makes the oil more stable.

2.3.4. Constituents of edible oils

The constituents of edible oils can be grouped into the saponifiable (triacylglycerols, free fatty acids, phosphatides) and the unsaponifiable (hydrocarbons, fatty alcohols, etc.) fractions. The unsaponifiable fraction accounts. In general, 0.5–1.5 % of the oils .

2.3.4.1. Saponifiable Fraction

The Saponifiable fraction accounts for 98.5–99.5 % of oils. The major part of this fraction are triacylglycerols and free fatty acids (Heidelberg, 2012), derivatives such as mono and diacylglycerols, phospholipids, waxes and sterol esters are also found.

2.3.4.2. Triacylglycerols

These compounds comprise 98–99 % of the oils. They are esters derived from the union of glycerol (1,2,3-propanetriol) and fatty acids generally, the fatty acids at the central position of the glycerol molecule are generally unsaturated, although saturated acids can be found at this position when the total concentration of saturated fatty acids in the oil is very high. The most abundant triacylglycerols found in olive oil are ooo (43.5 %), poo (18.4 %), ool (6.8 %), pol (5.9 %) and soo (5.1 %) (Being o: oleic acid; p: palmitic acid; s: stearic acid and l: linoleic acid) .

2.3.4.3. Mono and diacylglycerols

Jointly with triacylglycerols, edible oils also contain partial glycerols such as mono and diacylglycerols, comprising 0.2 and 1.3 % of total fatty acids, respectively. Their present in an olive oil is an index of low quality. For this reason, their determination is often used as an oil quality marker.

2.3.4.4. Free Fatty Acids

Their proportion in the oil depends on the hydrolysis degree of triacylglycerols, being their composition variable according to the botanical variety of oil, or, in the case of olive oil, according to the genetic variety, climatic conditions, fruit maturity and geographical origin of olives (Aparicio, *et al*., 1994; Boskou, 2002; Imperio, *et al*., 2007; Kotsifaki and koutsaftakis 1999). Major fatty acids in olive oils are oleic (55–85 %), palmitic (7.5–20 %), linoleic (7.5–20 %), stearic (0.5–5 %), palmit- oleic (0.3–3.5 %) and linolenic (0.0–1.5 %) acids, although traces of myristic, arachidic and margaric acids could be also found (Kohno, *etal* 2004).

2.3.4.5. Phospholipids

Phospholipids are found in small quantities in freshly produced olive oils (40–135 mg/kg), being their concentration lower with oil aging. The most important phospholipids in olive oil are phosphatidyl choline, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine .

2.3.4.6. Waxes

These compounds are esters of fatty alcohols with fatty acids. The main waxes detected in olive oils have a high and even carbon number, in particular, c36– c46 esters. Their amount is very low, not exceeding 35 mg/100 g (Pacheco-Palencia, etal 2008).

2.3.5. Usage of edible oil

Many vegetable oils are consumed directly or indirectly as ingredients in food a role that they share with some animal fats. Vegetable oils are used as an ingredient or component in many manufactured products. Many vegetable oils are used to make soaps, skin products, candles, perfumes and other personal care and cosmetic products. Some oils

are particularly suitable as drying oils, and are used in making paints and other wood treatment products. Dammar oil (a mixture of linseed oil and dammar resin), for example is used almost exclusively in treating the hulls of wooden boats. Vegetable oils are increasingly being used in the electrical industry as insulators.

Vegetable oil is used in production of some pet foods. In some poorer grade pet foods though, the oil is listed only as "vegetable oil", without specifyingthe particular oil. Vegetable oils are also used to make biodiesel, which can be used like conventional diesel. Some vegetable oil blends are used in unmodified vehicles but straight vegetable oil, also known as pure plant oil, needs specially prepared vehicles which have a method of heating the oil to reduce its viscosity. The vegetable oil economy is growing and the availability of biodiesel around the world is increasing. it is believed that the total net greenhouse gas savings when using vegetable oils in place of fossil fuel- based alternatives for fuel production, range from 18 to 100% .

margarine originated with the discovery by french chemist michel eugene chereul in 1813 of margaric acid (itself named after the pearly deposits of the fatty acid from greek (margaritēs / márgaron), meaning pearl-oyster or pearl, or (margarís), meaning palm-tree, hence the relevance to palmitic acid). Scientists at the time regarded margaric acid, like oleic acid and stearic acid, as one of the three fatty acids which, in combination, formed most animal fats. In 1853, the german structural chemist wihelm heinrich heintz analyzed margaric acid as simply a combination of stearic acid and of the previously unknown palmitic acid. Emperor Louis napoleon of France offered a prize to anyone who could make a satisfactory substitute for butter, suitable for use by the armed forces and the lower classes. French chemist hippolyte megemouries invented a substance he called oleomargarine, the name of which became shortened to the trade name "margarine".mege-mouries patented the concept in 1869 and expanded his initial manufacturing range of functionalities (Devon,*et al*., 1972).

2.3.6. Negative Health Effects of edible oils

Hydrogenated oils have been shown to cause what is commonly termed the "double deadly effect", raising the level of low density lipoproteins and decreasing the level of high density lipoproteins in the blood, increasing the risk of blood clotting inside blood vessels.

5 a high consumption of omega-6 polyunsaturated fatty acids, which are found in most types of vegetable oil (e.g. soyabean oil, corn oil– the most consumed in USA, sunflower oil, etc.) may increase the likelihood that postmenopausal women will develop breast cancer. A similar effect was observed on prostate cancer in mice. Plant

based oils high in monounsaturated fatty acids, such as olive oil, peanut oil, and canola oil are relatively low in omega-6 pufas and can be used in place of high polyunsaturated oils (Sharma, *et al*., 2007).

2.4. Sunflower oil

2.4.1. Description of plant

Sunflowers are botanically classified as helianthus annuus. The sunflower plants reach various heights, but most are from 1.52–2.1 m tall. The diameter of the flower heads is relatively large, typically between 7.62 -15.24 cm, although some can measure more than 30 cm. an exception is the dwarf varieties, which are only 0.91–1.22 m high and have smaller flower heads. A common characteristic of sunflowers is a tendency for their flowering heads to follow the movement of the sun during the day. This phenomenon, called heliotropism, has the benefit of reducing damage from birds and preventing the development of disease (Jean, 1994).

Sunflowers are a large plant and are grown throughout the world because of their relatively short growing season. Domesticated sunflowers typically have a single stalk topped by a large flower.This is significantly different from the smaller, multiply branched wild sunflower. During the growing season, the individual flowers are each pollinated. Seed development then begins moving from the outer rim of the flower toward the centre. It generally takes 30 days after the last flower is pollinated for the plant to mature .

2.4.2. Sunflower oil extraction

Sunflower oil is generally extracted in two stages. Initially, a fraction of the oil is mechanically extracted by screw- press expelling. The cake obtained from this pressing stage, containing 15–20% of oil, is later solvent extracted, usually with hexane. Oils obtained by pressing are considered to be of better nutritional quality than those obtained by solvent extraction. However, both fractions are generally blended prior to storage (Skorić *etal* 2008).

2.4.3. Sunflower oil characteristics

Sunflower oil has the following characteristics

- Liquid at room temperature
- Smoke point of refined oil 232 °C.
- Smoke point of unrefined oil 227 °C.
- Density (25 °C): 917 kg/m3.
- Refractive index (25 °c): ≈1.473.

- Viscosity (25 °C, unrefined): 0.04914 kg/(m*s).
- Refined sunflower oil color is pale yellow in color.
- Slightly fatty odor.

2.4.4. Composition of sunflower oil

Sunflower oil contains predominantly linoleic (48–7%), oleic (14–40%), palmitic (4–9%) and stearitic (1–7%) there are several types of sunflower oils produced, such as high linoleic, high oleic and mid oleic. high linoleic sunflower oil typically has at least 69% linoleic acid while high oleic sunflower oil has at least 82% oleic acid. The variation in the unsaturated fatty acids profile is strongly influenced by both genetics and climate

In the last decade, high stearic lines of sunflower oil have been developed in Spain to avoid the use of hydrogenated vegetable oils in the food industry. The conventional sunflower oil (high linoleic) is used for home cooking oil and margarine and for industrial use (paint, etc). The high oleic sunflower oil is used for cosmetics, gasoline blend and other purposes. Sunflower oil also contains lecithin, tocopherols, carotenoids, waxes and high vitamin E content . (Alfred , *et al*., 2002).

2.4.5. Modified properties of sunflower oil

Like other edible vegetable oils, sunflower oil may be chemically modified by hydrogenation or by interesterification with other vegetable and animal fats. A relatively good oxidative stability of regular sunflower oil results from the insignificant content of linolenic acid. To increase the stability of vegetable oils having a higher linolenic acid content, light hydrogenation is required to reduce the level of linolenic acid, while avoiding the formation of significant amounts of trans- isomers. In producer countries, regular sunflower oil is partially hydrogenated for use in the manufacture of shortenings and margarines (Yu Xiuzhu, *et al*.2007).

The behavior of sunflower oil in the hydrogenation process is similar to that of soybean oil, and does not require special reaction conditions. Nor does it contain any compounds that may interfere with the hydrogenation reaction, unlike the case with rapeseed oil. However, the use of partially hydrogenated sunflower oil for the manufacture of margarine spreads may lead to the appearance of graininess during storage, resulting from a strong tendency of partially hydrogenated sunflower oil to formcrystals.

2.4.6. Oxidative stability of commercial sunflower oil

Good oxidative stability results in a good storage quality of sunflower oil and related oilbased products. The oxidation reactions leading to the deterioration of both sunflower

oil and related products during their useful life are the same as for other edible vegetable oils (Toshiyuki, *et al*., 1999).

2.4.7. Uses of sunflower oil

Both regular and high- oleic sunflower oil may be used for the preparation of several food products, although the former is generally used because of the price difference between the two types. Other specific uses of sunflower oil are associated with the particular properties of different sunflower oil types, which differ in oil composition. In countries where sunflower oil is common edible oil, it is mainly used as salad dressing and cooking oil.Industrially, sunflower oil is used in frying applications and in the manufacture of mayonnaise and oil based dressings. Although high- oleic sunflower oil is also suitable for these applications, it is used predominantly as frying oil .

Both continuous and discontinuous deep- fat frying is normally used for the preparation of food products. Discontinuous frying is used according to consumer demand. Frying oil is exposed to the air as it is heated for relatively short and sometimes occasional cooking periods, and most often with infrequent oil replenishment. Continuous frying, used for the industrial processing of fried and pre- fried foods, the steam generated during the frying process constitutes a protective barrier against air exposure and oxidation. Oil decomposition products absorbed with the frying oil by the product affect not only the sensory and nutritional quality but also the useful life of fried foods such products include polar compounds such as triacylglycerol dimers and polymers, di and monoacylglycerols, free fatty acids and peroxidised compounds (Cox, Jeff April 1979).

CHAPTER THREE
Materials and Methods

3.1. Materials

3.1.1. Sampling

3.1.1.1. *Coriandrum sativum* L (coriander)

The agricultural researches authority in Shendi city allocated to the researcher a plot of agricultural land on one of its farm, which was planted with coriander seeds at the end of November 2020 under the supervision of the Agricultural Research Authority. The researcher then mentioned the crop throughout its growth period until harvest in mid-March 2021. The seeds were then collected and dried and grinded. Part of grinded made into powder and the rest of the crop is stored as raw material.

3.1.1.2. Sunflower oil

Edible oil (Sunflower oil) purchased from the Arabian oils company in Khartoum State, Sudan.

3.1.1.3. Bacteria species

Six types of bacteria (three Gram-positive and three Gram-negative) and fungi species were prepared in the laboratory of the faculty of medical laboratories at Shendi university.

3.1.2. Chemicals and reagents

- Acetic acid.
- Chloroform
- Ethyl Acetate
-Petroleum ether
-Ethanol
-Hexane
- Potassium iodide solution.
- Sodium thiosulphate
- Starch solution
- Ethyl alcohol
- Phenolphthalein indicator solution
- Potassium hydroxide.

- Alcoholic potassium hydroxide
- Hydrochloric acid
- Mueller–Hinton agar
- Normal saline
- Gentamicine (Positive control)
- Dimethyl sulphoxide (DMSO)

3.1.3. Equipment

- Standard thermometer
- Conical flasks
- Burette
- Separating funnel
- Propeller Stirrer
- Glass cells
- Needles
- Sterilized swab

3.1.4. Apparatus

- Gas chromatography-mass spectrometer (GC-MS)
- Distillation apparatus (steam distillation -aqueous)
- Lovibond tintometer
- Refractometer Abbe
- Pycnometer
- Balance
- Water bath

3.2. Methods

3.2.1. Extraction

Coriander seeds in powder form are dipped in Six solvents polarity range were used: chloroform (relatively non-polar), ethyl acetate (medium polarity), petroleum ether (non-polar), steam distillation (aqueous), ethanol (polar), and hexane (non-polar).

To obtain Six different solvent extracts using the hot extraction method.

3.2.1.1. Hot extraction

Coriander oil was extracted by Water distillation (aqueous) method. Mature coriander fruits were put into distillation instrument over water, then the water was heated and the steam passed through the coriander fruits and vaporized the volatile compounds. The vapors were flowed through a coil, where they were condensed back to liquid, which then was collected in the receiving vessel. At the end of the distillation process coriander

oil was separated from water depending on the difference in their density by using laboratory separating funnel. The oil of coriander was collected and kept at suitable temperature in dark pure bottle and had been ready for chemical profile analysis and to determine the chemical and physical tests.

3.2.1.2. Cold extraction

Coriander seeds were immersed in two different solvents; polar (ethanol) and non-polar (n-hexane). Then both solvents were subjected to two type of extraction hot and cold. After that two extracts were drying at room temperature and their percentages were calculated using this formula.

3.2.1.3. Calculation of percentage yield

Yield percentage = (Weight of extract x 100) / (Weight of sample)

3.2.2. Centrifugation (Biloia techenque- 2025)

Extracts were centrifuged at 4000–6000 rpm to separate fractions. The supernatant and residue will be collected for further chemical and biological testing.

3.2.3. Analytical techniques

3.2.3.1. Qualitative analysis

I. Phytochemical of extracted coriander oil (Color tests)

Different Phytochemical techniques were used to identification, separation and quantification according to Harborne procedure (1998). Preliminary screening for the detection of major classes of secondary metabolites was carried out using the following techniques:

a.Detection of tannins

2 ml of ethanol extract were placed in a test tube and then 2-3 drops 5% (W/V) ferric chloride solution were added. Appearance of blue or green color indicates the presence of tannins.

b.Detection of saponins

5 ml of ethanol extract were placed in a test tube and 5 ml of distilled water were added then the tube was corked and shaken vigorously.The existence of saponins was detected via formation of persistent foam, which remains stable for at least one hour.

c.Sterols and triterpenes

One gram of the powdered plant material was macerated with 20 ml of petroleum ether(.%99 V/V)for six hours, filtered and the ether was evaporated to dryness. The residue was dissolved in acetic anhydride (.%99 V/V)(2 ml), transferred to a test tube and cautiously concentrated sulphuric acid was added along the side of the

test tube. Possible presence of sterols or triterpenes was indicated by the immediate appearance of violet color in case of triterpenes, which change to green on standing in the case of the sterols.

d.Flavonoids

6 grams of the powdered material were extracted with 100 ml ethanol for 72 hours. The ethanol extract was evaporated to dryness using rotatory evaporator. The residue was dissolved in 50 ml of water and filtered. The aqueous extract was tested for the possible presence of glycosides as following.

