Physicochemical, Bioactive & Antimicrobial Property Analysis of Cardamom (*Elettaria cardamomum*) Seed and Husk and its Flavor Extracted by Different Extraction Methods

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Roll no: 0121/15

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University Chattogram-4225, Bangladesh

March, 2023

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Session: January – June/ 2021

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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PLAGIARISM VERIFICATION

Title of Thesis: **Physicochemical, Bioactive & Antimicrobial Property Analysis of Cardamom (*Elettaria cardamomum*) Seed and Husk and its Flavor Extracted by Different Extraction Methods.**

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DEDICATED TO MY RESPECTED AND BELOVED PARENTS AND TEACHERS

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The author

March 2023

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Abbreviation

%	: Percentage
&	: And
ANOVA	: Analysis of variance
AOAC	: Association of Official Analytical Chemists
FFA	: Free Fatty Acids
CHO	: Carbohydrate
dL	: Deciliter
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
et al	: Et alii/ et aliae/ et alia
etc.	: Et cetera
G	: Gram
GAE	: Gallic acid equivalent
TPC	: Total Phenolic Content
TFC	: Total Flavonoid Content
TE	: Trolox equivalent
ZOI	: Zone Of Inhibition
QE	: Quercetin equivalents
AD	: Alzheimer's Disease
PPM	: Parts per Million
m	: Meter
SD	: Standard Deviation
spp.	: Species
µg	: Microgram
SPSS	: Statistical Package for Social Science
^{o}C	: Degree Celsius
RSA	: Radical Scavenging Activity
DM	: Dry Matter

Abstract

Cardamom (*Elettaria cardamomum*) is a popular spice all over the world that has the potential to be classified as a functional food because it provides health benefits that go beyond its basic nutritional value. The current study attempted to investigate the nutritional properties of individual cardamom husks and seeds. The husk has a higher value of crude fiber (31.8%) and ash (15.4%) than the commonly used cardamom seed which contains 11.8% crude fiber and 4.6% ash. Cardamom husk has a higher concentration of minerals such as calcium (12.11%), magnesium (4.84%), and potassium (24.03%) where seed contains calcium (2.46%), magnesium (2.48%), and potassium (13.1%). Because cardamom has a high fat content, fatty acid analysis was performed to determine the nutritional value of the spice. Cis-oleic acid (C18:1) is found in the highest concentrations in both the seed (46.87%) and the husk (22.79%). Palmitic acid and linoleic acid are also more abundant as plant fatty acids. Cardamom, despite containing a certain amount of protein, did not produce a chromatogram for amino acid analysis. It could be a case for further research. Furthermore, cardamom oil is used for both food and pharmaceutical purposes. So, cardamom flavor was extracted by rotary evaporator, Liebig condenser and cabinet dryer in order to study its bioactive, antimicrobial, and radical scavenging activities. Though the yield of extract was highest from condensation method, the highest amount of bioactive compound recorded for husk sample of drying technique as 145.001mg QE/100gm. The RSA value ranged from (54.6-77.44) %, which has a greater impact when using cardamom for therapeutic purposes. On the other hand, cardamom seed and husk extract showed antimicrobial properties. There is potential for using cardamom extract as an alternative to chemical preservative in foods such as meat, which can be well blended with this spice.

Keywords: Bioactive compound, Radical Scavenging Activity, Antimicrobial properties, Chemical preservative

Chapter-1: Introduction

Cardamom, which is generally regarded as green cardamom, is an herbaceous plant in the ginger family. It is native to southern India and is the most common spice on the subcontinent. Usually, whole cardamom seeds are used as a spice. This species is native throughout tropical and subtropical Asia. Two main types of cardamom are found natively. These are black cardamom and green cardamom, and there is also a bleached version of green cardamom, which is called white cardamom. (Prabhakaran, 2011). The Zingiberaceae family's black cardamom (*Amomum subulatum*), commonly known as Nepal cardamom (badi or kali elaichi in India and Nepal), is a comparatively large pod (C.P. Khare, 2011). The Mughal kings who invaded India centuries ago loved cardamom and used it in rice, sauces, and meat dishes. Making masala chai, a form of milk-based, spiced black tea, requires it. One of the many spices included in the well-known garam masala spice blend is cardamom. When added to hydrating beverages, cardamom adds fantastic flavor and scent in addition to having several medical benefits. As a result, it is called the "Queen of Spices."

Cardamom is a wonderful source of nutrients and a good source of energy. According to the study, it contains 24.14% crude fiber, 9.29% crude protein, 10.025% oleoresin, 6.72% ash, and 41.08% total carbs. 2020 (Gebreyes *et al*.,2020) A good source of vitamins and minerals is cardamom. Cardamom contains large amounts of minerals like calcium, magnesium, and potassium. 100 g of this electrolyte contains 1119 mg (Vutakuri *et al.*, 2018). Cardamom has a high nutritional value.

One of the oldest essential oils in existence is cardamom oil. The seeds are steam-distilled to extract the oil. The flavor of cardamom oil is intense, aromatic, spicy, and warming. It can be a little bit burning, aromatic, and even have a hint of bitterness when used in large quantities. Cardamom capsules contain large amounts of flavonoids, carotenoids, and terpenes, among other bioactive metabolites (Ashokkumar *et al*., 2019). In numerous trials, including those involving cardamom capsules, a comparable sample weight of 20 g was employed, and 0.8–1.5 ml of essential oil was produced (Murugan *et al*., 2005; Ashokkumar *et al*., 2019). Cardamom is used to produce oil. It has fatty acids in it. Linoleic

(C18:2, 17.6%), palmitic (C16:0), and oleic (C18:1, 43.7%) Acids are the most abundant to note. (Hamdan *et al*., 2008)

Green cardamom showed total phenolic contents of 0.317± 0.00-1.66 ± 0.05 g/100 g and total flavonoids of 11.33±0.03-4.63±0.12g/100 g. (Bhatti *et al.,* 2020). It shows antioxidant property, which is also known as radical scavenging activity (RSA). Due to their antibacterial, anti-inflammatory, and antioxidant properties, essential oils are in high demand. Yet, it has been difficult to quickly get high-quality extracts while utilizing minimal energy. Green technologies are therefore emerging as potential substitutes. (Castillo *et al.,* 2023)

The antidiarrheal effect of cardamom extract was also seen by experimenting on lab mice (Zavala *et al*., 1998). Recent epidemiological studies have demonstrated a link between prolonged sickness, a higher risk of invasive disease, hospitalization, and increased mortality in humans infected with resistant Salmonella and E. coli (Molbak, 2004). In order to evaluate the potential of cardamom extracts as antibacterial agents, their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was measured.

Generally, the seeds of cardamom are used as spices. But in this study, the husk of cardamom is also used to measure its potential. Cardamom has a unique flavor that goes well with both savory and sweet foods. Ground cardamom is usually called for when the spice is added to batters and baked goods. Cardamom has the latent to act as a functional food due to its high concentration of nutrients, fatty acids, and bioactive compounds. Many experts recognize flavonoids, vitamin C, and essential oils as potential antioxidant compounds that are beneficial to health. As a result, it is thought that cardamom rhizome could be used as a component of functional food (Bhadra *et al.,* 2021)

Nowadays a term is mostly going that “food as medicine”. Considering the therapeutic value of food, it is considered as the replacement of drug as well as the antibiotics. Cardamom enriches diet not only by adding flavor but also taking modification in nutritional state. If we get habituated to a diet which is a combination of phytochemicals and antioxidant, it may consider as preventive measure which will definitely cut the cost

of cures literally medicine. Doctors are now learning that one of the best ways to reduce inflammation lies not in medicine cabinet but in refrigerator. This is the reason behind choosing this topic for research study. The research would have greater impact if cardamom is cultivated in Bangladesh in large scale.

Specific Objectives

1. To analyze the nutritive value of cardamom seed and husk.
2. To assess the antioxidant capacity, phytochemical components of extracted flavor from cardamom seed and husk.
3. To determine the antimicrobial property of ethanolic cardamom husk and seed extracts against *E. coli*. and *staphylococcus aureus*.

Chapter-2: Review of Literature

2.1 Overview of cardamom

Cardamom seeds have been used in the kitchen as a spice, flavoring agent, and medicinal herb since ancient times. Leafy shoots of the cardamom plant arise from 1.5 to 6 meters (5 to 20 feet) from the branching rootstock. Flowering shoots that are about 1 meter (3 feet) long and can be upright or sprawling bear numerous flowers that are about 5 cm (2 inches) in diameter and have greenish petals and a purple-veined white lip. The entire fruit, measuring 0.8 to 1.5 cm in length, is a green, three-sided oval capsule with 15 to 20 dark, reddish brown to brownish black, hard, angular seeds (Ravindran *et al.*, 2002).