To 2 ml of the extract in a test tube, 5 drops of 2% (WV) ferric chloride solution in methanol were added. Formation of a green color indicates the presence of flavonoids compounds. 1 ml of 1%(WV)aluminum chloride solution in methanol was added to 2 ml of the extract. Formation of a yellow color indicates the presence of flavonoid compounds.

Addition of 1 ml of 1%(WV) potassium hydroxide solution to 2 ml of the extract produced a bright yellow color which indicating the presence of flavonoid compounds (Kumar Tripathi,A. (2011).

e. Alkaloids

10g grams of powdered plant material were macerated with 10%(W/V) acetic acid in 50 ml 80%(V/V) aqueous ethanol, allowed to stand for 24 hours then filtered and concentrated. Small portions of the concentrated extract were placed in a separate test tube to which 2-3 drops of the Dragendroff reagent were added. The remaining portion of the extract with ammonia solution, allowed to stand for one hour, and filtered. The residue was air-dried and extracted with chloroform. The chloroform extract was evaporated to dryness and the residue was dissolved in a few ml of methanol, acidified with dilute hydrochloric acid and tested for presence of alkaloids.

e. Coumarin

10 ml of the aqueous extract were extracted twice with 10 ml petroleum ether (10º - 60ºC) and were then concentrated. Few drops were spotted on a piece of filter paper, dried and examined under ultraviolet light. The presence of coumarin compounds is indicated by the blue fluorescence, which changes to bluish green on exposure to ammonia vapor.

h. Carbohydrates

0.5ml of the filtrate, 0.5ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 min. Acharacteristic red colored precipitate indicates the presence of sugar.

II. GC-MS compounds

Coriander oil was analyzed by using a gas chromatography mass spectrometry (GCMS-QP 2010 plus), equipped with selective detector mass spectrometry. The qualitative analysis of the sample as carried out by using GC/MS technique (GCMS - QP 2010 ultra) from japans simadzu company, with serial number 020525101565SA and capillary column (Rtx - 5ms-30m ×0.25mm ×0.25um). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml /min, the temperature program was started from 60cwith rate 10c/min to 300c as a final temperature degree with 5 minutes hold time , the injection port temperature was 300c, the ion source temperature was 200c and the interface temperature was 250c . the sample was analyzed by using scan mode in the range of m/z 40-500charges to ratio and the total run time was 29 minutes identification of components for the sample was achieved by comparing their retention times and mass fragmentation. Patents with those were available in the library, the national institute of standards and technology (Amirav, *et al*., 2008).

3.2.3.2. Quantitative analysis

Anti-oxidant property of *Coriandrum Sativum L* on the edible oils was assessed by measuring chemical properties (peroxide value) of storage sunflower oil. The peroxide value was measured before and after addition of extract to sunflower oil. This was happened for the mixture (coriander and sunflower oil) beginning and through storage period.

I. Determination of Peroxide value (PV)

Principle

$$ROOH + KI \xrightarrow{H^+,\ heat} ROH + KOH + I_2$$

$$I_2 + \text{starch } (\textit{blue}) + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6 + \text{starch } (\textit{colorless})$$

Procedure

Five gram g of sample were weighed into a 250 ml stopper conical flask, 30 ml acetic acid and chloroform solvent mixture in the ratio of 3:2 was added and swirled until was dissolved, then 0.5 ml saturated potassium iodide solution was added with a Mohr pipette. The mixture solution was stand for 1min in dark with occasional shaking, after that about 30 ml of distilled water were added and also about 0.5 ml starch solution was added as indicator and then the reaction was initiated and continued with shaking vigorously until all iodine (I_2) was released from chloroform ($CHCl_3$) layer. The

liberated iodine was slowly titrated with 0.1 N sodium thiosulphate solution or (0.001 mol/l) $Na_2S_2O_3$, with vigorous shaking until yellow colour was a disappeared (Chakrabarty, *et al*., 2011).

Calculation

$$\text{Peroxide value} = \frac{\text{titer} \times N \times 100}{\text{Sample weight}}$$

Where,

Titre = ml of sodium thiosulphate used (blank corrected)

N = normality of sodium thiosulphate solution.

3.2.4. Bioactivity of coriander oil

3.2.4.1. Antibacterial activity of coriander oil

The culture media

The culture medium for bacteria that used for antibacterial screening was nutrient agar and nutrient broth.

Nutrient broth

Nutrient broth (30grams) was dissolved in one liter distilled water and sterilized by autoclaving at 121°C and at an atmospheric pressure of 15 pounds for 15 minutes.

Nutrient agar

Nutrient agar was prepared by dissolving 28grams of the medium in one liter distilled water and was sterilized by autoclaving at 121°C and an atmospheric pressure of 15 pounds for 15minutes.

Normal saline

Normal saline was prepared by dissolving 8.5grams of NaCl in one liter of distilled water. The solution was autoclaved in a way similar to that followed for the previous nutrients.

Control positive

One gram of antibiotic Streptomycin and anti-fungal Nystatine were dissolved in 1ml sterilized water and diluted into a prepared 40µg/ml concentration for antibiotic and 500µg/ml concentration for antifungal treatment (Damasius, *et al*., 2007).

Bacterial strains

The test organisms used were the standard strains obtained from the medical laboratories college, Shendi University. These strains are:

Preparation of standard bacterial suspensions

Sterilized nutrient broth 10 ml was inoculated with a loop full of the bacterial culture in a test tube and kept at room temperature for 24 hours. Bacterial growth was shown by turbidity of the nutrient broth. Dilution of the bacterial concentration

was carried out by taking one ml of bacterial suspension into 9 ml of normal saline in a sterile test tube. This suspension contains 108-109 colony forming units per ml (CFU/ml). A fresh stock suspension was prepared every two weeks intervals.

Assay for antibacterial activity

Cup plate diffusion method was used to assess the antibacterial activity of cumin extracts under investigation. The assay adopted for fungi was used to assay antibacterial activity. The only difference is that potato dextrose agar was replaced by nutrient agar (Delaquis, *et al*., 2002).

Procedure

The medium was cool to 45-50 C^0 placed in the plates, then allowed to adjust the surface level to a depth of about 4mm. Antibiotic drug stocks were kept at -20C^0. Cotton wool swab supplies are prepared on wooden rod sticks.2 ml mixture of bacterial suspension was prepared thoroughly uniformed with 250 mL nutrient agar at 45 C^0 .Agar inoculation (20-25 ml) was distributed vouchers in sterile Petri dishes. Leaves were left to harden and 4 wells (7mm in diameter) were made using sterile cork bore. Grains (12.5, 25, 50 and 100%) were prepared for oil extracted, as well as by dissolving the extracts and fractures in dimethyl sulfoxide (DMSO).The effects of coriander oil were considered after 24-48 hours by measuring the diameter of the inhibition region of each treatment. Three replicates were performed for each extract / fracture and control of living organisms tested.

Calculation

The relative percentage of test inhibition with respect to positive control using the following law:

$$\text{Percentage of relative inhibition of test extraction} = \frac{(X-Y)\times 100}{(Z-Y)}$$

Where:

X: Total area of inhibition of the test extract.

Y: Total area of inhibition of the solvent.

Z: Total area of inhibition of the standard drug.

3.2.4.2. Antifungal activity

The culture medium for fungi

Potato dextrose agar (PDA) media was prepared by dissolving 39grams and 500 mg chloromphenicol in one liter distilled water. 10 ml portions of the prepared medium were sterilized in 30 ml bottles. The fungal cultures were maintained in the PDA media as slopes and incubated for about two days. The fungal growth with washed

with 100ml sterile normal saline and then the suspension was kept at 4°C untile use (Damasius*, et al*., 2007).

Preparation of standard fungal suspension

The fungal cultures were incubated at 25ºC for two days. The fungal yield was harvested and washed with 100 ml sterile normal saline and then the suspension was stored in the refrigerator until used.

Assay for antifungal activity

The cup plate agar diffusion method was adopted to assess the antifungal activity of the cumin extracts. 2 ml of the standardized fungi suspensions were mixed thoroughly with 250 ml potato dextrose agar at 45 °C . Aliquots of the inoculated agar (20-25 ml) were distributed into sterile petri dishes. The agar was left to solidify and 3 wells (9mm in diameter) were made using a sterile cork borer (No 9). Two concentrations (100 and 200 mg/ml) were made for each crude cumin extracts and fractions, as well, by dissolving the extracts and fractions in dimethyl sulphoxide (DMSO). The effects of the cumin extracts were considered after 18-24 hours by measuring the inhibition zone diameter of each treatment. Three replicates were carried out for each extract/ fractions and control against the test organisms (Teshale, *etal*. (2013).

3.2.5. Statistical Analysis

Data was collected and entered into the statistical analysis program (SPSS) using the Windows computer system (IBM). The P value was considered significant at 0.05 and the maximum confidence was 95%.

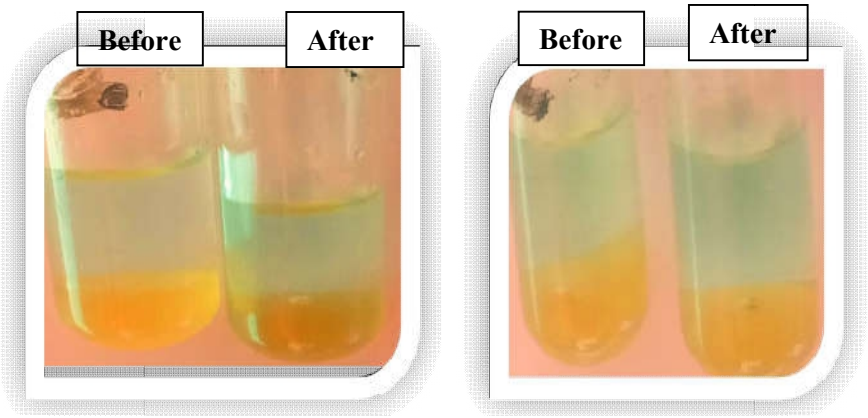
CHAPTER FOUR
4. Result and Discussion

The study focuses on employing a range of solvents to obtain coriander extracts under both cold and heated extraction conditions, with each solvent examined individually. Subsequently, the phytochemical profile, antioxidant potential, and biological activities of the extracts are evaluated. An innovative extraction approach utilizing a centrifuge is then applied to investigate these properties before and after centrifugation, thereby elucidating the influence of the centrifugation process on the chemical composition and bioactivity of the extracts.

4.1. Secondary metabolites

4.1.1. Chloroform extract

The qualitative phytochemical screening of *Coriandrum sativum* chloroform extract, under both cold and hot extraction conditions before and after centrifugation, revealed the presence of several classes of secondary metabolites including alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins, and saponins. Figures (4-1), (4-2), (4-3), (4-4), (4-5), (4-6) and Table (4-1) represent the main secondary metabolites of chloroform extract under four states (cold and hot before and after centrifugation.

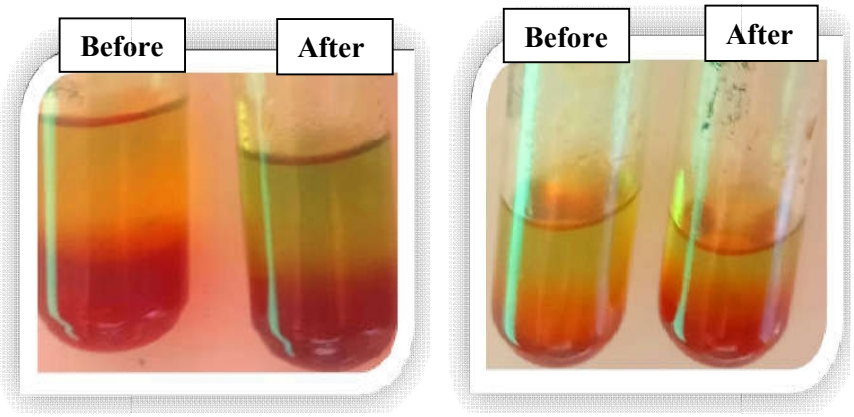


Cold extract Hot extract

Figure (4-3): Test for alkaloids

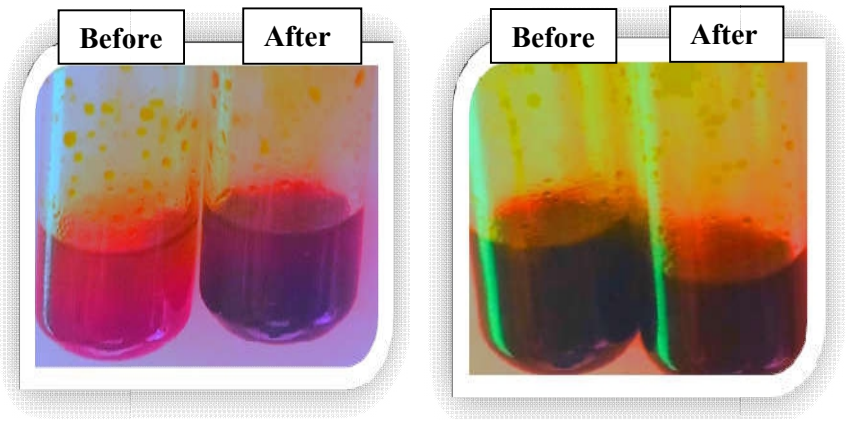
Cold extract Hot extract

Figure (4-4): Test for flavonoids



Cold extract Hot extract

Figure (4-5): Test for terpenoid

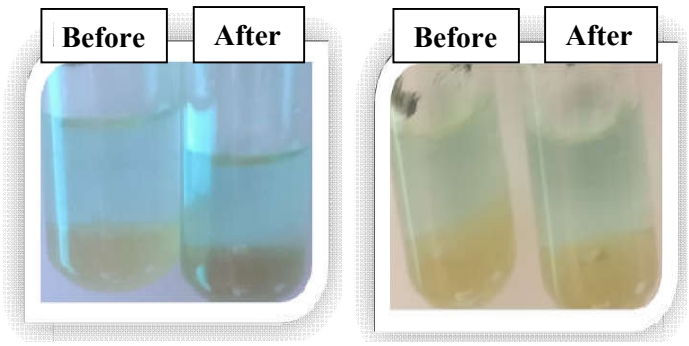


Cold extract Hot extract

Figure (4-6): Test for glycosides

Cold extract Hot extract

Figure (4-7): Test for tannins



Cold extract Hot extract

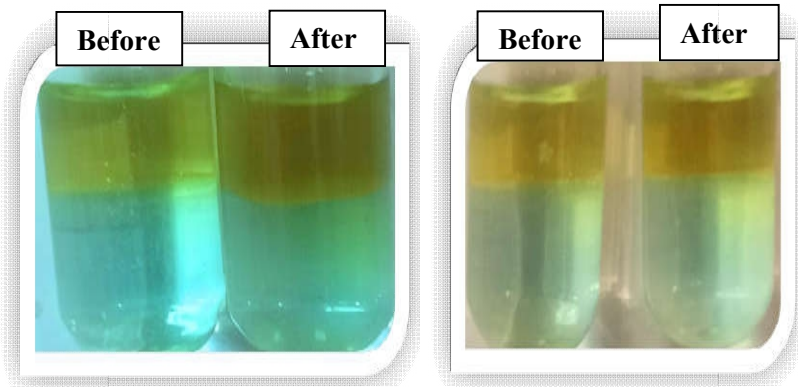
Figure (4-8): Test for saponin

Secondary	Method	Cold extract	Hot extract

Metabolite		Before	After	Before	After
Alkaloids	Mayer's test	+ + +	+ ++	+ +	+ +
Flavonoids	FeCl3 test	+ +	+ ++	+ +	+ + +
Terpenoids	Libermann Burchard	+ +	+ ++	+ +	+ +
Steroids	Salkowaski reaction	+ +	+ ++	+ +	+ +
Glycosides	Keller-Killiani test	+	+++	+ +	+ + +
Tannins	Ferric chloride test	- -	-	-	-
Saponins		-	-	+	+ +

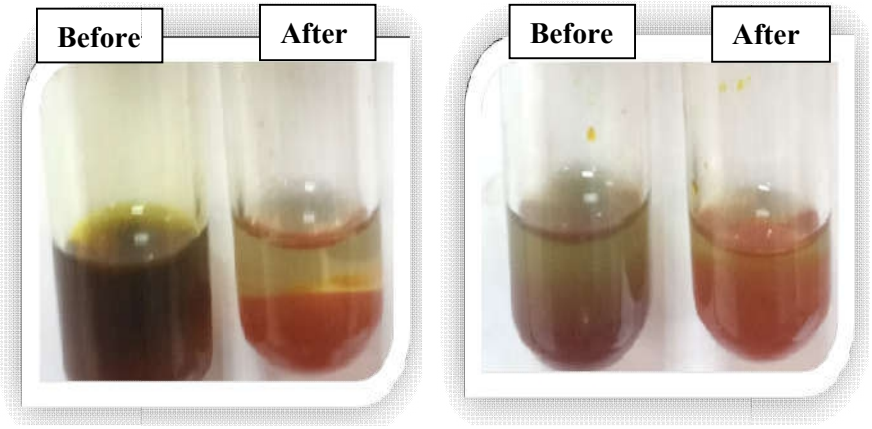
Table (4-1): Secondary metabolites of chloroform solvent extract before and after centrifugation

4.1.2. Ethyl acetate extract



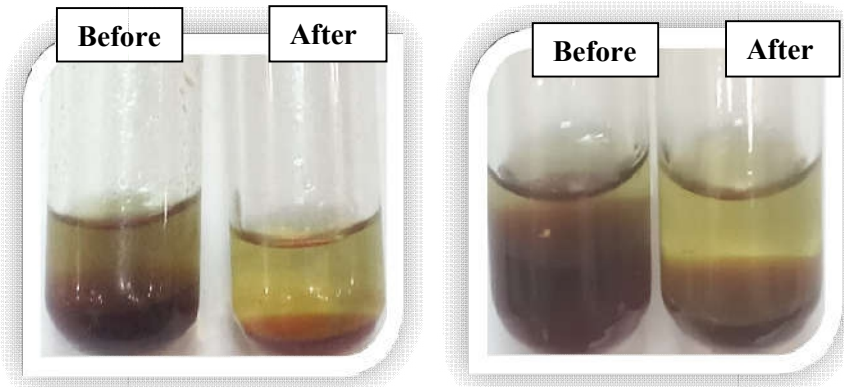
Cold extract **Hot extract**

Figure (4-9): Test for alkaloids



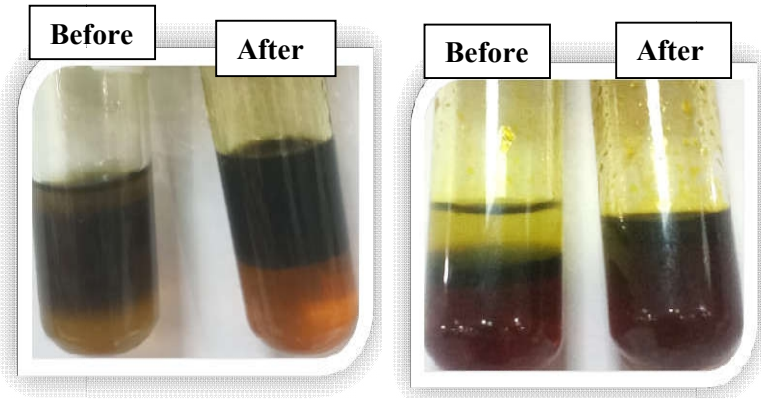
Cold extract Hot extract

Figure (4-10): Test for flavonoids



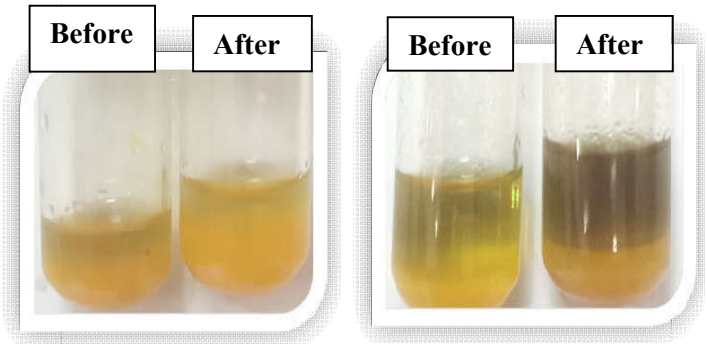
Cold extract Hot extract

Figure (4-11): Test for terpenoid



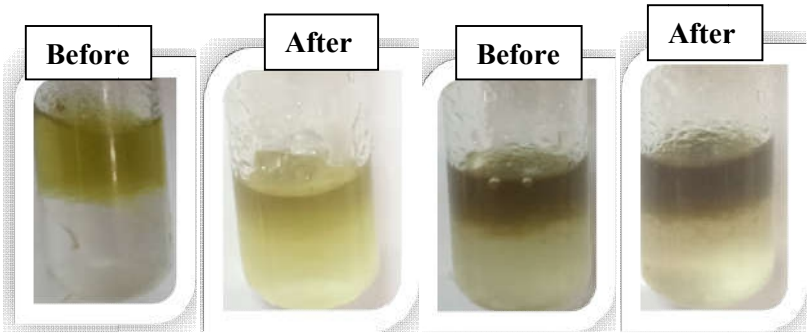
Cold extract Hot extract

Figure (4-12): Test for glycosides



Cold extract **Hot extract**

Figure (4-13): Test for tannins



Cold extract **Hot extract**

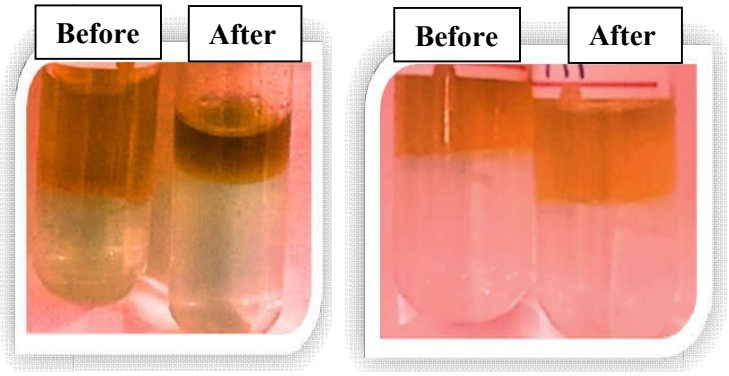
Figure (4-14): Test for saponins

Table (4-2): Secondary metabolites of ethyl acetate solvent extracts

Secondary Metabolite	Method	Cold extract		Hot extract	
		Before	After	Before	After
Alkaloids	Mayer's test	+	++	+	+
Flavonoids	FeCl3 test	+	+ +	+	+ +
Terpenoids	Libermann Burchard	+	+ +	+ + +	+ +
Steroids	Salkowaski reaction	-	-	-	-
Glycosides	Keller-Killiani test	++	+++	+ +	+ +
Tannins	Ferric chloride test	+	+++	- -	_
Saponins		++	+++	++	++

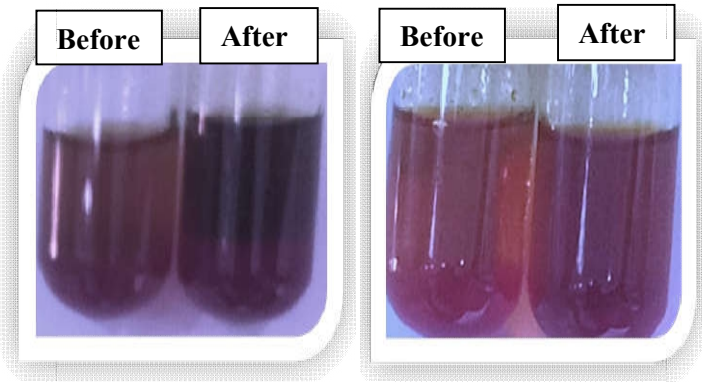
4.1.3. Petroleum ether extract

The qualitative phytochemical screening of the petroleum ether extract before and after centrifugation under both cold and hot extraction conditions revealed distinct differences in metabolite profiles, next figures and table (4-7) revealed that.



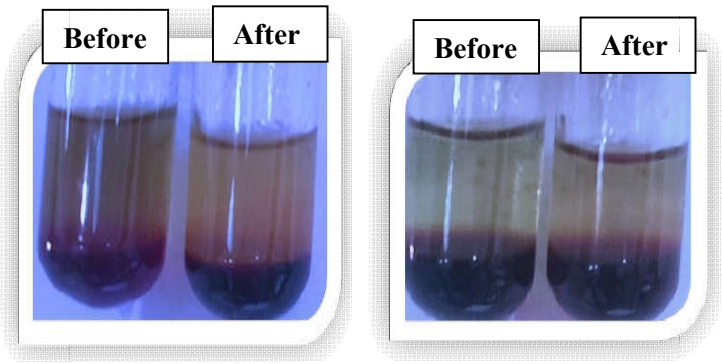
Cold extract **Hot extract**

Figure (4-15): Test for alkaloids



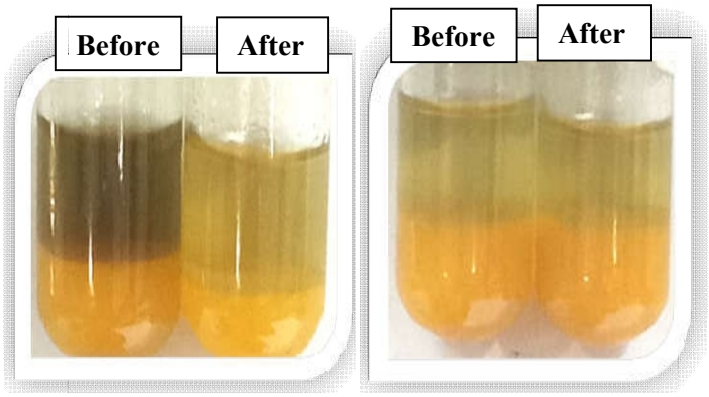
Cold extract **Hot extract**

Figure (4-16): Test for flavonoids



Cold extract Hot extract

Figure (4-17): Test for terpenoid



Cold extract Hot extract

Figure (4-18): Test for tannins

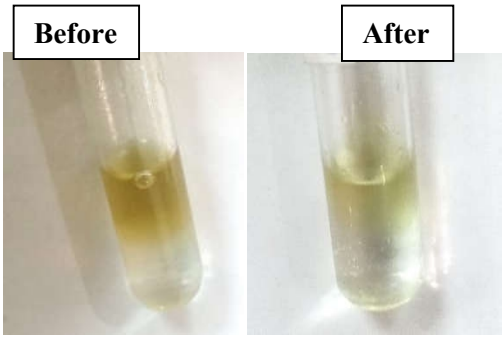


Figure (4-19): Test for saponins

Table (4-3): Secondary metabolites of petroleum ether extracts

Secondary Metabolite	Method	Cold extract		Hot extract	
		Before	After	Before	After
Alkaloids	Mayer's test	-	+	+	+ +
Flavonoids	FeCl3 test	+	+ +	+ +	+ + +
Terpenoids	Libermann Burchard	+	+ +	+ +	+ ++
Steroids	Salkowaski reaction	-	-	-	-
Glycosides	Keller-Killiani	++	++	+ +	+ + +
Tannins	Ferric chloride	+	-	- -	_
Saponins		+	+	-	-

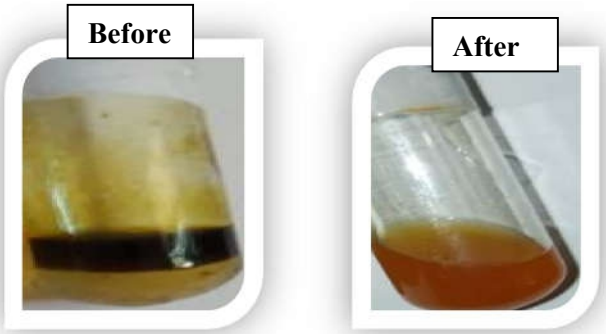


Figure (4-20) Alkaloids before and after centrifugation

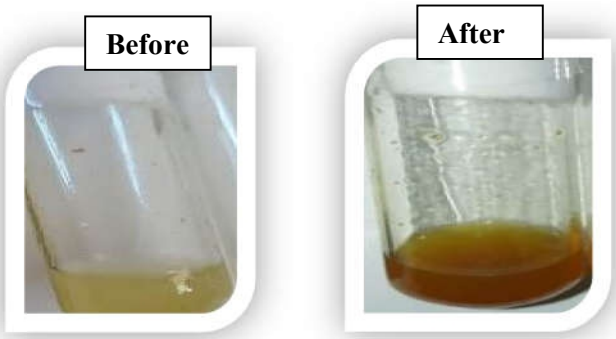


Figure (4-21) Flavonoids before and after centrifugation

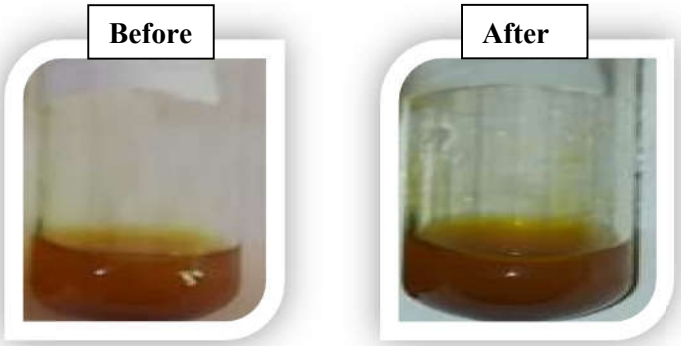


Figure (4-22) Glycosides before and after centrifugation

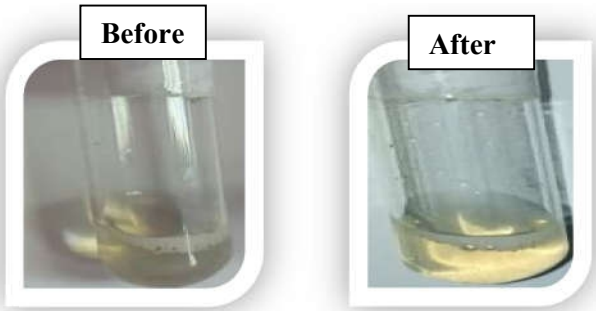


Figure (4-23) Saponins before and after centrifugation

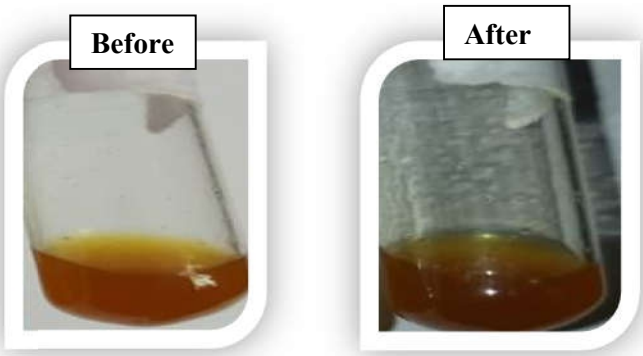


Figure (4-24) Tannins before and after centrifugation

Table (4-4): Secondary metabolites of steam distillations solvent extracts

Secondary Metabolite	Method	Before Centrifugation	After centrifugation
Alkaloids	Mayer's test	+ +	+ ++
Flavonoids	FeCl3 test	+ +	+ +
Terpenoids	Libermann Burchard	+ +	+ ++
Steroids	Salkowaski reaction	+ +	+ ++
Glycosides	Keller-Killiani test	+	+
Tannins	Ferric chloride test	- -	- -
Saponins		-	-