Though cardamom is mostly popular in south India, distinct languages have different names for it, including Elachi (Hindi), Elam (Tamil), Huba Alhal (Arabic), Phalazee (Burmese), Kardamom (German), Ts'ao-k'ou (Chinese), Kapulaga (Indonesia), and Nucșoară (Romania).

Figure 2.1: **Cardamom plant**

2.2 Origin and Taxonomy of cardamom

The Zingiberaceae family includes the cardamom plant (*Elettaria cardamomum*). Elettaria cardamomum is the most important spice in the order's largest family, Zingiberaceae, which has 56 genera and over 1,300 species. Ginger is among the important spices in the family. cardamom is thought to have originated from an area extending from southern India. Cardamom fruits can be collected from wild plants native to southern India's moist forests, but the majority of cardamom is cultivated in India, Sri Lanka, and Guatemala (Prabhakaran, 2021).

Table 2.1: The taxonomy of cardamom is as follows, according to ITIS (Integrated Taxonomic Information System):

Kingdom:	Plantae
Sub Kingdom:	Viridiplantae
Infra kingdom:	Streptophyta
Super division:	Embryophyta
Division:	Tracheophyta
Sub division:	Spermatophytina
Class:	Magnoliopsida
Super order:	Lilianae
Order:	Zingiberales
Family:	Zingiberaceae
Subfamily:	Alpinioideae
Species:	Cardamomum
Binomial name:	***Elettaria cardamomum***

Source: ITIS (Integrated Taxonomic Information System)

2.2.1 Cross section of cardamom seed

A cross section is the result of cutting a section along its length or through the middle. The cut is made perpendicular to the axis. It can be a drawing, photograph, or diagram that depicts how a plant appears when cut from one side to the other with a perpendicular line running through the center (James, 2011).

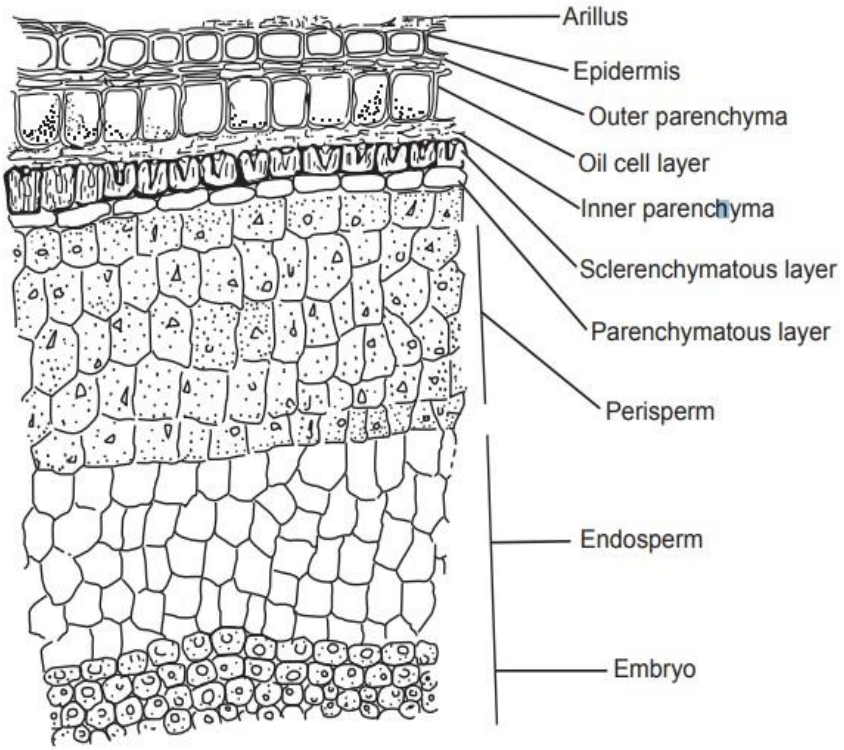


Figure 2.2: Cross sectional view of cardamom seed

The essential oil is found in large parenchyma cells beneath the seed coat's epidermis. The essential oil content ranges between 2 and 10%; its main components are cineole and -terpinyl acetate (kumar *et al*., 2013).

2.3 Production and cultivation history of cardamom

Cardamom was derived from wild plants in southern India's Western Ghats. The plants grew so abundantly in this area that it became known as Cardamom Hills. During their travels, the Vikings discovered this spice and brought it back to Scandinavia. Plantations of cardamom were established by British colonists during the nineteenth century, and this is where much of the green and black cardamom that we use today comes from. Guatemala is the world's largest commercial producer of cardamom. It is considered a more valuable crop than coffee in some parts of Guatemala (Prabhakaran, 2011).

2.3.1 Cultivation pattern in Bangladesh and sub-continent

Karnataka is India's largest cardamom producer. Cardamom was first mentioned in Sumerian and Indian Ayurvedic literature. Tanzania, Malaysia, and Guatemala are now among the countries that grow it. Prior to World War I, German coffee planter Oscar Majus Kloffer introduced Indian cardamom to Guatemala; by the year 2000, Guatemala had surpassed India as the world's largest producer and exporter of cardamom. (Varadarasan, 2019)

According to a report by S. Dilip Roy of the Daily Star published in 2012, a woman has successfully farmed this spice item in her nursery at Fulgacchii village in Lalmonirhat sadar upazila for the first time in Bangladesh. Local farmers are encouraged to farm this item on their lands, and as a result, Asma Begum of Fugacchi village began farming cardamom in her nursery in 2012. In her nursery, she planted 50 plants. In 2013, she harvested two kilograms of cardamom. She merely gave routine care to her cardamom plants instead of using pesticides or fertilizers.

The ideal growing conditions for this crop are clay soil and shade. A mature cardamom plant can produce at least one kilogram of product annually. Mushtaq Ahmed Lennin, the scientific officer at the Lalmonirhat species research subcenter, claims that this is cardamom of the black kind. And even in the Bogra region, this has developed on the hill tract. Dry and humid conditions are ideal for growing cardamom. According to Allaudin Khan, the scientific officer in charge of the spices research subcenter, the soil in Fulgacchi village is ideal for growing spices.

2.4 parts of whole Cardamom

2.4.1 Cardamom Husk

It is the green skin of the green cardamom. It has the aroma, flavor, and taste of green cardamom. It can be used to grind a wide range of ingredients, including spices and sweets.

Figure 2.3: Husk of cardamom separated from seed

2.4.2 Cardamom seed

Cardamom seed is the inner part of the whole cardamom that is black in color and circular in shape. The flavor of the seeds is warm, slightly spicy, and intensely aromatic, almost camphor-like. Cardamom seed and shells can be used to flavor cappuccinos and tea beverages, as well as stews, sweets, and meat dishes. Cardamom can also be taken as a supplement for its health benefits (Davidson, 2013).

Figure 2.4: Seed of cardamom separated from husk

2.5 Comparison within black and green cardamom

Amomum subulatum, also known as black cardamom, Hill cardamom, or Nepal cardamom, is an herbaceous plant in the Amomum subulatum family. Two common variety of cardamom can be compared on following basis (Sing *et al.*, 2008).

• **Appearance**: Black cardamom capsules, which are truly dark brown in color, are larger than green cardamom pods, which have a slight green tinge.

• **Flavor**: Green cardamom has a pleasant eucalyptus flavor, which makes it a popular spice for both sweet and savory dishes, including desserts. Black cardamom, on the other hand, has a smokiness and menthol notes that make it popular in soups and sauces.

• **Growing regions:** green cardamom is grown in southwest India and in parts of Central America. Nepal, India, and China, on the other hand, are the major producers of black cardamom (all around the Himalayas).

• **Medicative applications**: All seasonings have numerous health advantages and are commonly used in Indian and Chinese cuisine. Asthma has been treated with black cardamom, which is said to aid in digestion. Green cardamom, on the other hand, is just a conventional sleeping pill.

• **Processing**: Green cardamom is harvested at the beginning of the growing season, utilizing the pretty much the entire shells and the seeds. Harvesters, on the other hand, pick black cardamom late in the planting period. Production companies toss aside a whole seed pods after browning them on to an open flame (to imbue those with such a musky odor).

2.6 Reason of using green cardamom

When compared to black types, they have a better flavor. Green cardamom, on the other hand, is much more common in Bangladesh than black cardamom.