4.1.5. Ethanol extract

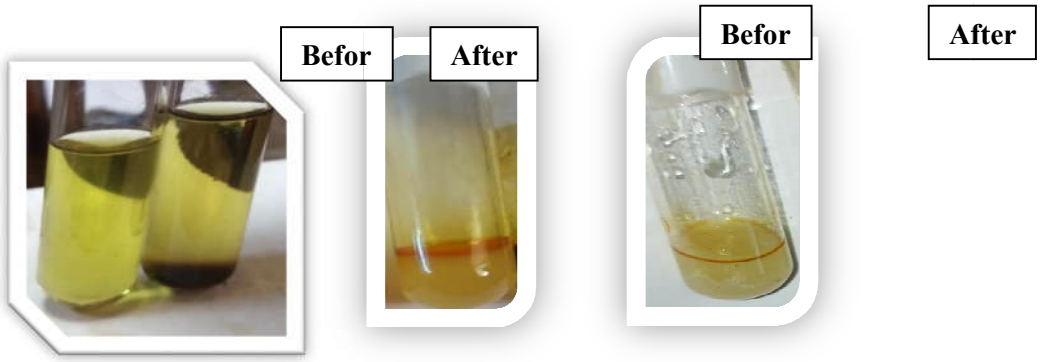


Figure (4-25): Test for alkaloids before and after centrifugation

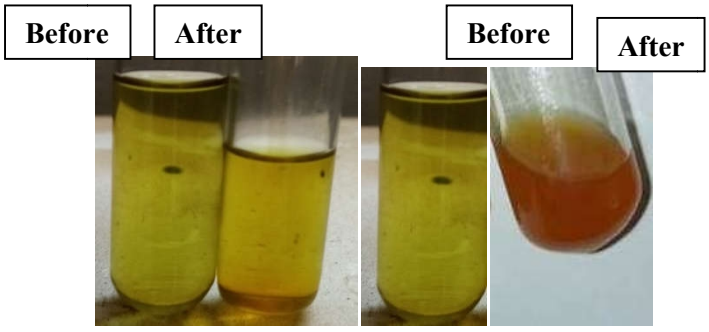
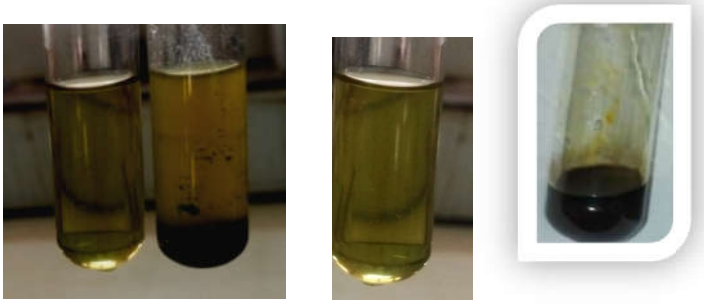
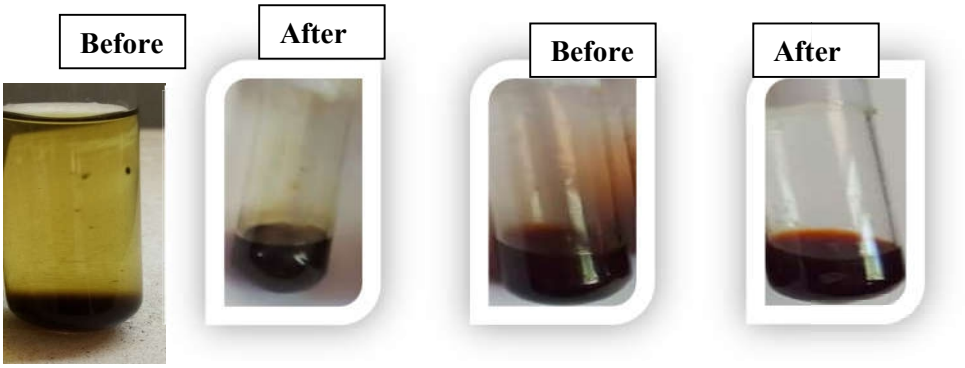


Figure (4-26): Test for flavonoids before and after centrifugation



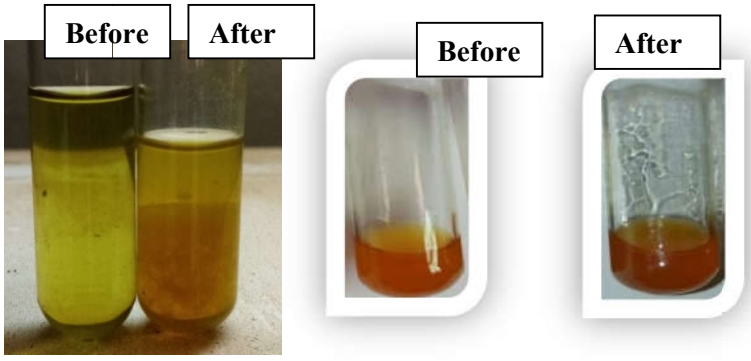
Cold extract **Hot extract**

Figure (4-27): Test for glycosides before and after centrifugation



Cold extract **Hot extract**

Figure (4-28): Test for terpenoid before and after centrifugation



Cold extract **Hot extract**

Figure (4-29): Test for tannins before and after centrifugation

Before

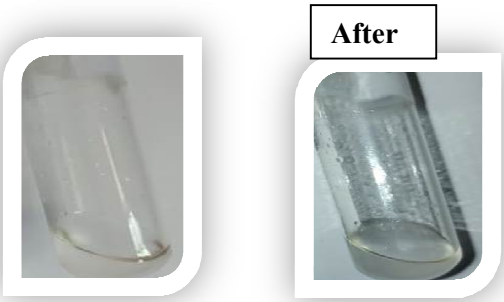


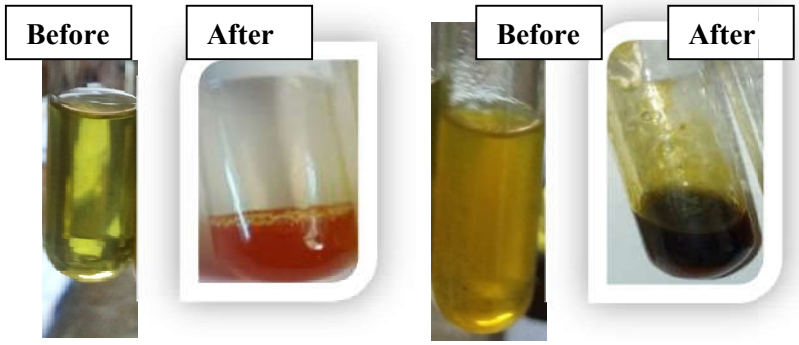
Figure (4-30): Test for saponins before and after centrifugation

Table (4-5): Secondary metabolites of ethanol extracts for cold and hot extract before and after centrifugation

Secondary Metabolite	Method	Cold extract		Hot extract	
		Before	After	Before	After
Alkaloids	Mayer's test	+ +	+ ++	+ +	+ ++
Flavonoids	FeCl3 test	+ +	+ ++	+	+ +
Terpenoids	Libermann Burchard	+ +	+ ++	+ +	+ ++
Steroids	Salkowaski reaction	+ +	+ ++	+ +	++ +
Glycosides	Keller-Killiani	+	+	+	+
Tannins	Ferric chloride	- -	- -	- -	- -
Saponins		-	-	_	_

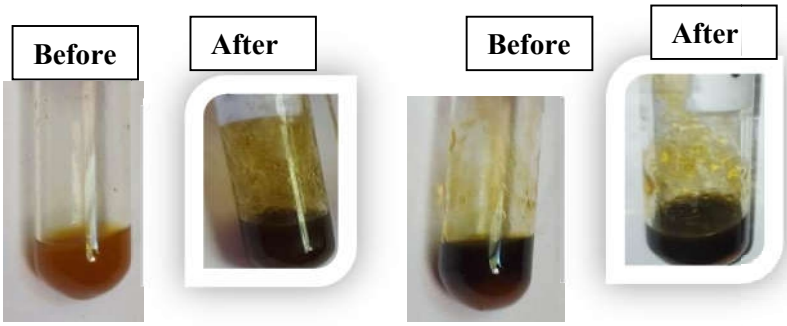
4.1.6. Hexane extract

The following figures and table (4-6) elucidate the phytochemical of *Coriandrum sativum* extract by hexane.



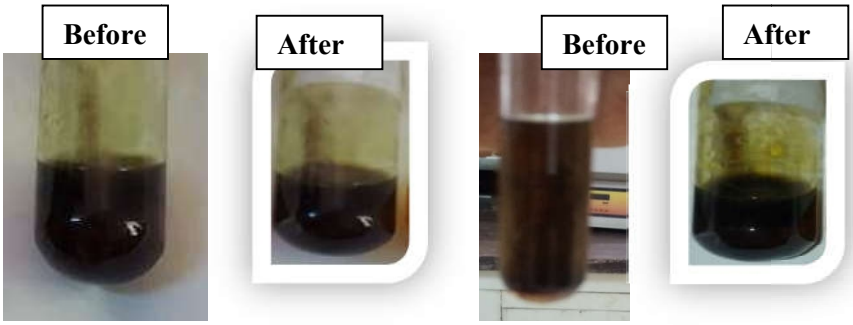
Cold extract Hot extract

Figure (4-31): Test for alkaloids before and after centrifugation



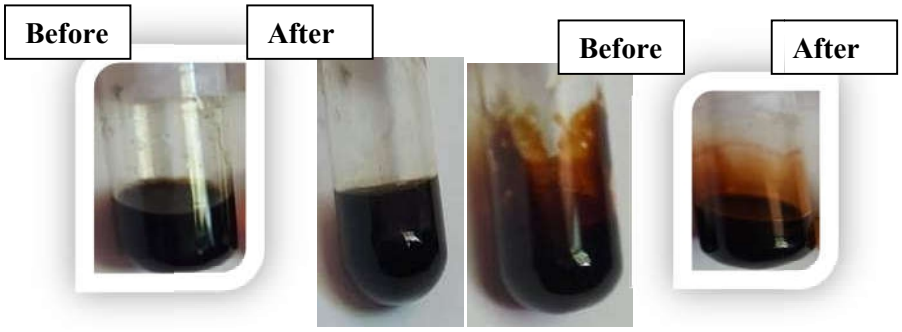
Cold extract Hot extract

Figure (4-32): Test for flavonoids before and after centrifugation



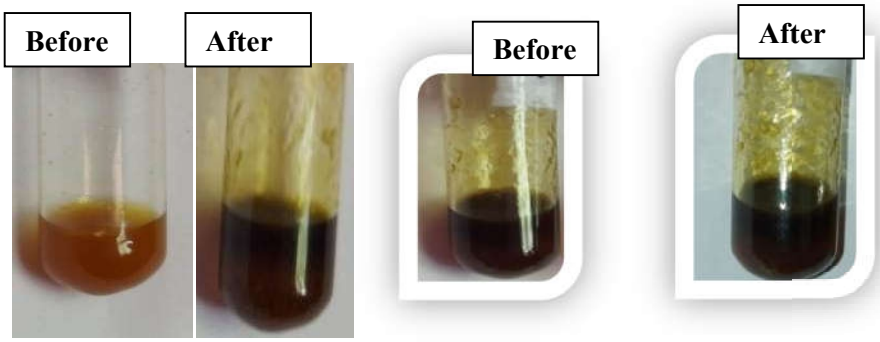
Cold extract Hot extract

Figure (4-33): Test for glycosides before and after centrifugation



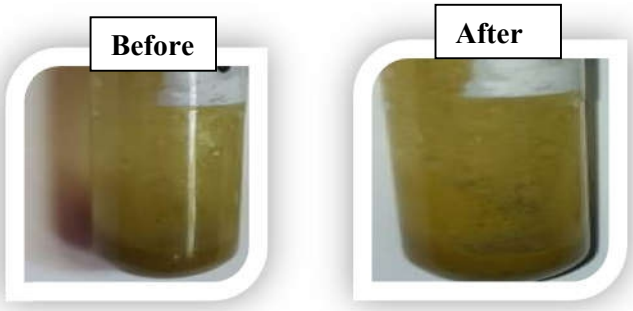
Cold extract Hot extract

Figure (4-34): Test for terpenoid before and after centrifugation



Cold extract Hot extract

Figure (4-35): Test for tannins before and after centrifugation



Cold extract Hot extract

Figure (4-36): Test for Saponins before and after centrifugation

Table (4-6): Secondary metabolites of cold and hot hexane extract before and after centrifugation

Secondary Metabolite	Method	Cold extract		Hot extract	
		Befor	After	Before	After
Alkaloids	Mayer's test	+	+	+	+
Flavonoids	$FeCl_3$ test	+	+ +	+	+ +
Terpenoids	Libermann Burchard	+	+	+	+
Steroids	Salkowaski reaction	+ +	+ +	+ +	+ +
Glycosides	Keller-Killiani test	+	++	+	+ +
Tannins	Ferric chloride test	+++	+++	++	+++
Saponins		-	-	-	-

Discussion of Phytochemical screening of secondary metabolites

This study aims to evaluate the effect of solvent type, extraction temperature (cold vs. hot), and centrifugation (before and after) on the profile of secondary metabolites in extracts of coriander (Coriandrum sativum). Six solvents representing a wide polarity range were used: chloroform (relatively non-polar), ethyl acetate (medium polarity), petroleum ether (non-polar), steam distillation (aqueous), ethanol (polar), and hexane (non-polar). The results revealed significant variation in the content of alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins, and saponins across these extracts, reflecting the decisive role of the three aforementioned factors in determining the final chemical profile of the extract.

Effect of solvent type and polarity on extraction selectivity

The choice of solvent is the most critical factor in determining the nature of extracted compounds, based on the principle of (like dissolves like). The results showed that:

Non-polar solvents (petroleum ether and hexane) were selective for lipophilic and hydrophobic compounds such as steroids and terpenoids. In the hexane extract, a high intensity of steroids (++) and terpenoids (+) was observed across all treatments, which is expected given the lipophilic nature of these compounds. The hexane extract also recorded a very high concentration of tannins (+++), an unusual result for a non-polar solvent, which may indicate the presence of specific forms of tannins soluble in non-

polar media or the formation of complexes with lipophilic compounds that enhanced their solubility.

Medium-polarity solvents such as chloroform and ethyl acetate showed an ability to extract a wide spectrum of compounds. Chloroform extracted alkaloids, flavonoids, terpenoids, steroids, and glycosides, while ethyl acetate was particularly effective in extracting glycosides, saponins, and terpenoids, with a complete absence of steroids. This could be due to unsuitable solvent polarity for extracting this group or concentrations below the detection limit.

Polar solvents (ethanol and steam distillation) were effective in extracting compounds with polar groups. Ethanol extracted all compounds except tannins and saponins, which were completely absent, possibly due to poor solubility in ethanol or interference from other substances. Steam distillation gave positive results for alkaloids, flavonoids, terpenoids, and steroids, while tannins and saponins were absent due to precipitation by high heat or their non-volatile nature.

Effect of Extraction Temperature (Cold vs. Hot)

Comparison between cold extraction (room temperature) and hot extraction (using heat) showed a clear impact on the extraction efficiency of specific compound groups:

Cold extraction was preferable for heat-sensitive compounds such as alkaloids and terpenoids in the chloroform extract, where they appeared at higher intensity in the cold extract compared to the hot one. Cold extraction also preserved the integrity of tannins in the ethyl acetate extract (+++) after centrifugation while they disappeared in the hot extract, confirming the heat-labile nature of these compounds. Similarly, the cold ethanol extract gave higher flavonoid intensity before centrifugation compared to the hot extract.

Hot extraction led to better release of some compounds through cell wall disruption, increased permeability, and enhanced diffusion, thereby boosting the extraction of glycosides and saponins in chloroform, terpenoids in ethyl acetate (+++) before centrifugation, and alkaloids in petroleum ether. We also observed increased flavonoid intensity after centrifugation in the hot ethanol extract compared to the cold extract, suggesting that heat may help extract specific flavonoids but brings along more impurities that are removed by centrifugation.

Effect of Centrifugation (Before and After)

Centrifugation played a prominent role in improving the clarity and intensity of chemical reactions for most compounds, which can be explained by the following mechanisms:

Removal of impurities and insoluble materials: Centrifugation sediments cell debris, macromolecules, and colloidal materials, resulting in a clearer supernatant with reduced turbidity. This improvement in purity allows for more distinct color development in chemical tests and increases detection sensitivity, as observed with flavonoids and glycosides in most extracts (e.g., chloroform, ethyl acetate, ethanol).

Precipitation of high-molecular-weight compounds: Some compounds, such as tannins and saponins, may precipitate under centrifugal force due to their large molecular size or tendency to aggregate. This explains the disappearance or reduction of tannin intensity after centrifugation in the hot petroleum ether extract, as well as the absence of saponins and tannins in the steam distillation supernatant after centrifugation. Conversely, the stability of tannins in the hexane extract after centrifugation (+++) indicates that they exist in a molecular form that does not precipitate in this medium.

Concentration of compounds in the supernatant: In some cases, centrifugation may increase the relative concentration of soluble compounds in the supernatant after removal of the solid pellet, positively affecting reaction intensity, as seen with alkaloids in the cold petroleum ether extract, which appeared only after centrifugation.

Analysis of major compound groups across different solvents

Alkaloids: They appeared in all extracts with varying degrees, with a notable increase after centrifugation in most cases (especially in cold chloroform, cold ethyl acetate, and petroleum ether). Their relative stability in hot extracts suggests heat resistance, while the improved detection after centrifugation is attributed to the removal of interfering substances. Their absence in the cold petroleum ether extract before centrifugation and subsequent appearance after it confirms the importance of centrifugation in releasing them from complexes or concentrating them.

Flavonoids: They showed a clear response to centrifugation, with intensity increasing after centrifugation in all extracts (except steam distillation where it remained ++). They were more prominent in polar (ethanol) and medium-polarity solvents (chloroform, ethyl acetate), while they appeared in non-polar solvents (hexane) after centrifugation, possibly indicating the presence of aglycone forms (non-glycosylated) more soluble in non-polar media. Heat negatively affected some flavonoids (e.g., cold ethanol gave higher intensity before centrifugation than hot) but positively affected others after impurity removal.

Terpenoidsand Steroids: Their strong presence was associated with non-polar and medium-polarity solvents. Terpenoid intensity increased after centrifugation in cold chloroform and hot ethyl acetate, reflecting their release from lipophilic impurities.

Steroids were completely absent in the ethyl acetate extract despite appearing in others, which could be due to differences in the chemical structure of steroids present in the plant or the effect of solvent polarity. In the hexane extract, steroids appeared with constant intensity (++), confirming their preference for non-polar media.

Glycosides: Notable increases in intensity were observed after centrifugation, especially in cold extracts (chloroform, ethyl acetate, hexane), while they sometimes remained constant in hot extracts. This could be because heat causes partial hydrolysis of glycosidic bonds or structural changes, whereas centrifugation removes substances that interfere with detection. Their appearance in the hexane extract (non-polar) might seem unusual but can be explained by the presence of glycosides with lipophilic moieties (e.g., saponins) or their adhesion to lipophilic compounds.

Tannins: The results varied significantly:They were completely absent in chloroform, ethanol, and steam distillation extracts.They appeared strongly in the hexane extract (+++) and remained stable after centrifugation, an atypical result that may point to a specific nature of coriander tannins soluble in non-polar solvents.In the ethyl acetate extract, they appeared strongly in the cold extract after centrifugation but disappeared in the hot extract, confirming their heat sensitivity.In petroleum ether, they appeared before centrifugation but disappeared afterward, indicating precipitation due to centrifugation because of their high molecular weight.These variations can be explained based on tannin polarity (they are polyphenolic and range from polar to non-polar depending on the degree of polymerization and conjugation), their tendency to precipitate, and their heat sensitivity.

Saponins: They appeared mainly in extracts containing polar or medium-polarity solvents (hot chloroform, ethyl acetate, and cold petroleum ether) but were absent in hexane, ethanol, and steam distillation. This is explained by their amphiphilic nature (having both hydrophilic and lipophilic parts), making them soluble in medium-polarity solvents. Centrifugation improved their appearance in some cases (cold ethyl acetate) but led to their precipitation in others (hot petroleum ether).

The results collectively confirm that there is no single ideal solvent for extracting all active compounds from coriander; instead, the solvent should be chosen according to the desired target. Furthermore, cold extraction preserves heat-sensitive compounds (like tannins and some alkaloids), while hot extraction may increase the yield of some heat-resistant compounds (like glycosides and terpenoids) due to cell wall disruption. Centrifugation is an essential step to improve extract purity and increase detection sensitivity, especially when performing qualitative chemical tests. The results indicate

that chloroform (cold), hexane, and ethanol (after centrifugation) extracts are the richest in a diverse array of bioactive compounds, supporting the use of coriander as a potential source of antioxidants and therapeutic agents. Further quantitative and chromatographic studies are recommended to confirm these findings and identify the compounds more precisely.

4.2. Antioxidant activity on commercial sample

The peroxide value (PV) is a reliable indicator of the primary oxidation level in edible oils. Commercial sample refers to a sunflower oil sample that was obtained directly from the company and used in a peroxide value test to determine its condition before being used in the cooking process. In this experiment, the peroxide values of sunflower oil were monitored for five consecutive weeks under storage conditions, both for the commercial oil (control) and for samples supplemented with *Coriandrum sativum* chloroform extracts obtained under different extraction conditions (cold/hot, before/after centrifugation).

4.2.1. Chloroform extract

Table (4-7): Peroxide value of commercial sunflower sample before and after addition chloroform extract

Storage period Period	Peroxide value				
	Commercia sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	10	10	11	10	10
After 1st week	11	11	12	11	11
After 2nd week	12	11	12	11	11
After 3rd week	13	11	12	11	12
After 4th week	14	10	12	10	11
After 5th week	15	9	13	8	12
P value	< 0.05				

4.2.2. Ethyl acetate extract

Table (4-8): Peroxide value of commercial sunflower oil before and after addition of ethyl acetate extract

Storage Period	Peroxide value				
	Commercial sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	10	10	10	10	10
After 1st week	11	11	11	11	11
After 2nd week	12	12	12	12	12
After 3rd week	13	13	13	12	13
After 4th week	14	13	14	12	14
After 5th week	15	13	14	12	14
P value	< 0.05				

4.2.3. Petroleum ether

Table (4-9): Peroxide value of commercial sunflower oil before and after addition petroleum ether

Storage Period	Peroxide value				
	Commercial sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	10	10	10	10	10
After 1st week	11	11	11	11	11
After 2nd week	12	12	12	12	11
After 3rd week	13	12	13	12	10
After 4th week	14	12	13	12	10
After 5th week	15	12	13	11	9
P value	< 0.05				

4.2.4. Steam distillations extract

Table (4-10): Peroxide value of sunflower oil before and after steam distillations extract addition

Storage period	Peroxide value		
	Commercial sample	Before	After
Before storage	10	10	10
After 1st week	11	11	11
After 2nd week	12	10	10
After 3rd week	13	11	11
After 4th week	14	10	10
After 5th week	15	9	9
P value	< 0.05		

4.2.5. Ethanol extract

Table (4-11): Peroxide value of sunflower oil before and after addition of ethanol extract

Storage Period	Peroxide value				
	Commercial Sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	10	10	10	10	10
After 1st week	11	11	11	11	11
After 2nd week	12	11	11	11	11
After 3rd week	13	10	10	11	11
After 4th week	14	9	9	11	10
After 5th week	15	9	8	10	9
P value	< 0.05				

4.2.6. Hexane extract

Table (4-12): Peroxide value of commercial sunflower oil before and after addition of hexane extract

Storage Period	Peroxide value				
	Commercial sample	Cold extrac		Hot extract	
		Before	After	Before	After
Before storage	10	10	10	10	10
After 1st week	11	11	11	11	11
After 2nd week	12	12	12	12	11
After 3rd week	13	12	11	12	11
After 4th week	14	11	11	11	10
After 5th week	15	11	10	11	10
P value	**< 0.05**				

Discussion of peroxide value for commercial sample

The peroxide value (PV) is a primary indicator for measuring the initial stage of deterioration in oils due to the formation of hydroperoxides, the primary products of oxidation. This part of the study aims to evaluate the efficiency of various crude extracts prepared from coriander (Coriandrum sativum) using different organic solvents (chloroform, ethyl acetate, petroleum ether, ethanol, hexane, in addition to steam distillation) in inhibiting the oxidation of sunflower oil over five weeks. The study also examined the effect of centrifugation (before and after) on enhancing antioxidant activity, comparing cold and hot extracts.

Chloroform Extract (Table 4-7),The results clearly showed the superiority of the extract prepared by the hot extraction method with centrifugation, which maintained the lowest peroxide value (11–12 meq O_2/kg) until the end of the fifth week, compared to the control sample which increased to 15 meq O_2/kg. This is attributed to increased solubility of active compounds such as terpenoids, steroids, and flavonoids in the organic phase due to heating.

Removal of polar impurities and pro-oxidant factors (such as heavy metals or water residues) during centrifugation, leading to a purer extract with a higher concentration of

active substances.In contrast, the cold, non-centrifuged extract showed a slight increase in PV, suggesting the presence of impurities that may promote oxidation.

Ethyl Acetate Extract (Table 4-8),The hot extract before centrifugation recorded the best performance, maintaining a PV of 12 meq O_2/kg in the fifth week, lower than the control (15) and other treatments (13–14). This performance correlates with phytochemical analysis, which showed the highest concentration of terpenoids (+++) in this extract, enhancing its ability to scavenge free radicals in fresh oil.Notably, the hot extract before centrifugation was more effective than the extract after centrifugation, suggesting that some antioxidant compounds might precipitate or be lost during centrifugation.

Petroleum Ether Extract (Table 4-9), Samples treated with the centrifuged extract (hot or cold) showed very low PV values (11–12 meq O_2/kg), while the control reached 15 meq O_2/kg. This is attributed to removal of suspended particles and heavy non-polar substances that might catalyze oxidation.Concentration of active non-polar compounds such as lipophilic terpenoids and flavonoids.The hot, centrifuged extract was the best, with PV reaching only 9 meq O_2/kg in the fifth week, indicating a high capacity to inhibit oxidation or even decompose formed peroxides.

Steam Distillation Extract (Table 4-10),Samples treated with the distilled extract (before and after centrifugation) were distinguished by a decrease in PV starting from the third week, reaching 9 meq O_2/kg in the fifth week, which is lower than the initial value (10). This behavior indicates to a dual effect such as inhibition of new peroxide formation and decomposition of some pre-existing peroxides, reflecting the high efficacy of volatile compounds such as essential oils and terpenoids present in the steam extract.This result supports the hypothesis that active compounds in the steam extract possess the ability to scavenge free radicals and terminate the chain reactions of oxidation.

Ethanol Extract (Table 4-11), the cold ethanol extract (before and after centrifugation) recorded the best results overall, with PV decreasing to 8–9 meq O_2/kg in the fifth week. This is attributed to the high concentration of effective polar compounds such as flavonoids and phenolics, which dissolve well in ethanol, and the heat sensitivity of some active compounds that may degrade or volatilize during hot extraction, explaining the weaker performance of the hot extract compared to the cold one.This result confirms that cold ethanol is the optimal solvent for extracting antioxidant compounds from coriander, especially those capable of decomposing peroxides and not just inhibiting their formation.

Hexane Extract (Table 4-12),the hexane extract showed moderate efficacy, with treated samples maintaining PV values (10–12 meq O_2/kg) in the fifth week, with a slight superiority for the hot, centrifuged extract. This is attributed to the non-polar nature of hexane, which selectively dissolves only lipophilic compounds (such as sterols and tocopherols) and leaves out most polar phenolics.The limited diversity of active compounds compared to ethanol or chloroform.

Statistical analysis and general comparison showed all results showed significant differences ($P < 0.05$) between treated samples and the control, confirming that the antioxidant effect is real and not due to chance.The efficiency of the extracts can be ranked in descending order based on PV reduction in the fifth week; Cold Ethanol (8–9 meq O_2/kg), Steam Distillation (9 meq O_2/kg), Petroleum Ether (Hot + Centrifuged) (9 meq O_2/kg), Chloroform (Hot + Centrifuged) (11–12), Ethyl Acetate (Hot) (12) and Hexane (10–12)

Possible mechanisms of antioxidant action attributed to free radical dcavenging; due to the extracts containing phenolics and flavonoids that donate hydrogen atoms to lipid free radicals, terminating the chain reactions of oxidation. Some compounds (e.g., flavonoids) can chelate metals like iron and copper, preventing them from catalyzing oxidation reactions.

In some treatments (especially cold ethanol and steam distillation), the PV decreased below the initial value, indicating the ability of these extracts to decompose hydroperoxides into harmless secondary products.

Some factors may be influencing efficacy such aspolar solvents (ethanol) dissolve a broader range of phenolic compounds, while non-polar solvents (hexane) dissolve only lipophilic compounds,hot extraction increases the solubility of some compounds but may destroy heat-sensitive ones andimproves efficacy by removing pro-oxidant impurities but may also remove some active compounds if they precipitate.Extracts showed higher efficacy in fresh oil compared to previously used oil (results not fully detailed), meaning that oxidized oils might require higher doses of antioxidants.

In conclusion, the results confirm that *Coriandrum sativum* extracts prepared with organic solvents, especially cold ethanol, possess an excellent ability to inhibit the oxidation of sunflower oil. Centrifugation and thermal extraction can either enhance or reduce this efficacy depending on the nature of the target compounds. This research represents an important step towards developing effective natural antioxidants for food applications.