Figure 2.5: Black and green cardamom

2.7 Nutritional Attributes

Research says that cardamom seed contains Saturated fat is 1 g, 5 5% of total fat (7 g), and trans-fat is 0 g.% of total fat (7 g), and trans-fat is 0g Sodium is 18 mg. 1%; lipids 0 mg. 0% Potassium: 0%, 0 mg. 68 g of total carbohydrates. 25%, or 28 g, of dietary fiber. (Krishnakumar *et al*., 2002)

There are 28 grams of dietary fiber per 100 grams of cardamom, which can prevent constipation. Many disease-preventing phytochemicals can be found in cardamom. Moreover, it's a wonderful supplier of vital electrolytes like magnesium, potassium, calcium, and phosphorus. Together with this, it also contains a wealth of vital nutrients like riboflavin, niacin, and vitamin C. (Vitamin B2) (Hamdan *et al.,* 2008)

2.8 Fatty acids of cardamom

The basic elements of fat in the human body as well as the substances we consume are called fatty acids. Saturated, monounsaturated, polyunsaturated, and trans fats are the four main kinds of fatty acids. Trans fats and saturated fatty acids both raise the likelihood of developing coronary artery disease. In a study, it was shown that cardamom extract containing khoa showed 0.60% oleic acid as free fatty acid (FFA) (Patel *et al.,* 2023) Oleic (18:1), linoleic (18:2), and -linolenic (18:3) acids are the three C18 species that make up the majority of the unsaturated fatty acids (UFAs) in plants. Those basic substances serve a variety of vital functions in plants and are thus key to the economic viability of oil seeds.

2.9 Cardamom flavor

Cardamom flavoring is primarily added to processed foods via water-infused cardamom oil or liquid cardamom volatile oil (Govindarajan *et al*., 1982). A greenish oleoresin containing at least 70% essential oil is obtained by steam distilling cardamom-flavored blended seeds. It tastes like it's been seasoned. At high temperatures, the turbulent oils may change. Terpenoids, which comprise the oils in cardamom, are more likely to occur in harsh environments such as those with acids, sunlight, air, or temperature. The appealing flavor of the spice is due to an increase in p-cymene, a terpene with a perilla fragrance, at

the expense of its primary component, terpinyl acetate (Brennand and Heinz, 1970). By enclosing fine dust of a base material within a constant polymeric membrane

2.10 Cardamom essential oil

Cardamom seeds have a wide range of therapeutic applications in the Ayurvedic and Unani medical traditions. Its oil is used in food preparation, fragrance, healthier alternatives, medicines, and beverages as a fragrant, demulcent, and stimulating agent. It is used as both a preventative measure and a treatment for sore throats, lungs tightness, eye irritation, digestive issues, and respiratory illness (Kaur & Mahajan, 2013). Anorexia, kidney stones, asthma, bronchitis, and urinary tract infections all have medical applications. It is commonly used to treat constipation, spastic colon, and digestive system disorders (Bakhru, 2001, Korikanthimathm *et al*., 2000). Many of cardamom's pharmacological properties include antioxidants, pro, bactericidal, antitumor, antimicrobial, and pesticidal action (Ashokkumar *et al.*,2019).

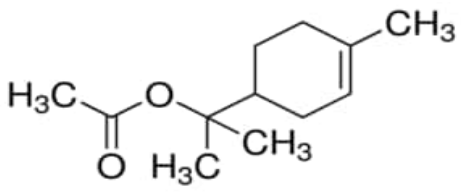


Figure 2.6: chemical structure of alpha terpinyl acetate

2.11 Bioactive compounds

Bioactive compounds are defined by the National Cancer Institute of the USA as a type of chemical that can be found in trace amounts in plants and certain foods (such as fruits, vegetables, nuts, oils, and whole grains). The actions of bioactive compounds in the body may promote good health.

2.11.1 Flavonoids

A vast class of phenolic plant components called flavonoids is included. They are advertised as 2-phenylbenzopyrone derivatives. In flavonoid compounds, the carbon atoms

are organized in two benzene rings, which are joined by an oxygen-containing pyrene circle. Flavonoids such as quercetin, kaempferol, and quercitrin can be found in approximately 70% of plants. Flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanins, calichons, catechins, and leucoanthocyanidins are some of the other flavonoids. Cardamom also contains phenolic compounds. Most herbs, fruits, crops, and foliage contain phytoconstituents, molecules called flavonoids, that have possible uses in medical science. Flavonoids have a variety of health advantages, such as viral, antitumor, and antioxidant properties, but they also have anti-inflammatory characteristics. Cardamom is a good source of flavonoids.

2.11.2 Phenolic compound

Phenolic substances are secondary plant metabolites that result from the metabolism of phenylpropanoid in pentose phosphate and plant shikimic acid. These can be anything from straightforward phenolic compounds to polymeric compounds, all of which contain benzene rings with one or more hydroxyl substituents. Vanillic acid, gallic acid, caffeic acid, -hydroxybenzoic acid, gentistic acid, protocatechuic acid, and -coumaric acid are the primary phenolic chemicals found in cardamom (Variyar, Bandyopadhyay, & Thomas, 1998)

2.12 Antioxidant activity

One of the ways the body fights peroxidation is by producing antioxidants either internally (endogenous antioxidants) or externally (via diet) (exogenous antioxidants). Antioxidants aid in the prevention of illness by scavenging excessive oxidative stress, shielding tissues against its adverse effects, and also eliminating free radicals Antioxidants aid in the prevention of illness by scavenging excessive oxidative stress, shielding tissues against its adverse effects, and also eliminating free radicals. Antioxidant supplements are compounds that are either chemically manufactured or derived from natural sources. They naturally differ chemically from the natural antioxidants present in meals. There is controversy over whether antioxidant pills offer the same health benefits as antioxidants contained in food. The evidence is still ambiguous, even though antioxidant supplementation is a contentious issue and is growing in popularity in so many wealthy countries. Antioxidants might

benefit numerous chronic illnesses, according to epidemiologic studies, but systemic supplement use has been constrained by a variety of factors, including a dearth of randomized and control studies, lengthy impacts, and concentration requirements for varied disorders. If used in amounts far exceeding the suggested nutrient intake (RDI), supplements can potentially have pro-oxidant effects, such as causing peroxidation (Pham-Huy et al., 2008). Cardamom contains a total phenolic concentration of between 0.316 and 1.66 g per 100 g, a phenolic content of between 11.33 and 14.63 g per 100 g, and an antioxidant capacity of between 85 and 90 %, which is enough to avoid oxidative damage (Bhatti *et al*., 2019).

2.13 Antimicrobial effect

Remarkable antimicrobial property has been demonstrated by cardamom extracts, which makes them highly helpful in the search for new drugs. Cardamom crude extract has long-lasting antimicrobial properties towards S. typhi, Escherichia coli, Bacillus cereus, and Staphylococcus aureus germs (Singh et al., 2008; Kaushik et al., 2010). Likewise, cardamom extract in both methanol and ethanol suppressed the activities of the food-borne fungus Aspergillus spp., indicating that it has potent antifungal properties. Streptococci mutans and Candida albicans, two oral germs, were resistant to the antibacterial effects of cardamom extracts. Cardamom may therefore be used to reduce cavities because it enhances saliva production (Aneja and Radhika, 2009).

2.14 Uses of cardamom seed in different aspects

Cardamom seed has been used extensively as a culinary spice throughout history. In addition to being a food spice, it has also been used as a traditional treatment for stomachaches. It is sometimes used to make handwash and mouthwash. In addition, many people in several Arabic countries, including Saudi Arabia, chew cardamom seeds like tobacco. As a result, it appears that the cardamom seed's oral toxicity for humans is neither significant nor ignored. This behavior prompted researchers to investigate the antibacterial properties of cardamom seeds, particularly in the control of germs that can cause tooth, skin, and hair problems such as dandruff, acne, and dental caries (Isao Kubo, Hisae Muroi, and Masaki Himejima). One of the oldest spices in the world is thought to be cardamom.

This spice has been used for cooking for at least 4,000 years. Cardamom was utilized extensively in ancient Egyptian rites, medicine, and even embalming. Because of its strong scent, cardamom was used by the Greeks and Romans. It was a key component of fragrances and aromatic oils.

2.14.1 Culinary

Cardamom is a staple flavor in Middle Eastern as well as Indian cuisines and therefore is frequently employed in those cuisines' customary spice mixes. This spice is frequently used to prepare the classic beverage cardamom teas in Asia. In addition, it is a highly-liked spice in many Scandinavian dishes, including wine and glogg. It is a staple ingredient in all breads and delicious pie meals.

2.14.2 Health benefits

Cardamom has been used medicinally for thousands of years. It has been most commonly used to treat indigestion, asthma and bad breathing problem. Memory problems and memory impairment are hallmarks of the progressive degenerative condition known is Alzheimer's disease (AD). Free - radical, cholinergic enzyme, or -amyloid (A) peptide are a few therapeutic candidates connected to AD. Phytochemical component derived from plants offer a huge variety of chemicals as just a source of novel medications. In order to explore the illness benefits in AD, Elettaria cardamomum L. Maton. extract and its active associated with a greater alphaterpinyl acetate were utilized. The latest research was a great success in proving alpha-terpinyl acetate's therapeutic effects because it unites to various drug objectives and inhibits acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), A-induced neurotoxicity, hydrogen peroxide-induced oxidative, antioxidant properties, as well as amyloidogenicity.