4.3. Reused sunflower oil sample

It is a sample of sunflower oil that was used in repeated frying processes, separated by cooling periods at room temperature, which led to an increase in peroxide value. The sample was purified from cooking residues and subjected to determine its peroxide value at the beginning, during and at the end of storage period, as well as after the addition of the extract in the four conditions as shown in the following tables

4.3.1. Chloroform extract

Table (4-13): Peroxide value of reused sunflower sample before and after addition of chloroform extract

Storage period	Peroxide value				
	Reused sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	22	22	22	22	22
After 1st week	23	22	23	22	23
After 2nd week	24	22	23	21	23
After 3rd week	26	21	25	20	24
After 4th week	27	20	26	20	25
After 5th week	28	20	27	19	26
P value	< 0.05				

4.3.2. Ethyl acetate extract

Table (4-14): Peroxide value of reused sunflower oil before and after ethyl acetate extract addition

Storage Period	Peroxide value				
	Reused Sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	22	22	22	22	22
After 1st week	23	23	23	23	23
After 2nd week	24	24	24	23	24
After 3rd week	26	25	25	24	25
After 4th week	27	24	24	23	24
After 5th week	28	24	23	22	23
P value	< 0.05				

4.3.3. Petroleum ether

Table (4-15): Peroxide value of reused sunflower oil before and after petroleum ether extract addition

Storage Period	Peroxide value				
	Reused Sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	22	22	22	22	22
After 1st week	23	23	23	22	22
After 2nd week	24	24	24	23	21
After 3rd week	26	25	24	24	21
After 4th week	27	26	23	25	20
After 5th week	28	26	22	24	20
P value	< 0.05				

4.3.4. Steam distillation extract

Table (4-16): Peroxide value of reused sunflower oil before and after steam distillation extract addition

Storage period	Peroxide value		
	Commercial sample	Before	After
Before storage	22	22	22
After 1st week	23	23	23
After 2nd week	24	23	23
After 3rd week	25	22	22
After 4th week	27	21	21
After 5th week	28	19	19
P value	< 0.05		

4.3.5. Ethanol extract

Table (4-17): Peroxide value of reused sunflower oil before and after addition of ethanol extract

Storage Period	Peroxide value				
	Reused sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	22	22	22	22	22
After 1st week	23	22	22	22	22
After 2nd week	24	23	23	22	22
After 3rd week	26	23	23	22	22
After 4th week	27	22	22	21	20
After 5th week	28	20	19	19	19
P value	< 0.05				

4.3.6. Hexane extract

Table (4-18): Peroxide value of reused sunflower oil before and after addition of hexane extract

Storage Period	Peroxide value				
	Reused sample	Cold extract		Hot extract	
		Before	After	Before	After
Before storage	22	22	22	22	22
After 1st week	23	23	23	23	23
After 2nd week	24	24	23	23	23
After 3rd week	26	25	24	24	23
After 4th week	27	23	22	22	22
After 5th week	28	22	21	21	20
P value	< 0.05				

Discussion of reused sunflower oil sample

Reused frying oils pose a significant challenge in the food industry, as they undergo complex oxidation reactions due to repeated exposure to high temperatures, leading to the formation of harmful compounds such as hydroperoxides and aldehydes, as well as the accumulation of pro-oxidant metal ions from cookware and food. The peroxide value (PV) is a critical indicator for measuring the initial stage of oxidation, reflecting the concentration of formed hydroperoxides. This study aims to evaluate the effectiveness of coriander (*Coriandrum sativum*) extracts prepared with different organic solvents (chloroform, ethyl acetate, petroleum ether, ethanol, hexane) in addition to the steam distillation extract, in inhibiting the oxidation of reused sunflower oil, while studying the effect of extraction temperature (cold/hot) and centrifugation on the antioxidant activity.

The experiments were conducted on reused sunflower oil (with a high initial peroxide value of 22 meq/kg) compared to fresh commercial oil (10 meq/kg). The evolution of the peroxide value was monitored over five weeks, and the results showed significant differences ($P < 0.05$) between the different treatments, allowing for an in-depth analysis of the underlying biochemical mechanisms.

The P-values < 0.05 in all tables indicate that the differences between the control samples and the treatments were statistically significant, confirming that the observed effects were not random but resulted from the extracts' efficacy. All extracts showed the

ability to inhibit the increase in peroxide value to varying degrees, and in some cases, even reduced the value below the initial level, indicating advanced mechanisms beyond mere radical scavenging.

Chloroform Extract (Table 4-13),the results showed that the cold chloroform extract before centrifugation was the most effective in inhibiting oxidation, maintaining the peroxide value at 20 meq/kg throughout the storage period, compared to its rise to 28 in the control sample. This is attributed to the ability of polyphenolic and flavonoid compounds soluble in chloroform to donate hydrogen atoms to neutralize lipid free radicals (ROO•) and break the chain reaction of oxidation. The hot extract was less effective, indicating the thermal degradation of some active compounds during extraction. It is noted that centrifugation improved the extract's purity by removing catalytic residues, but the main effect was due to extraction temperature.

Ethyl Acetate Extract (Table 4-14), the performance of this extract was remarkable, as its effect was not limited to inhibiting oxidation but led to a decrease in peroxide values after the third week. For example, the value in the hot extract sample before centrifugation decreased from 24 in the third week to 22 in the fifth week. This phenomenon reflects the extract's ability to decompose pre-existing hydroperoxides into stable, non-radical secondary products. Furthermore, the presence of compounds such as terpenoids and saponins in the ethyl acetate extract may contribute to chelating pro-oxidant metal ions, thereby breaking the chain propagation of free radicals.

Petroleum ether extract (Table 4-15), the results showed that centrifugation played a crucial role in improving the effectiveness of this extract, especially in samples treated with the hot extract after centrifugation, where the peroxide value decreased to 20 meq/kg in the fifth week. Petroleum ether dissolves non-polar compounds such as phospholipids, tocopherols, and some terpenoids. Centrifugation removes insoluble materials that could act as pro-oxidants, thereby concentrating the thermally stable antioxidant compounds. This confirms that mechanical purification enhances the functional performance of the extract even in degraded oil systems.

Steam distillation extract (Table 4-16), this extract was remarkably effective, reducing the peroxide value in reused oil from 22 to 19 after five weeks. Steam distillation extracts volatile oils and semi-volatile compounds such as limonene, linalool, and carvone, which possess antioxidant activity through multiple mechanisms: radical scavenging, chain-breaking oxidation, and hydroperoxide decomposition. The results also showed that centrifugation removes heavy non-polar compounds, but the crude extract (before centrifugation) was more effective, suggesting that some active

compounds might be associated with mucilaginous materials that were removed by centrifugation, or that the process caused the loss of some volatile components.

Ethanol extract (Table 4-17), ethanol is a polar solvent that extracts a wide range of flavonoids, terpenoids, and glycosides. The results showed that the ethanol extract (cold and hot) was highly effective, maintaining low peroxide values (20-19) in the fifth week. Notably, the samples before and after centrifugation gave similar results, indicating that the active compounds in ethanol are highly soluble and not substantially affected by centrifugation. This reinforces the idea that ethanol extracts a diverse set of potent antioxidants that work synergistically to prevent oxidation and decompose peroxides.

Hexane extract (Table 4-18), hexane is a non-polar solvent, extracting lipids, sterols, and tocopherols. The results showed that the extracts after centrifugation (especially hot ones) were the most effective, reducing the peroxide value to 20 meq/kg. This indicates that centrifugation removes non-polar impurities that might interfere with antioxidant activity, allowing active compounds such as tocopherols and carotenoids to function efficiently. Hexane may also extract some slightly polar phenolic compounds that contribute to oxidation inhibition.

By comparing the final results in the fifth week, the extracts can be ranked in descending order of effectiveness in reducing the peroxide value in reused oil: Ethanol extract (reached 19-20) with hot ethanol after centrifugation giving 19, steam distillation extract (reached 19), ethyl acetate extract (reached 22-23), hexane extract (reached 20-22), petroleum ether extract (reached 20-22) and lastly ischloroform extract (reached 20-27).

This disparity can be explained by the nature of the extracted compounds: Ethanol and steam distillation extract a wide range of highly effective polar and semi-polar antioxidants. Non-polar solvents extract a limited but important set of compounds like tocopherols. Chloroform may extract chlorophyll and some compounds that might be heat-sensitive.

The results generally showed that cold extraction was better than hot extraction in most cases, especially with volatile solvents like chloroform and petroleum ether. High temperatures during extraction may lead to the degradation of heat-sensitive phenolic compounds or the volatilization of some essential oils, thereby reducing antioxidant activity. However, in the case of the hexane extract, hot extraction was slightly better, possibly due to increased solubility of some sterols or bound tocopherols.

Centrifugation played a dual role;In non-polar solvents (petroleum ether, hexane), centrifugation significantly improved effectiveness by removing suspended particles and insoluble materials that could act as pro-oxidants or dilute the concentration of active

substances.In polar solvents (ethanol), the effect was minimal because the active substances were fully dissolved.In steam distillation, centrifugation led to a slight increase in values, possibly due to the loss of some volatile compounds or mucilaginous materials carrying the activity.This indicates that centrifugation is an important purification step, but its effect depends on the nature of the solvent and the extracted compounds.

Based on the results, the previous concurrent mechanisms can be proposed (radical scavenging and hydroperoxide Decomposition). This suggests the ability of certain compounds to decompose hydroperoxides into non-radical secondary products (such as alcohols and ketones), a process that does not generate new free radicals. This advanced property is found in potent antioxidants like some sulfur and selenium compounds, but may also exist in some terpenoids.Some compounds may regenerate other antioxidants (vitamin E by vitamin C) thereby enhancing overall activity.

These findings hold significant importance for the food industry, especially in the food service sector and restaurants where frying oils are reused. The ability to stabilize degraded oils and extend their functional life using natural extracts (instead of synthetic antioxidants like BHT and BHA) represents an achievement in the field of food safety and sustainability. Ethanol extract and steam distillation extract stand out as promising candidates for developing effective natural additives.

In general, all studied coriander extracts showed antioxidant activity to varying degrees in reused sunflower oil, with a clear superiority of polar extracts (ethanol and steam distillation).Cold extraction was better than hot extraction for preserving heat-sensitive compounds.Centrifugation improved the effectiveness of non-polar extracts by removing impurities, while its effect was limited on polar extracts.The proposed mechanisms include radical scavenging, hydroperoxide decomposition, metal chelation, and compound synergy.The results are statistically significant ($P < 0.05$) and support the potential application of these extracts as natural antioxidants in edible oils and fats.

4.4. Gas Chromatography–Mass Spectrometry (GC-MS)

For the GC-MS analysis of three *Coriandrum sativum* (coriander) extracts were detected, In addition to water distillation extract, two organic solvent extracts with different polarities were examined (ethanol and hexane).

4.4.1. Water distillation extract

Table (4-19): The GC-MS profile of water distillation extract

Peak	Compound name	R.Time	Area%
1	Alpha.-Pinene	3.326	1.29
2	o-Cymene	4.492	2.42
3	Eucalyptol	4.609	1.29
4	1-Octanol	5.064	0.51
5	trans-Linalool oxide (furanoid)	5.153	6.76
6	2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.al	5.374	6.44
7	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	5.546	61.16
8	(+)-2-Bornanone	6.236	5.14
9	(3R,6R)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-p	6.518	1.54
10	(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-p	6.568	1.17
11	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,	6.660	0.56
12	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	6.748	2.58
13	.alpha.-Terpineol	6.835	0.98
14	Decanal	6.963	0.38
15	Citronellol	7.272	0.46
16	Propanal, 2-methyl-3-phenyl-	7.540	0.29
17	D-Carvone	7.586	0.63
18	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	7.644	0.72
19	2-Decenal, (E)-	7.750	0.83
20	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	7.907	1.07
21	exo-2-Hydroxycineole	8.571	0.35
22	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	9.065	0.34
23	n-Decanoic acid	9.121	0.36
24	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, ac	9.361	1.60
25	Alloaromadendrene	10.255	0.24
26	2-Dodecenal, (E)-	10.441	0.20
27	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-tri	11.972	0.23
28	(-)-Globulol	12.056	0.45
Total percentage		**100.00**	

A total of 28 chemical constituents were identified, including alcohols, terpenoids, aldehydes, ketones and cyalic compounds. The main three compounds with higher percentages are:

I. Cyclohexanol 2-methyl-5-(1-methylethenyl recorded the highest peak area (61.16%), including it is the most abundant constituent in the extract. This compound is a type of oramtic alcohol kmown for its antimicrobial and anti-inflammatory properties, as well as its role as an antioxidant.

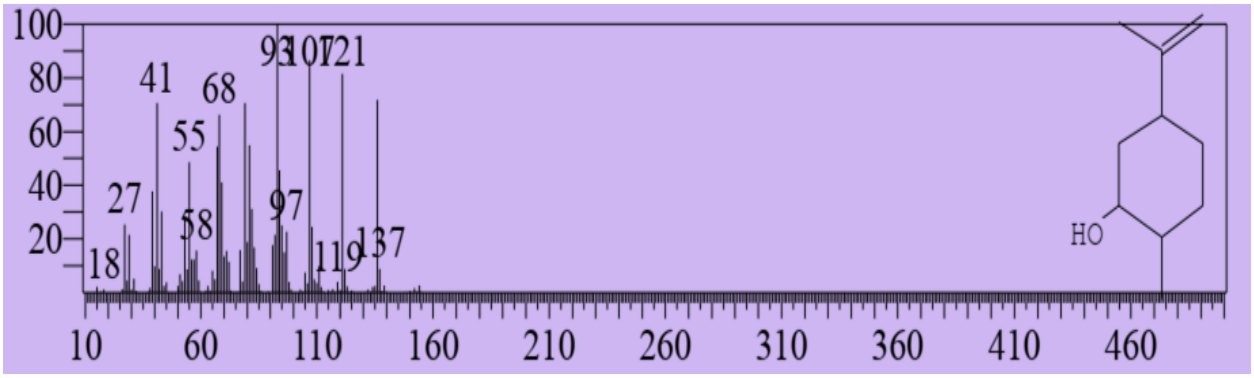


Figure (4-34): Cyclohexanol, 2-methyl-5-(1-methylethenyl)

II. Furanoid is a natural aromatic compound (a type terpenoid), found in some flowers and aromatic plants. Chemically it contains a five b-membered ring similar to furan and belongs to terpene oxides. It is derivative of linalool, acommon fragrance compounds in essential oils. It has a pleasant floral scent, often describe as lavender-like or with a hint of fresh citrus. Frequently it used in perfumes and personal care products for its appealing aroma.

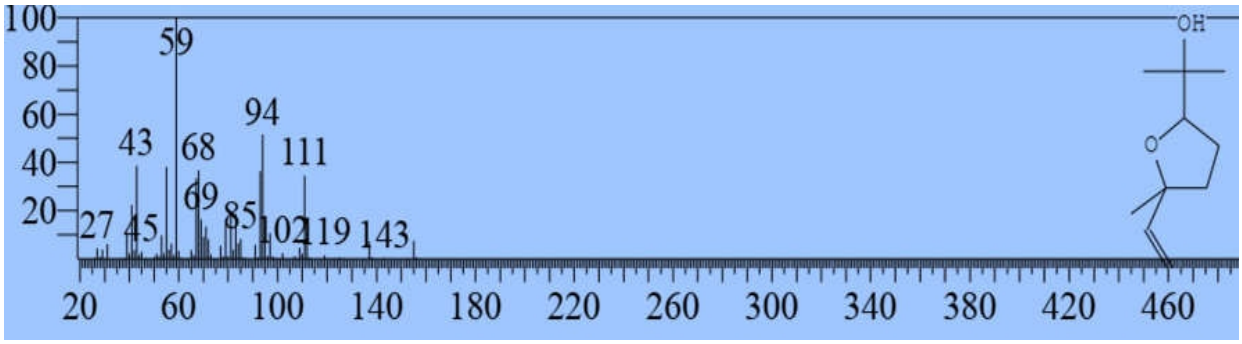


Figure (4-35): *trans*- Linalool oxide (furanoid)

III. 2-furanmethanol is an alcohol that contains a furan ring attached to a methanol group. It is colorless to light yellow liquid. Used in resin production, paints and coatings, adhesives, fuel additives. Also used in food industry in trace amounts as a flavoring agent.

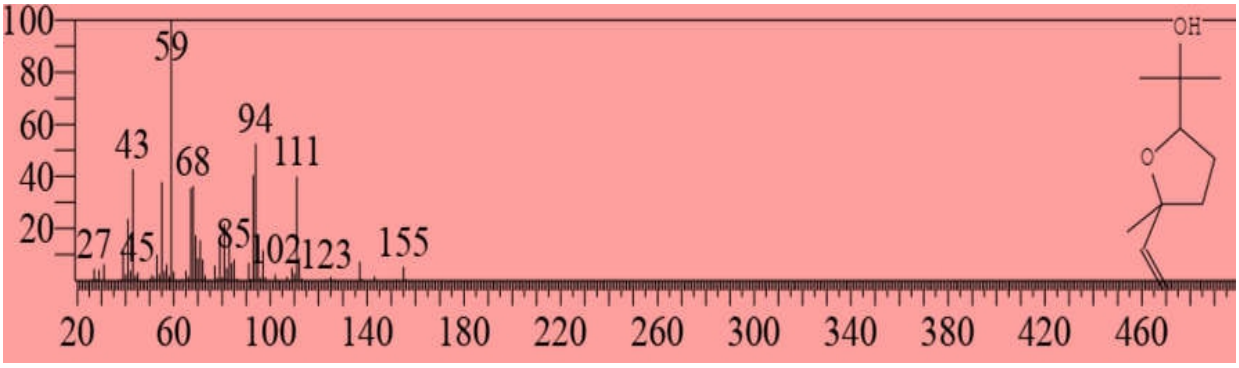


Figure (4-36): 2-furanmethanol, 5-ethenyltetrahydro

Several terpenoid compounds were identified in significant quantities, including: αterpineol (0.98%), trans- linalool oxide (6.78%) and α- pinene (1.29%). These compounds are commonly associated with aromatic and therapeutic properties, especially antioxidant and antibacterial activity.

The extract also contained other biologically significant compounds such as; D-carvano known for its antifungal and antibacterial activity which used in pharmaceutical and food industries. Citronellol is fragrant compounds with insect-repllent activity. Decanal and 2-decanal are aldehydes known for their antimicrobial potential.

The biological activity of the coriander extract is likely not attributed to a single compound, but rather the synergistic interaction of multiple constituents. The diversity in chemeical classes (terpenes, alcohols, aldehydes, etc.) offers a broad spectrum of biological functions, supporting the traditional and potential medical uses of coriander.

The GC-MS analysis of the coriander of the water distillation extract demonstrates chemically rich and diverse profile. The presence of multiple bioactive compounds highlights its potential as an antimicrobial, antioxidant and aromatic agent. In addition their application as natural source in pharmaceutical, nutracetical, and food industries.

4.4.2. Ethanol extract

The results of gas chromatography-mass spectrometry analysis of the cold ethanolicectract of coriander (high percentage) revealed the presence of wide rang of bioactive compounds Table (4-20).

Table (4-20): GC-MS analysis of ethanol extract

Peak	Name	R.Time	Area%
1	Formamide, N-methoxy-	3.649	7.38
2	Undecane, 1-bromo-	4.079	5.85
3	1,3,5-Triazine-2,4,6-triamine	5.129	4.05
4	3-Mercaptopropionitrile	5.307	4.27
5	Acetic acid, diethoxy-, ethyl ester	5.342	2.51
6	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	6.735	8.29
7	(2R,5S)-2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahyd	7.900	4.95
8	2-Furancarboxylic acid, tetradecyl ester	9.075	6.23
9	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	9.988	26.41
10	Phenol, 2,6-bis(1,1-dimethylethyl)-	11.017	4.26
11	Dodecanoic acid, ethyl ester	11.952	3.21
12	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d	15.602	18.59
13	7-Octadecenoic acid, methyl ester	17.233	4.01
Total			**100.00**

The GC–MS analysis of the ethanolic extract of *Coriandrum sativum*(Table 4.20) revealed a complex chemical composition consisting of various bioactive compounds belonging to several functional groups such as esters, fatty acids, aldehydes, alcohols, and heterocyclic compounds. This diversity highlights the high extraction efficiency of ethanol as a polar solvent, capable of dissolving both polar and moderately non-polar constituents (Singh, *et al*., 2020).

At lower retention times, volatile and low-molecular-weight compounds such as Formamide, N-methyl- and 1-Bromoundecane were identified. These compounds have been reported to possess antimicrobial and metabolic regulatory properties (Wang, et al, 2004).

The detection of sulfur-containing compounds such as 3-Mercaptopropionic acid, ethyl ester derivatives indicates the presence of potent antioxidant and antibacterial agents, as sulfur heterocycles are known to inhibit oxidative stress and microbial growth. Medium retention peaks correspond to oxygenated compounds, notably acetic acid, hexyl ester

and 2-Furancarboxylic acid derivatives, which contribute significantly to the characteristic aroma and flavor of coriander. These compounds are widely recognized for their preservative and antimicrobial roles, often enhancing food quality and stability. Their detection supports the traditional culinary and medicinal uses of coriander as a natural flavoring and therapeutic plant. (Almeida and C. Magalhães, 2015)

At higher retention times, long-chain fatty acid esters such as Dodecanoic acid ethyl ester, and 7-Octadecenoic acid methyl ester were identified. These compounds represent the lipid-soluble fraction of the extract and are associated with anti-inflammatory, emollient, and antioxidant activities (Saranya D and Sekar. J.2016).

The relatively high area percentages of these fatty acid esters suggest that the ethanolic extract is rich in lipidic and esterified compounds that contribute to its pharmacological potential.

Overall, the GC–MS analysis confirms that the ethanolic extract of *Coriandrum sativum* contains a diverse range of bioactive constituents that collectively contribute to its pharmacological and nutraceutical value. The dominance of fatty acid esters and heterocyclic compounds aligns with previous studies demonstrating that coriander possesses antioxidant, antimicrobial, and anti-inflammatory activities. Consequently, these findings substantiate the traditional and industrial applications of coriander as a natural source of bioactive molecules suitable for therapeutic and food-related purposes.

4.4.3. Hexane extract

The following table illustrate the major compounds identified by GC-MS analysis for hexane extract, arranged according to their relative abundance in the extract. These results highlight the most prominent constituents that contribute to the chemical profile and potential biological activity of the sample.

Table (4-21): GC-MS analysis of hexane extract

Peak	Name	R.Time	Area%
1	p-Benzoquinone	3.091	2.40
2	Decane	4.067	6.40
3	Undecane	5.436	7.68
4	2[1-(R)-Pantetheinyl]-myristoyl-glycinamide	5.527	1.19
5	Decanal	6.953	1.69
6	1H-1,2,3,4-Tetrazole-1-acetamide, N-(5-methyl-3-	7.500	1.59
7	2-Decenal, (E)-	7.742	0.84
8	Hydroquinone	7.841	11.42
9	Undecanal	8.349	4.82
10	1-Decanol, 2-hexyl-	8.937	1.35
11	Dodecanal	9.691	7.01
12	Tetradecanal	10.970	1.69
13	2-Dodecenal, (E)-	11.685	9.96
14	(E)-Tetradec-2-enal	12.875	8.33
15	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,	15.596	1.66
16	6-Octadecenoic acid, methyl ester, (Z)-	17.232	3.09
17	3,6-Dodecadienoic acid, methyl ester	18.986	4.28
18	Bicyclo[10.8.0]eicosane, cis-	19.051	1.65
19	7-Tetradecenal, (Z)-	19.199	2.84
20	2H-Pyran, 2-(2-heptadecynyloxy)tetrahydro-	19.368	5.91
21	10-Methyldodec-2-en-4-olide	19.517	3.77
22	9-Octadecenal, (Z)-	20.433	4.59
23	Hexadecanoic acid, n.-octyl ester	21.168	1.06
24	Oleoyl chloride	22.082	3.60
25	Eicosane	23.476	1.21
	Total		**100.00**

The GC–MS analysis of the hexane extract of Coriandrum sativum in above table revealed a wide range of volatile and semi-volatile components predominantly composed of hydrocarbons, aldehydes, esters, and oxygenated compounds. The predominance of non-polar constituents in this extract aligns with the low polarity of hexane, which selectively dissolves lipophilic compounds such as alkanes and long-chain aldehydes.

Early-eluting peaks correspond to low-molecular-weight aliphatic hydrocarbons including Decane, Undecane, and Dodecane, representing the major fraction of the volatile oil. These compounds are typically associated with hydrophobic and aromatic

properties that contribute to the plant's distinctive fragrance profile. Hydrocarbons of this nature are also reported to exhibit mild antibacterial and antioxidant potential by stabilizing free radicals and enhancing membrane interaction (Harris,*et al*., 1999).

Compounds such as Dodecanal, 2-Dodecenal (E), and (E)-Tetradec-2-enal, which appeared at moderate retention times with relatively high peak areas (7.01%, 9.96%, and 8.33%, respectively), are unsaturated and oxygenated aldehydes that play a vital role in defining the characteristic citrus-like aroma of coriander seeds. Beyond their sensory contributions, these aldehydes possess strong antimicrobial and antifungal activities, likely due to their ability to interfere with microbial cell wall synthesis and enzymatic function (Hussain *et al*., 2019). The high abundance of these aldehydic compounds suggests their functional significance in both the flavor and defensive chemistry of coriander.

The detection of Hydroquinone (11.42%), a phenolic compound, is of particular interest, as it represents one of the dominant peaks in the chromatogram. Hydroquinone is a potent antioxidant and reducing agent, widely recognized for its ability to neutralize reactive oxygen species (ROS) and to contribute to the stabilization of lipid components within the extract. Its significant presence reinforces the antioxidant potential of coriander's non-polar fraction.

At later retention times, several fatty acid derivatives and esters, such as Hexadecanoic acid, n-octyl ester, Oleoyl chloride, and Eicosane, were detected, representing the higher-molecular-weight components of the extract. These long-chain lipophilic molecules are known for their emollient, anti-inflammatory, and antimicrobial activities, and are commonly reported in essential oils of aromatic herbs (Mochida,*etal* (2006). Their occurrence further indicates that the hexane extract retains compounds of pharmacological and nutraceutical interest despite its non-polar nature.

Overall, the GC–MS chromatographic profile of the hexane extract of *Coriandrum sativum* demonstrates a distinct predominance of non-polar and aromatic aldehydic hydrocarbons, complemented by phenolic antioxidants and fatty acid esters. Compared with the ethanolic extract, the hexane extract is richer in volatile hydrocarbons and unsaturated aldehydes, which are largely responsible for the characteristic aroma, preservative potential, and antimicrobial efficacy of coriander. The results collectively affirm that coriander's bioactivity arises from a synergistic interaction between hydrophobic aroma compounds and oxygenated functional molecules, highlighting the plant's value in both therapeutic formulations and flavoring industries.

A brief comparison between the three GC-MS profiles of coriander extracts; water (Table 4-19), ethanol (Table 4-20), and hexane (Table 4-21)) based on the obtained data. Water distillation extract (), the dominant compound is Cyclohexanol, 2-methyl-5-(1-methylethenyl) at (61.16%), a terpene compound (from the terpineol family). The main groups arehighly concentrated in terpenes and oxygenated terpenoids such as Eucalyptol, Linalool oxide, and Bornanone. General characteristics are rich in volatile and aromatic compounds, making it primarily responsible for the distinctive aroma of coriander. High diversity but dominated by one major compound.

In the ethanol Extract, the dominant compound is 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl) at (26.41%), a polyol (polyhydric alcohol). The main groupscharacterized by the presence of polar compounds and esters, such as 7,9-Di-tert-butyl1-oxaspiro(4,5) deca-6,9-diene-2, (18.59%), Formamide (7.38%), and Dodecanoic acid, ethyl ester. General characteristics of this extract tends to contain higher molecular weight and more polar compounds than the water extract. The presence of compounds like `Triazine` (a melamine derivative) suggests it may contain nitrogenous compounds.

While the hexane extract reflect the dominant compound is hydroquinone at (11.42%), a phenolic compound; however, the cumulative total of long-chain aldehydes and hydrocarbons surpasses it. The main groups is predominantly (hydrocarbon and fatty) in nature. Contains high proportions of alkanes (Decane, Undecane, Eicosane), long-chain aldehydes (Dodecanal, 2-Dodecenal), and fatty esters.This extract is non-polar (since hexane is a non-polar solvent), so it extracts fixed oils, waxes, and heavy hydrocarbon compounds.

Water extract isideal for studying flavor, aroma, and light volatile compounds, while ethanol extractis provides a comprehensive picture of semi-polar and polar compounds and may be the most biologically active due to its diversity (presence of phenols, esters, and acids). In contrast, hexane extract reflects the content of fats and hydrocarbons, useful for studying non-polar compounds that may be responsible for viscous or emulsifying properties.

4.5. Biological assay of extracts

4.5.1. Antimicrobial activity

The antibacterial activity of coriander oil was tested according to (Teshale,*et al* 2013) with miner modification. The following figures represent influence of six extracts on selected antimicrobial. Due to the limited space available on the petri dish for conducting the antimicrobial test, the extracts were divided into two groups.

4.5.1.1. First group

This group includes ethanol, petroleum ether and chloroform extracts, in addition standard reagent (gentamycin). The following figures (4-37), (4-38), (4-39), (4-40), (4-41) and (4-42) illustrate that for *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, Salmonella *and Candida respectively.*

Figure (4-37) effect of extracts on *Staphylococcus aureus*

Figure (4-38) effect of extracts on *Pseudomonas aeruginosa*

Figure (4-39) effect of extracts on *Escherichia coli*

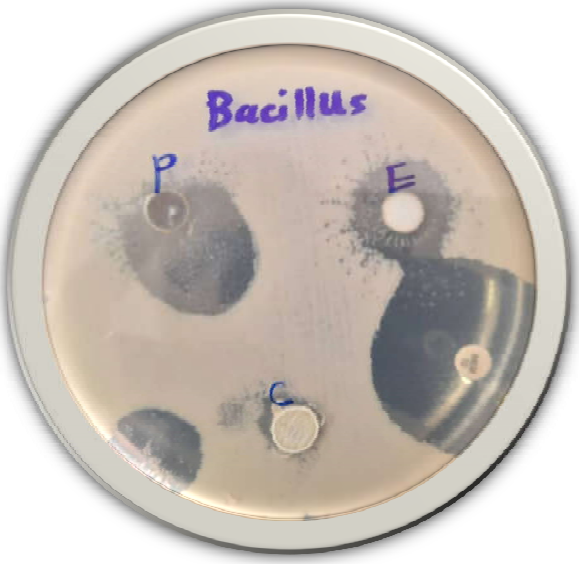


Figure (4-40) effect of extracts on *Bacillus cereus*

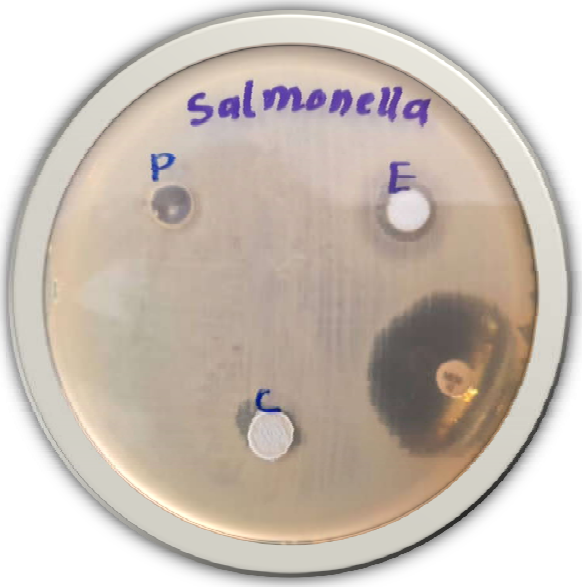


Figure (4-41) effect of extracts on *Salmonella*

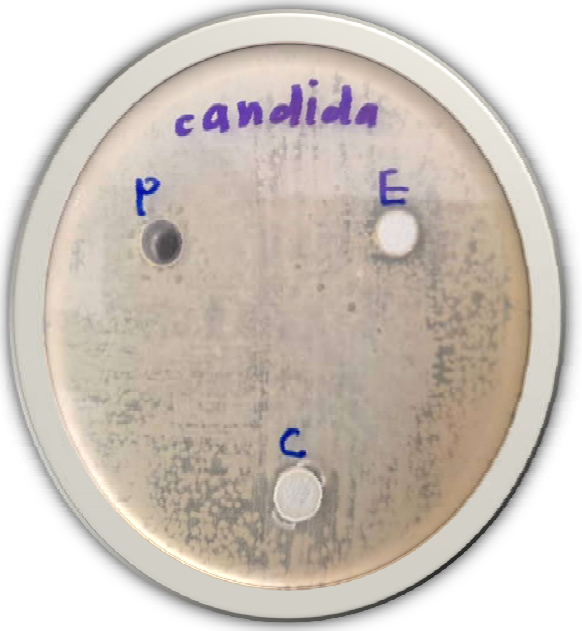


Figure (4-42) effect of extracts on *Candida*

4.5.1.2. Second group

This group includes the reminder extracts; hexane and water distillation, the next figures show their effect on the same bacteria and fungi.