2.15 Dosage of cardamom oil

The use level of this oil in food is strongly dependent upon the further processing of the food (high temperatures for baked goods, etc.) but it would be about 0.20 to 0.50% while the minimum perceptible is 0.04 to 0.05 mg % for a good and true cardamom oil. (Bhatti *et al*.,2020)

Chapter 3: Materials and Methods

3.1 Study Area

The experiment was conducted in the laboratory of the department of Applied Food Science and Nutrition, Applied Chemistry and Chemical Technology, Department of Food Processing and Engineering, Poultry Research and Training Center (PRTC), Department of Animal Science and Nutrition, Department of Physiology, Biochemistry and Pharmacology of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram. Sample was also tested at Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. A test is also done at the Biomaterials Research Laboratory of University of Chittagong.

3.2 Study Duration

The experiment was conducted for a period of six months from 15st August 2022 to 10th February, 2023.

3.3 Experimental Design

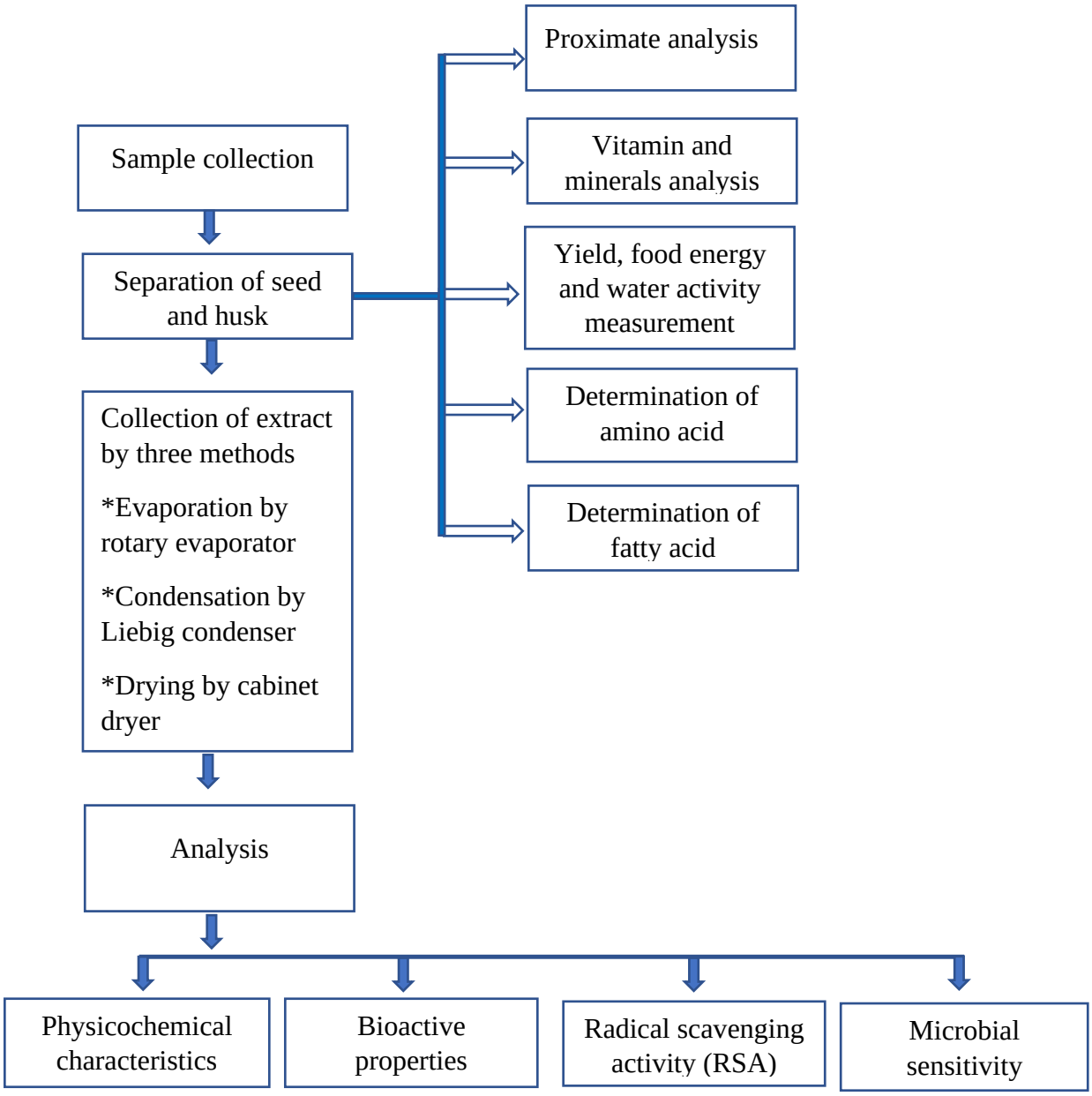


Figure 3.1 Stepwise design for the experiment

3.4. Collection of Sample Materials

Mature Elaach (*Elettaria cardamomum*) was collected from Riaz Uddin Bazar, local market of Chattogram. These all were imported from India. The green colored cardamom was chosen due to its enhancive flavor. Other necessary supplies for the experiment were obtained from the laboratories.

3.5 Proximate analysis

The quantitative analysis of macromolecules in food is referred to as proximate analysis. Protein, fat, moisture, ash, and carbohydrate levels are determined using a combination of techniques such as extraction, Kjeldahl. (James, 2015)

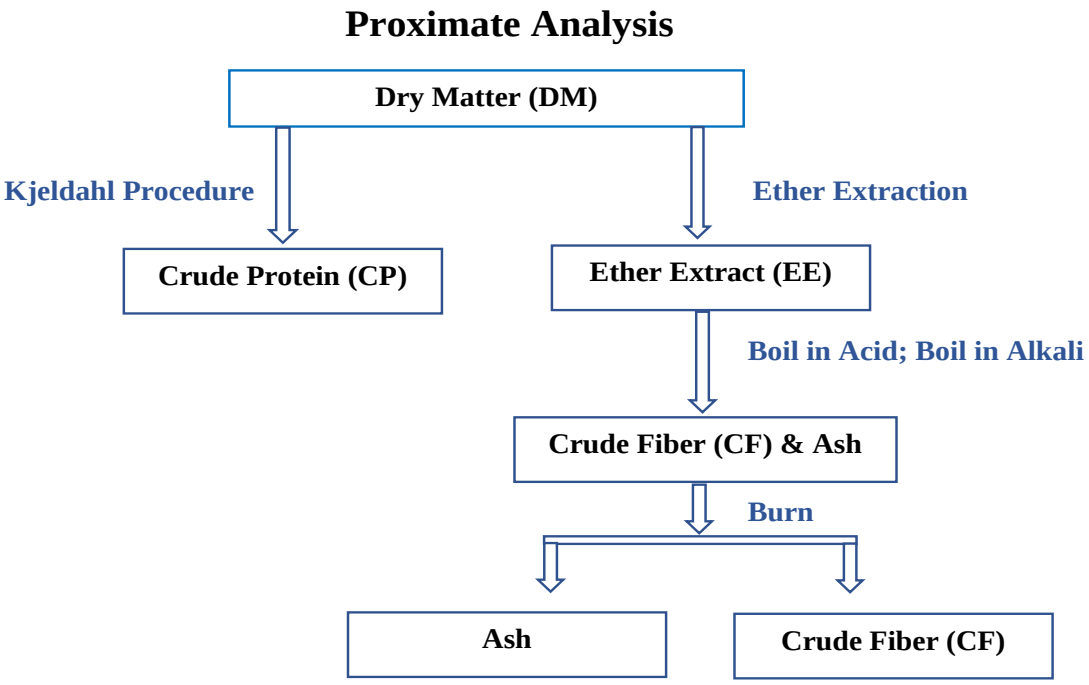


Figure 3.2: Flow diagram of proximate analysis

3.5.1 Moisture Content

One of the most significant and frequently used metrics in the production and monitoring of meals is water analysis. The water content is directly important economically to both the producer and the buyer since the quantity of dry mass in a bit of food is inversely related

to the amount of water it has. Yet, the impact of moisture on food stability and quality is considerably more significant. The Association of Official Analytical Chemists' standard technique was used to identify the moisture level (AOAC, 2016).

Procedure:

• An empty crucible was weighed
• Powdered sample of 5 gm was placed in the crucible
• The weight of the crucible with the sample was noted.
• The crucible was then dried in a hot air oven at 105°C for 48–72 hours.
• After removing the crucible from the oven, it was placed in a desiccator to cool.
• The crucible's final weight was determined.

Calculation: The percent of moisture was calculated as follow

Moisture % = {(Initial weight – Final weight)/ Sample weight} × 100

3.5.2 Total Solids/ Dry matter (DM)

Dry matter is what is left over after water has been removed, while water content indicates how much water is in the food item. Dry matter is the substance that remains after water has been removed, while moisture content indicates how much water is actually in the feed item. The DM part of food contains the elements needed for upkeep, growth, pregnancy, and lactation. (Buckmaster, D. 2005)

Calculation: AOAC procedures were used to calculate total solid (2016). And use the information gathered during the measurement of moisture, the proportion of the overall solid content got determined:

% Total solids = 100 – % moisture content.