Figure (3- 43): Effect of ethanol extract on *Staphylococcus aureu*

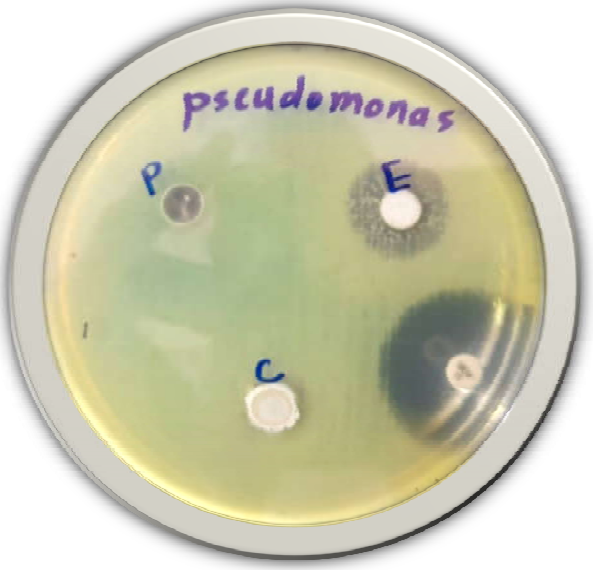


Figure (4-44) effect of extracts on *Pseudomonas aeruginosa*

Figure (4-45) effect of extracts on *Escherichia coli*

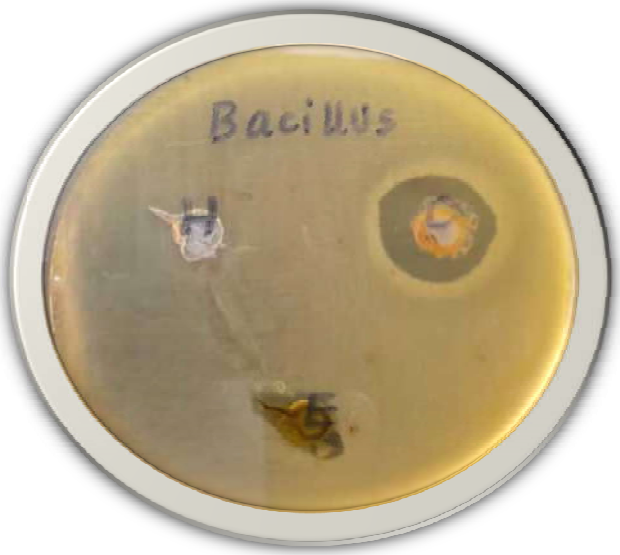


Figure (4- 46): Effect of ethanol extract on *Bacillus cereus*

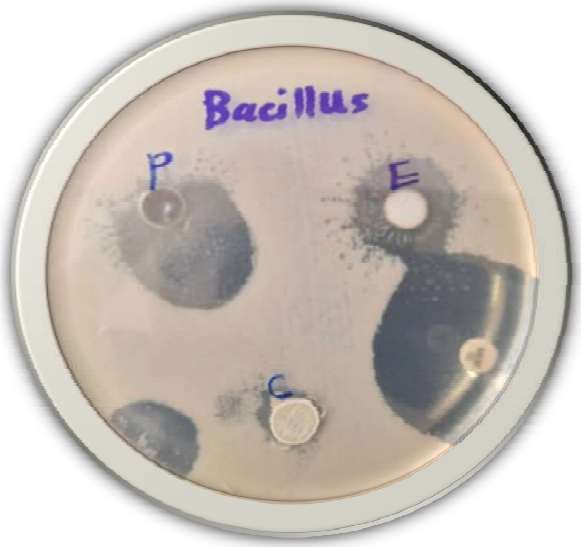


Figure (4-47) effect of extracts on *Bacillus cereus*

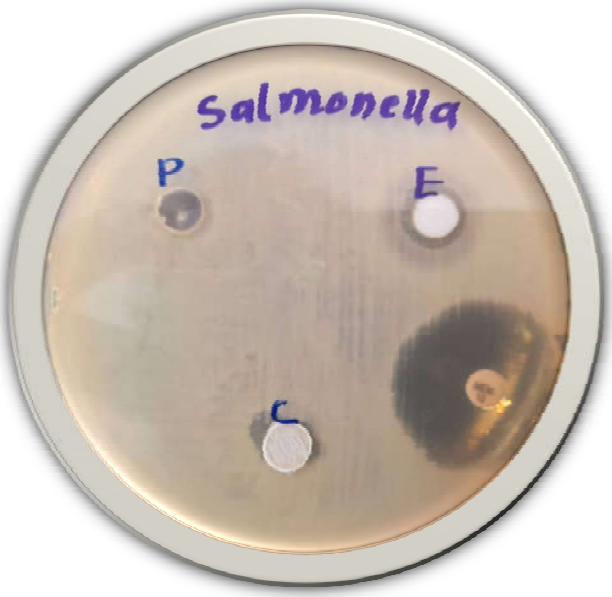


Figure (4-48) effect of extracts on *Salmonella*

Figure (3- 49): Effect of ethanol extract on *Candida albicans*

The provided data evaluates the antimicrobial efficacy of various solvent extracts of coriander (*Coriandrum sativum*) against a panel of Gram-positive bacteria, Gram-negative bacteria, and a fungal species. The activity is quantified by measuring the Mean Inhibition Zone Diameter (MIZD in mm) and is compared against a standard antibiotic, Gentamicin (Genta.), for the bacterial strains and MEM for fungi, table (4-22) reflects that

Table (4-22): Antimicrobial activity of coriander as antibacterial and antifungal

Antimicrobial	MIZD in mm						
	P. ether	E. acetate	Chlor.	Ethanol	Hexane	Water	Standard
E. coli	7	7	7	7	7	19	22
P. aeruginosa	7	15	7	7	7	20	21
B. cereus	20	17	7	30	7	30	27
Salmonella	7	10	7	7	7	19	25
S. aureus	7	7	7	7	7	20	30
Candida	7	7	7	7	7	13	-

The most striking observation is the superior and consistent antimicrobial activity of the coriander extract across all tested microorganisms. In contrast, the other solvent extracts (petroleum ether, ethyl acetate, chloroform, ethanol, hexane) demonstrated

predominantly weak or negligible activity, with an MIZD of 7 mm, which typically represents the baseline or no significant inhibition.

This finding strongly suggests that the active antimicrobial compounds in coriander are most effectively extracted by the methods used to produce its essential oil. These compounds are likely lipophilic (oil-soluble) secondary metabolites, such as terpenes (e.g., linalool) and phenolic compounds, which are known to disrupt microbial cell membranes. The general inefficacy of the other solvents indicates that either the concentration of active compounds in these extracts was too low, or the specific solvents were not suitable for dissolving the potent antimicrobial principles present in the plant material.

The data reveals a notable difference in susceptibility between the bacterial types. The Gram-positive bacterium, *Bacillus cereus*, exhibited significant sensitivity to multiple extracts. Notably, it was highly susceptible to the ethanol extract (30 mm) and the petroleum ether extract (20 mm), in addition to the oil (30 mm). Its inhibition zone for ethanol was even larger than that for the Gentamicin control (27 mm).

In contrast, the Gram-negative bacteria (E. coli, P. aeruginosa, Salmonella) and the other gram-positive bacterium (S. aureus) were largely resistant to all non-oil extracts, showing inhibition only from the oil and gentamicin. The exception was P. aeruginosa, which showed intermediate susceptibility to the ethyl acetate extract (15 mm).

This pattern aligns with established microbiological knowledge. The complex outer membrane of gram-negative bacteria, rich in lipopolysaccharides, presents a formidable permeability barrier to many antimicrobial compounds. Gram-positive bacteria, with their single, more accessible peptidoglycan layer, are generally more susceptible to plant-derived antimicrobials.

Bacillus cereus was the most susceptible organism, showing significant inhibition from three different extracts (oil, ethanol, petroleum ether). This broad susceptibility makes it a prime target for coriander-based antimicrobial applications. *Pseudomonas aeruginosa* notoriously resilient pathogen showed intermediate susceptibility to the ethyl acetate extract, which is noteworthy given its inherent resistance to many antibiotics. This suggests that coriander may contain compounds that can partially overcome its defense mechanisms. *Staphylococcus aureus* was resistant to all extracts except the oil and was highly susceptible to gentamicin (30 mm). This highlights the specificity of the coriander oil's active components. Antifungal activity (*Candida* sp.) was weak and only observed with the oil extract (13 mm). The absence of a gentamicin control is

appropriate, as it is not an antifungal agent. This indicates that while coriander oil has some antifungal properties, they are less potent than its antibacterial effects.

In all cases, the gentamicin control produced substantial inhibition zones, confirming the susceptibility of the tested strains to a known antibiotic. The coriander oil, however, demonstrated remarkably competitive activity. For B. cereus, the oil's MIZD (30 mm) exceeded that of gentamicin (27 mm). For other strains like S. aureus and Salmonella, the oil's activity, while strong, was less than that of the standard drug. In conclusion, the data unequivocally demonstrates that the antimicrobial property of coriander is primarily concentrated in its oil fraction. The extracts prepared with common organic solvents were largely ineffective, with the notable exception of certain activities against B. cereus and P. aeruginosa. The broad-spectrum, and in some cases potent, activity of coriander oil validates its traditional use in food preservation and herbal medicine.

These findings suggest that coriander essential oil holds significant promise as a natural antimicrobial agent, particularly against foodborne pathogens like B. cereus. Further research is warranted to identify the specific bioactive compounds within the oil, to determine their minimum inhibitory concentration (MIC), and to explore their mechanism of action and potential synergy with conventional antibiotics.

CHAPTER FIVE
5. Conclusion and Recommendations

5.1. Conclusion

This study provides a thorough investigation into optimizing the extraction of bioactive compounds from *Coriandrum sativum* and evaluating their functional properties. The core methodology involved employing six solvents of varying polarities under cold and heated conditions, with a novel emphasis on evaluating the impact of centrifugation as a purification step. The qualitative analysis phytochemical profile confirmed that coriander is a rich source of diverse secondary metabolites. Key findings indicated that solvent polarity selectively extracted different compound classes; for instance, chloroform and petroleum ether were effective for semi-polar and non-polar compounds like terpenoids and steroids, while ethanol extracted a broader range. Centrifugation consistently enhanced the detectable intensity of metabolites by removing insoluble plant debris and interfering impurities, leading to a purer and more concentrated extract. Heat generally improved extraction efficiency for most metabolites by enhancing solubility and diffusion rates.

The antioxidant potential, measured by the ability to suppress the rise in peroxide value (PV) in sunflower oil, was profoundly significant. All extracts showed antioxidant capacity, but the efficacy was highly dependent on the extraction method. Hot extraction followed by centrifugation consistently yielded the most potent antioxidant extracts across all solvents. These extracts were exceptionally effective in both fresh oil, where they prevented oxidation, and, more notably, in reused (thermally abused) oil, where they actively reduced the PV, indicating an ability to decompose existing hydroperoxides. This "curative" antioxidant effect highlights their potential for stabilizing used frying oils.

The chemical composition underlying these activities was elucidated by GC-MS. The ethanol extract was rich in esters and fatty acid derivatives, the hexane extract was dominated by aldehydes and hydrocarbons like dodecenal and hydroquinone, and the water-distilled essential oil was characterized by high concentrations of oxygenated terpenes such as linalool oxide. This diversity explains the broad-spectrum bioactivity of coriander.

The antimicrobial assay revealed that the water-distilled coriander essential oil was the most effective, exhibiting strong inhibition against Gram-positive (*Bacillus cereus*,

Staphylococcus aureus) and Gram-negative (*coli*, *Pseudomonas aeruginosa*, *Salmonella*) bacteria, as well as the fungus Candida. In contrast, most organic solvent extracts showed weak activity, underscoring that the potent antimicrobial principles are predominantly volatile, lipophilic compounds concentrated in the essential oil fraction.

In conclusion, this research successfully identifies extraction protocols, particularly heated extraction with centrifugal purification, to maximize the recovery of bioactive compounds from coriander. The results solidly position coriander extracts, especially the essential oil, as viable natural alternatives to synthetic antioxidants and antimicrobials in the food and pharmaceutical industries.

5.2. Recommendations

Based on the significant findings of this research, the following recommendations are proposed for future research and application:

- The food and pharmaceutical industries should consider adopting hot extraction coupled with centrifugation as a standard procedure for producing high-potency, clarified coriander extracts intended for use as natural antioxidants.

- Application in food preservation, given their superior efficacy in stabilizing reused frying oil, coriander extracts (particularly from chloroform, ethyl acetate, and ethanol) should be further developed as natural additives for the food service industry to extend the shelf-life and improve the safety of repeatedly used oils.

- For antimicrobial applications, future work should focus on the coriander essential oil (water-distilled extract). Research should aim to determine the minimum inhibitory concentration (MIC), identify the specific active compounds, and explore its synergy with conventional antibiotics.

- Further research is warranted to elucidate the precise mechanisms behind the "curative" antioxidant effect observed, particularly the ability of certain extracts to reduce pre-formed hydroperoxides. This could involve studying the redox chemistry of the dominant compounds identified in the GC-MS profile.

- A study should be conducted to explore the synergistic effects of combining different extracts (e.g., ethanolic and hexane) to create a broad-spectrum, multi-functional natural preservative with enhanced antioxidant and antimicrobial properties.

- Before commercial application, comprehensive toxicological studies and sensory analyses are essential to ensure the safety and consumer acceptability of food products fortified with these coriander extracts.

- Future efforts should be directed towards scaling up the optimized extraction process and developing stable formulations (e.g., powders, encapsulated oils) for easy integration into various food and pharmaceutical products.

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- بلولة,الماحي محمد احمد ,تقنية بلولة,111م/2025/م ك, مجلس حماية حق المؤلف والحقوق المجاورة السودان2025م (Biloia techenque- 2025)