3.5.3 Ash Content

AOAC procedures were used to determine the ash content (2016). The inorganic residue left over after organic stuff is destroyed is known as ash content.

Procedure:

• A pre-dried, weighted crucible containing 5 grams of powdered sample was taken

• Afterwards it was turned into charcoal.

• The charcoal was then placed in a muffle furnace to be heated for 4 hours at a temperature of about 650°C to remove all of the charcoal.

• The crucible was removed from the furnace.

• It was properly cooled in a desiccator before being weighed.

Calculation: The below phrase was used to determine the ash content.

Ash % = (Amount of ash supplied by sample/ sample weight) × 100

3.5.4 Estimation of Crude Fiber

The liquid portion of carbohydrates known as "crude fiber" is mostly made up of cellulose, hemicelluloses, and lignin. The AOAC technique was used to determine the crude fiber (2016).

Procedure:

• A measured quantity (5gm) of fat-free foodstuff in a mild acidic medium (1.25% H_2SO_4) for 30 min was heated

• Heating was also done in a low alkaline medium (1.25% NaOH) for 30 minutes at fixed volume,

• Afterwards subtracting ash from the residue generated, it was calculated by digestion. The AOAC technique was used to determine the crude fiber (2016).

• The leftovers were then heated to 550–600 °C (or white ash) in a muffle furnace.

Calculation

Calculation of the crude fiber percentage as follows:

% crude fiber = {(w- w1)/w2} × 100

Here,

W= Weight of crucible, crude fiber and ash

W1=Weight of crucible and ash

W2= Weight of sample

3.5.5 Estimation of Crude Fat

Different foods are dissolved in polar compounds (such as methanol or chloroform) to determine their fat content, and the supernatant is then separated by filtering. The filtrate is split into various funnel, the mix is left to dry to quantify the extracts, after which the anticipated fat percent is calculated. The crude fat content of the samples was ascertained using AOAC (2016) techniques and a Soxhlet equipment.

Procedure:

- Powdered cardamom sample (5gm) was taken in a thimble
- Hydrolysis of sample was done with HCl
- Extraction of hydrolyzed lipid materials with ether
- Evaporation of ether
- The lipid residue was heated constantly at 100
- Residue was expressed as % crude fat

Calculation: The percentage of crude fat was expressed as follows expression.
Fat % = (weight of the extract / weight of the sample) × 100

3.5.6 Estimation of Crude Protein

Both organic and inorganic samples can have their nitrogen concentration determined using the Kjeldahl method. Again, for purpose of calculating the crude protein, Kjeldahl nitrogen is measured in foods and beverages, flesh, feeds, grains, and forage crops. The Kjeldahl method is also used to determine the nitrogen content of soil, wastewater, and other substances. It is a recognized procedure that is defined in various prescriptive sources, including (AOAC, 2016). Digestion of sample was done by using a digestion mixture of sodium sulphate (Na_2SO_4), mercuric oxide (HgO) and concentrated sulphuric acid (H_2SO_4)

Procedure

- A clean and dry kjehldahl flask was used to collect 1 gm sample which was wrapped in an ash free filter paper

• 10 ml concentrated sulphuric acid (H_2SO_4) with a digestion mixture of sodium sulphate (Na_2SO_4), mercuric oxide (HgO) and concentrated sulphuric acid (H_2SO_4) in (1:1) ratio was added

• Digestion was done for 6 hours

• After that the beaker was let to cool and transferred to volumetric flask

• Then 10 ml of 50% NaOH and 2.5 ml of 15% of $Na_2S_2O_3$ mixture was taken in that flask

• Distillation was done for 10 minutes

• Distillate was collected with 2% Boric acid with an indicator

• The solution was titrated with 0.02N HCl

• At the same time a blank digestion was carried out.

Calculation

The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process. In the equations below, "N" represents normality. "ml blank" refers to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution. When boric acid is used as the receiving solution the equation is

Nitrogen % = {(ml of standard acid – ml of blank) × N of acid × 1.4007}/sample weight

3.5.7 Determination of Total Carbohydrate

For estimating the differences between both the Nitrogen Free Extractive and the carbohydrate content (NFE). The gap between 100 and the sum of the other proximal parts was presented as the answer.

Calculation: Hence it was calculated using the formula below-

% CHO = 100% - % (Protein + Fat + Fiber + Ash + Moisture content)

3.6 Vitamin and Mineral Determination

Vitamins and minerals are micronutrients that the body requires to perform a variety of normal functions. These micronutrients, however, are not produced by our bodies, so they must be obtained from the food we consume. Vitamins are organic compounds that can be classified as either fat- or water-soluble.

3.6.1 Determination of Vitamin C

Vitamin C is a water-soluble vitamin that can be found in citrus fruits and vegetables. Determination of vitamin C are dependent on chemical assays. Vitamin C concentration in plant or animal extracts is often assessed by its lowering effect on the dyes 2, 6 dichloride phenol indophenols. In this case, the color dye oxidizes the vitamin C to create dehydro-ascorbic acid. The dye is also changed into a colorless substance at the same time. It is simple to establish the reaction's termination point. (AOAC, 2016).

Reagent Requirement

1. Dye Solution (2, 6-dichlorophenol indophenols)
2. Metaphosphoric acid solution (3%)
3. Standard ascorbic acid solution

Procedure

• The dye solution was taken up to 0 marks in the burette.
• A conical flask was then filled with a 5 mL solution of vitamin C.
• The dye was added drop by drop to the conical flask using the burette.
• Titration was complete when pink appeared for 20 seconds and then vanished.
• At least three readings were taken.
• The same procedure was followed for an unknown concentration of ascorbic acid solution.
• The result was given in milligram percentage (mg%).

3.6.2 Determination of calcium

The formation of a color complex between calcium and o-cresolphtalein in an alkaline medium is used to determine the amount of calcium in the sample. The intensity of the color formed is proportional to the concentration of calcium in the sample.

Reagent Requirement

A. Buffer solution
- Ethanolamine
- Chloroform
- Methanol

B. Chromogen solution

Procedure

- Adjust the instrument to zero with distilled water
- Pipette into a cuvette standard, sample and reagent
- Mix and incubate for 5 min at 37c
- Read the absorbance of sample (A) and standard against the blank by spectrophotometer at 570 nm. The color is stable for 40 minutes.

Calculation

Amount of calcium(mg/dL) = [Sample (A) – Blank (A)] / [Standard (A) - Blank (A)] × 10

3.6.3 Magnesium determination

The method is based on the specific binding of a metallochromic indicator called calmagite. Magnesium at alkaline pH, resulting in a shift in the complex's absorption wavelength. The intensity of the formed chromophore is proportional to the magnesium concentration in the sample.

Reagent Requirement

A. Chromogen
B. Magnesium standard

Procedure

- Bring reagents and samples to room temperature.
- Pipette into labelled test tubes of reagent and sample
- Let the tube sit for 2 minutes at room temperature.
- Read the absorbance of samples(A) and the standard at 520 nm by colorimeter against the reagent blank.

Calculation

Amount of magnesium (mg/dL) = (A sample / A standard) × Absorbance of reagent blank

3.6.4 Phosphorus determination

Inorganic phosphate reacts with ammonium molybdate in an acid medium to form a phosphomolybdic complex that is measured at 340 nm.

Reagent Requirement

A. Molybdate reagent
B. Phosphorus standard

Procedure

- Pre incubate the working reagent, samples and standard
- set the photometer to 0 with the reagent blank
- pipet into a cuvette
- Read the absorbance of samples(A) and the standard at 340 nm nm by photometer against the reagent blank.

Calculation

Amount of phosphorus (mg/dL) = (A sample / A standard) × Absorbance of reagent blank

3.6.5 determination of potassium

Principle of determination of potassium based on the reaction takes place with sodium tetraphenyl boron in a specifically prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample.

Reagent Requirement

A. Potassium reagent
B. Potassium standard

Procedure

- Pipette into clean dry test tubes labelled as Blank (B), Standard (S) and Test (T)
- Mix well and incubate for 5 mins
- Measure the all absorbance

Calculation

Potassium in mmol/l = (Abs. T / Abs. S) × 100

3.7 Yield, Energy content and water activity analysis

The seed-to-husk ratio is calculated to determine the percentage of yield from 100 g of whole cardamom. From proximate analysis, the energy content per 100g of sample can also be determined. Besides water activity, food stuffing is an important issue for processing or storage purposes.

3.7.1 Yield percentage

The outer covering of a grain or seed is called a husk. To determine the yield of individual seed and husk from 100gm of whole cardamom, the seed husk ratio was measured.

Procedure

- Whole cardamom was taken
- Measurement of 100gm of cardamom
- Splitting off husk from seed
- Weight of husk and seed was taken

Calculation

The measurement taken as a percentage was used to calculate.

3.7.2 Energy content

The energy content of each sample was calculated using the following equation (Baer *et al.,* 1997)

Energy = (protein × 4.1) + (Fat × 9.2) + (Carbohydrate × 4.1)

3.7.3 water activity

The water activity (a_w) of a food is the ratio of the food's vapor pressure when in complete equilibrium with the surrounding air medium and the vapor pressure of distilled water under identical conditions (Gustavo *et al.,* 2020)**.** Water activity of seed and husk powder was measured directly by water activity meter.

Procedure

- Powdered sample was collected.
- In the plastic plate provided by the apparatus, approximately 5g was taken.
- Placing the plate in the meter-adjusted hole
- Fixing the meter's cover and turning on the power
- The measurement was displayed after a few seconds.

Figure 3.3: Novasina Digital Water activity meter (Model: Lab start, swift and touch)

3.8 GC Analysis of Anino Acids (AA)

There are two methods for sequencing amino acids: mass spectrometry and Edman degradation with a protein sequencer. Automated Edman amino acid sequencers are useful for analyzing polypeptides up to 50 amino acids in length (Russel 1986). In this study the method used for this assay was based on chromatographic technique.

Sample preparation

- First, 200 to 250 mg of sample was taken.
- The solution was then dissolved in 500 mL of hydrolysis solution (300 mL of 37% HCL, 200 mL of DI water, and 0.5 g phenol).
- The samples were kept at 120 0 c for 24 hours after soaking and mixing.
- Following that, the pH has been adjusted between 2.9 and 3.1.
- The sample volume was reduced to 250 mL after the pH was adjusted.
- Then, from this 250 mL sample stock, 100 µL of sample was taken and filtered through a 0.45M Syringe filter.
- 900 µL of sample dilution buffer (Na-acetate buffer, pH = 2.9 to 3.1) was added to this 100 µL of sample.
- Then it was time to go.

Here two special types of columns were used-

(a) Pre column for ammonia capturing

(b) Post column for amino acid separation (Sodium Column- 4.6 x 150 mm)

When samples were kept in an automatic sampler. 100 L of the sample was taken. Then the sample passed through the reaction chamber, where it reacted with the reagent ninhydrin. The base line for the isolation of amino acids was done with two different buffers with varied pH, one with an acidic 1.9 to 3.1 pH and the other with 10.5 to 11.85 pH.

3.9 GC Analysis of fatty acids

The basic elements of fats in the human body as well as the substances we consume are called fatty acids. Saturated, monounsaturated, polyunsaturated, and trans fats are the four main kinds of fatty acids. An elevated risk of coronary heart disease is linked to trans fats and saturated fatty acids. The body cannot generate essential fatty acids (EFAs), hence both humans and animals must consume them in order to maintain good health. Alpha-linolenic acid, an omega-3 fatty acid, and linoleic acid are the only two fatty acids recognized as essential for humans (an omega-6 fatty acid). Saturated or unsaturated fatty acids are non-essential fatty acids. Saturated fatty acid chain is made up entirely of single bonds between carbon atoms. Because it can cause atherosclerosis as well as heart disease, saturated fat is a harmful fat.

Oleic (18:1), linoleic (18:2), and -linolenic (18:3) acids are the three C18 species that make up the majority of the unsaturated fatty acids (UFAs) in plants. Those basic substances serve a variety of vital functions in plants and are thus key to the economic viability of oil seeds.

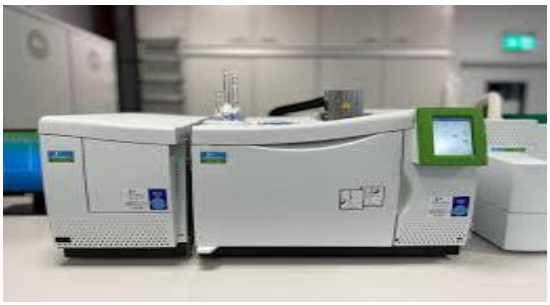


Figure 3.4: Gas chromatography for fatty acid determination

Procedure of analysis of Fatty Acids (FA)

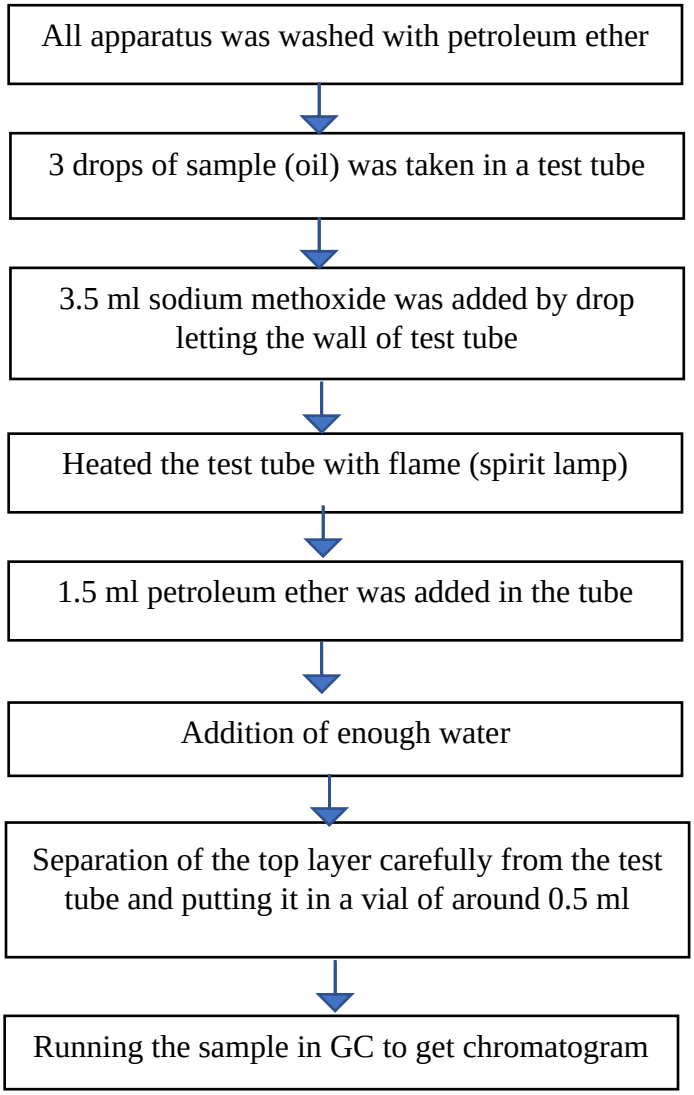


Figure 3.5: Gas chromatography procedure for fatty acid determination

3.10 Preparation of ethanolic extract

Absolute ethanol was employed to prepare the extract in a sample-to-solvent ratio of 1:10. The FDA and the Bureau of Alcohol, Tobacco, and Firearms control the use of ethanol in meals since it is a very pure type of alcohol. Ethanol is a substance that the FDA has classified as generally recognized as safe (GRAS) for use in food items. (U.S. Food and Drug Administration, 2022)

3.10.1 Preparation of *E. cardamomum* ethanolic extract from seed.

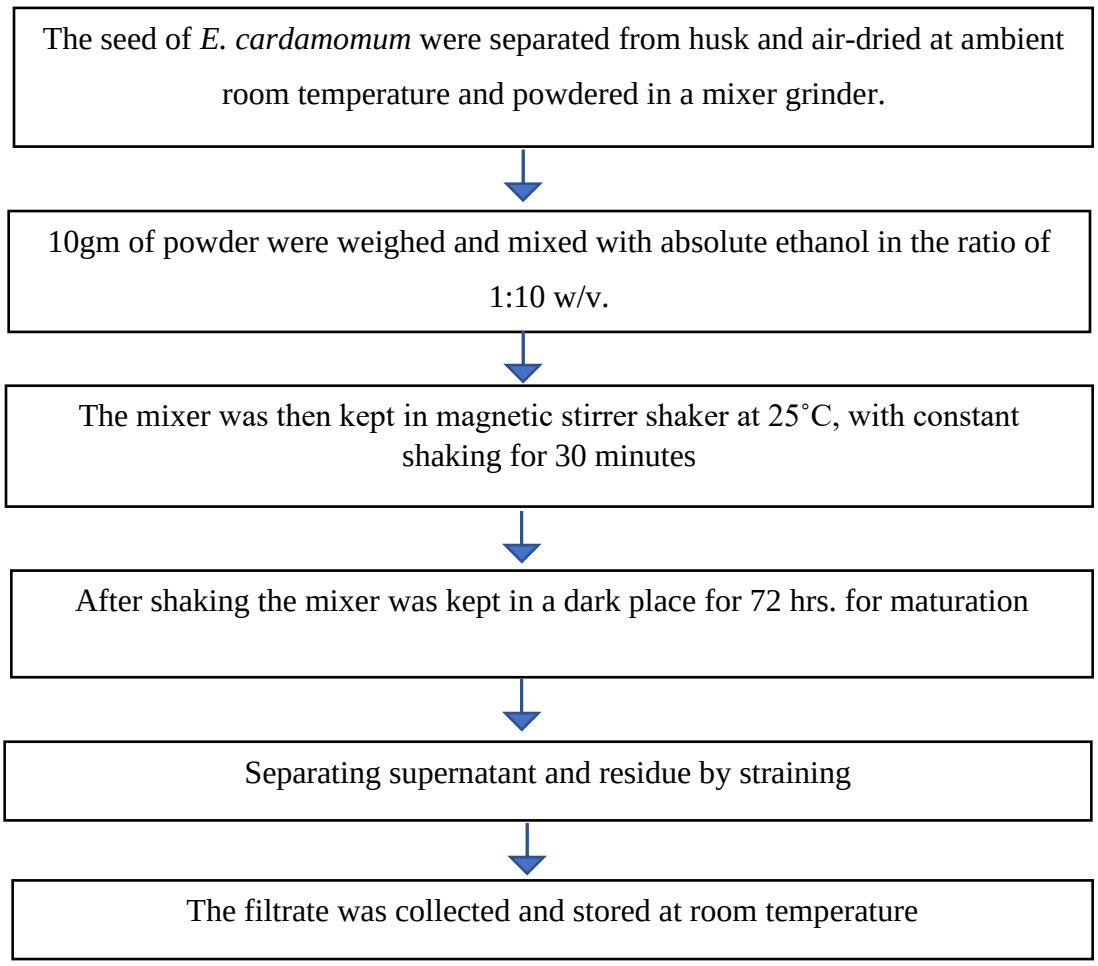


Figure 3.6: Ethanolic extract preparation flow sheet of cardamom seed

Figure 3.7: Ethanolic extract of cardamom seed powder

3.10.2 Preparation of *E. cardamomum* ethanolic extract from husk.

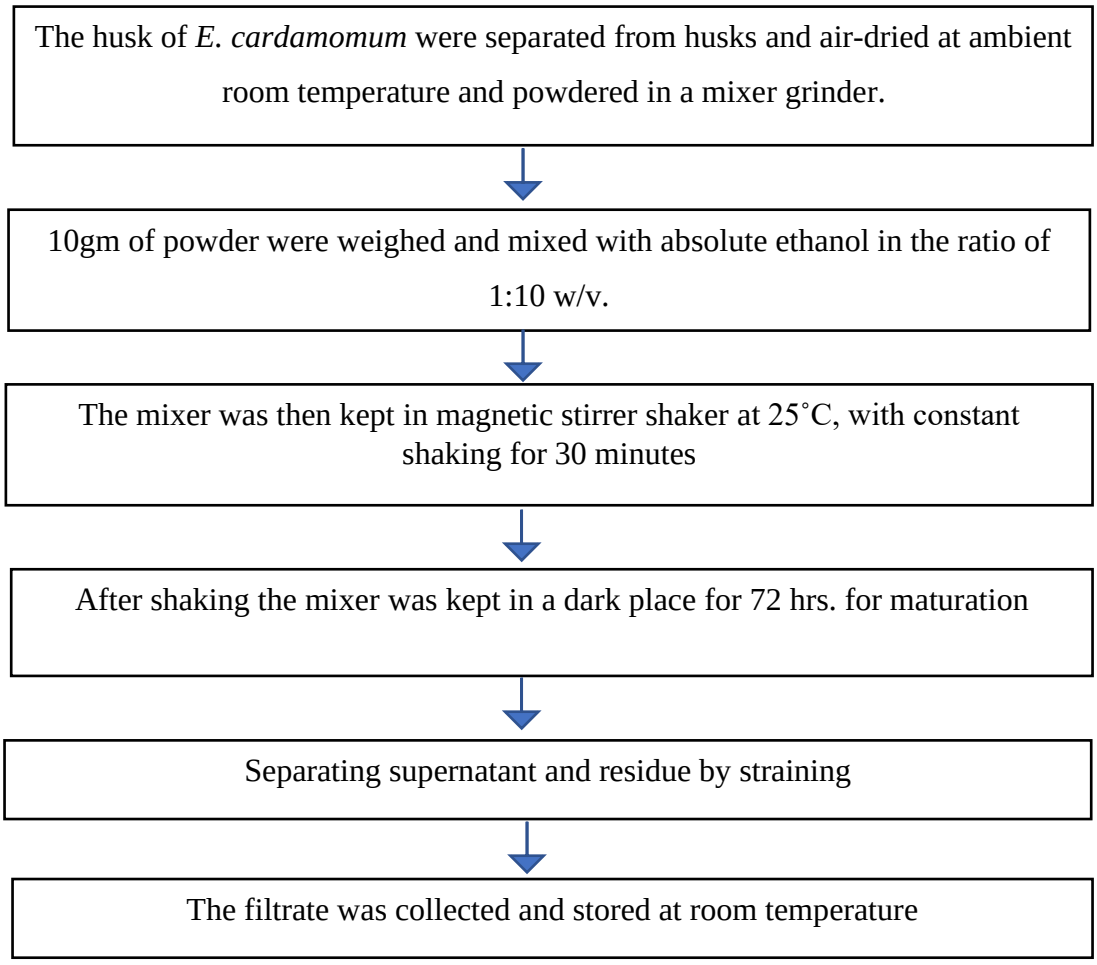


Figure 3.8: Ethanolic extract preparation flow sheet of cardamom husk

Figure 3.9: Ethanolic extract of cardamom husk powder

3.11 Extraction Procedures of cardamom flavor

Extraction of flavor compound from cardamom husk and seed has been done by three different methods. These are

- Solvent extraction method by using Rotary evaporator or evaporation method
- Condensation method by using Liebig condenser
- Drying method by using Cabinet dryer

Preparation of ethanolic extract was common step made for all three methods.

3.11.1 Rotary Evaporator

It is also known as rotovaps rotary, are machines being used to effectively evaporate solvents. Because of its outstanding separation and condensation capabilities, the rotary evaporation of water is among the most popular techniques of solvent evaporation.

Specification

Model	**Hei-VAP Value Digital**
Rotation speed setting	Scale
Drive	Brushless DC motor with electronic speed control
heating capacity (W)	1,300
Temp. range of the heating bath (°C)	20 – 210
Height adjustment Rotary Evaporator	155 mm
Cooling surface rotary evaporator	1200 qcm
Lift Rotary Evaporator	Hand lift

Principle of Operation

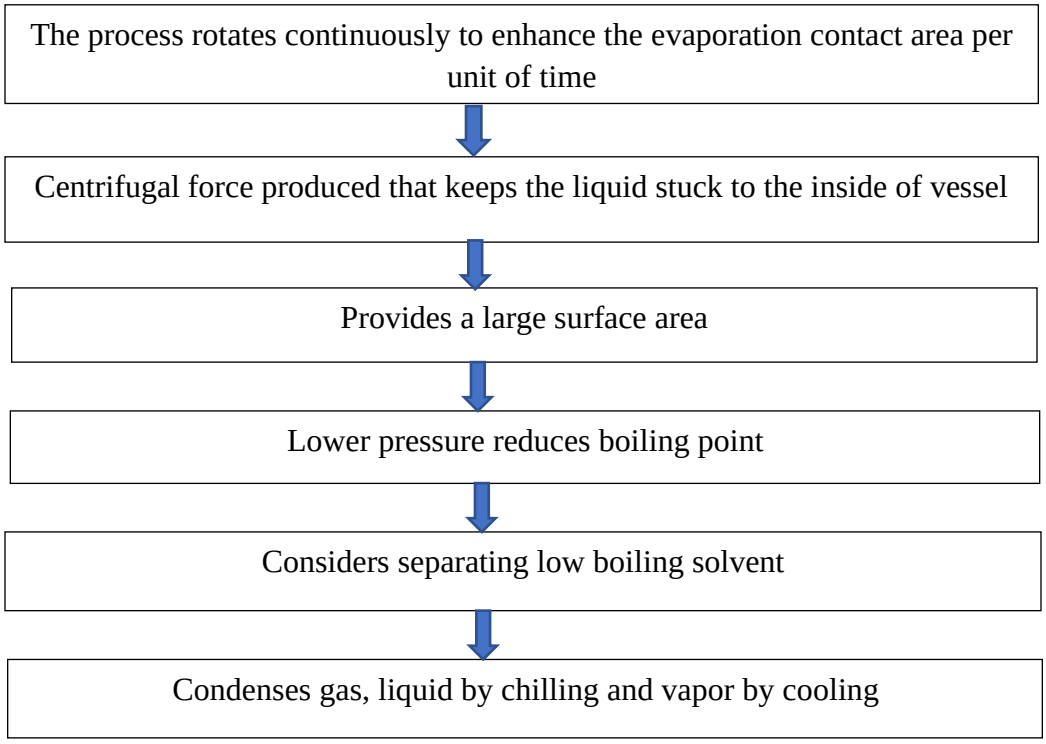


Figure 3.10: Operation flow chart of working principle of rotary evaporator

Figure 3.11: Rotary evaporator (category: Heidloph) with sample

Procedure of evaporation

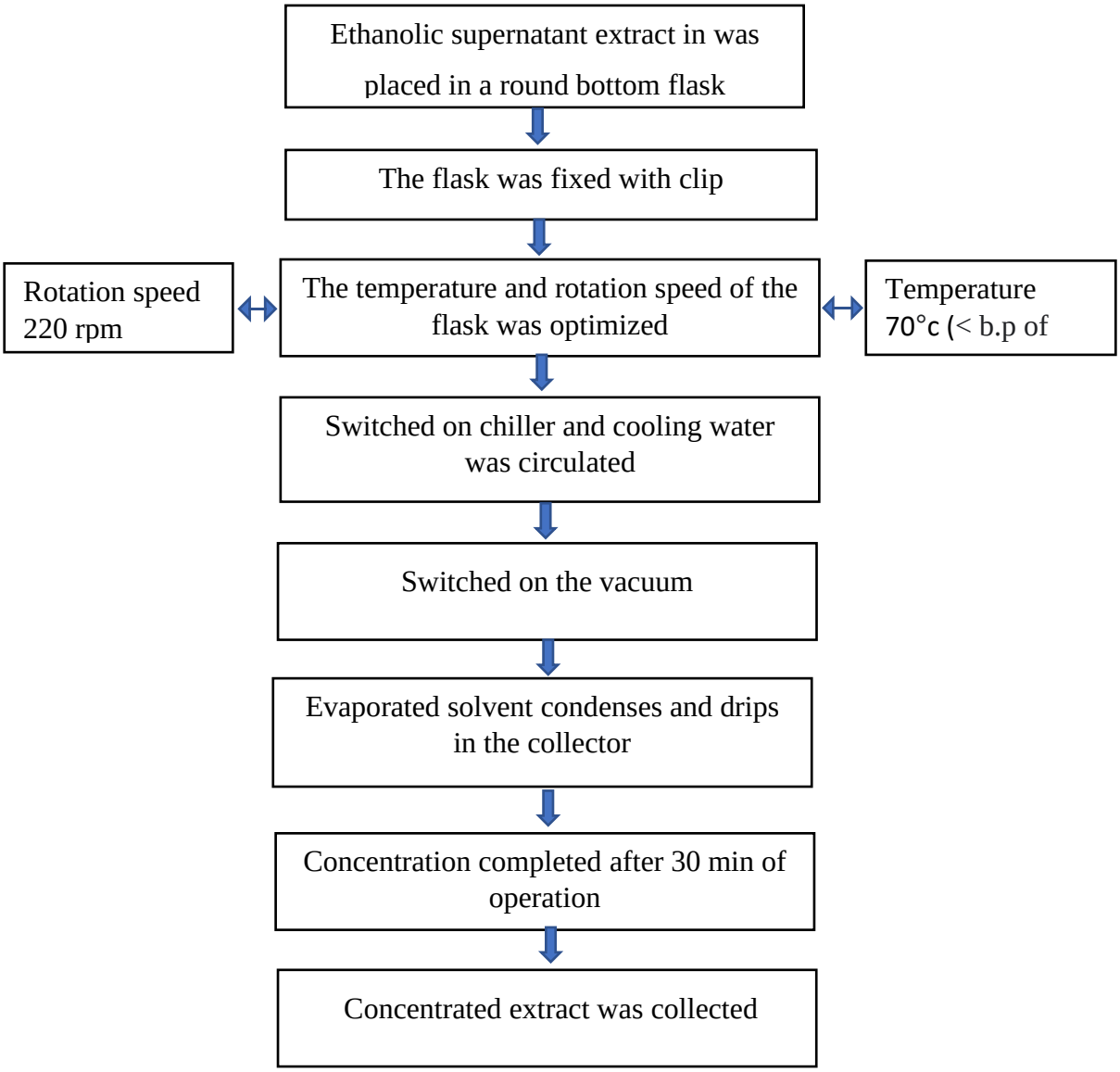


Figure 3.12: Working procedure of evaporation with rotary evaporator

3.11.2 Liebig condenser

In tribute of a German organic chemist Justus von Liebig (1803–1873), the word "Liebig condenser" is typically used to refer to refute, laboratory-scale water condensers made of

two concentric tubes: an inner distillation tube and an outer cooling jacket through which a constant process of distillation is taking place.

Specification

Usage/Application	Laboratory
Material	Borosilicate
Brand	Adarsh International
Color	Transparent
Size/Dimension	250 mm,300 mm,350 mm,400 mm,500 mm

Principle of Operation

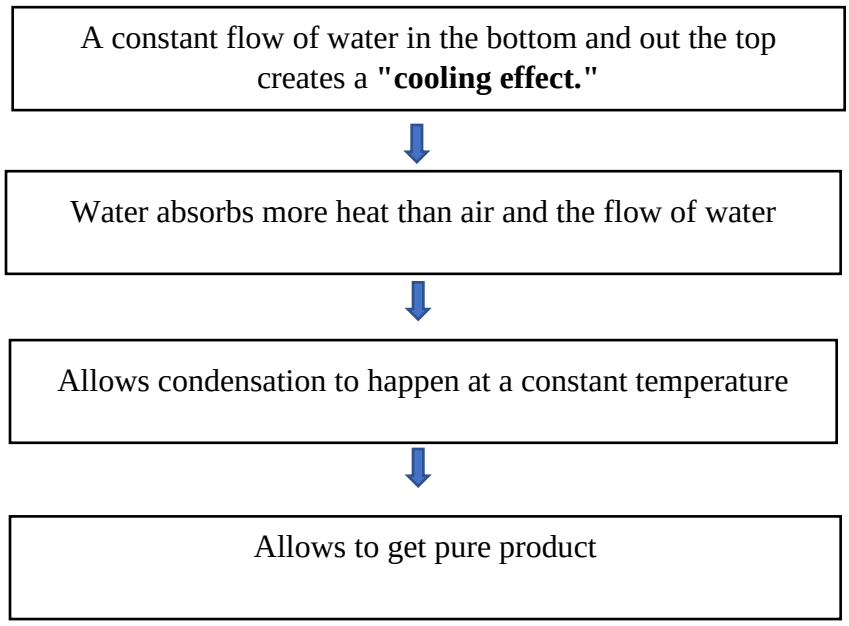


Figure 3.13: Operation flow chart of working principle of Liebig Condenser

Figure 3.14: Liebig Condenser with sample

Procedure of condensation

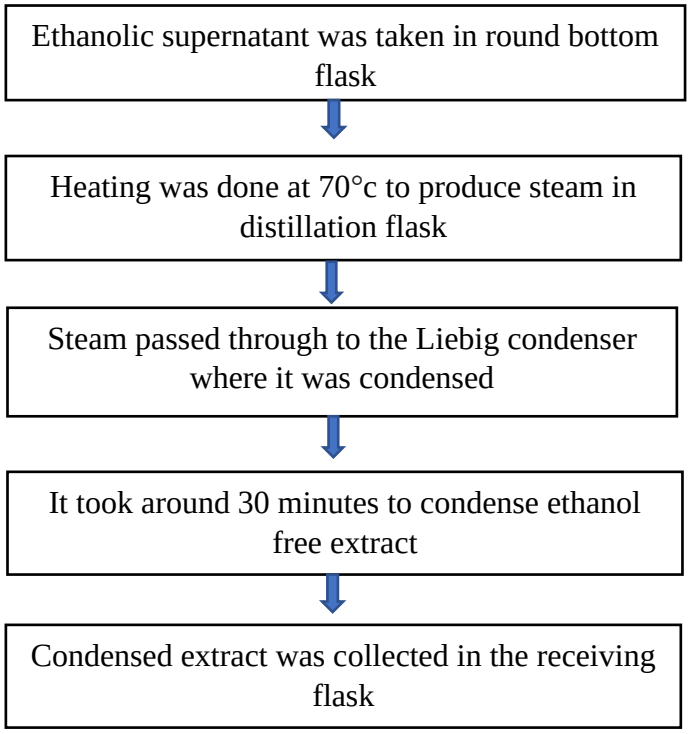


Figure 3.15: Working procedure of Liebig Condenser

3.11.3 Cabinet dryer

Nowadays, a drying cabinet is typically an electrical device made to speed up the drying of things. Inside a cabinet dryer, the feeds are placed upon tray that are then placed onto trolley or into the drying chamber, depending on the quantity of material that needs to be processed and, thus, the capacity of the dryer. The mechanism is sealed when the door is

shut. Dry closets are made out of an enclosed space where trays made of stainless steel are put with the products to be dried. These drying techniques lower the relative humidity, allowing the moisture to swiftly evaporate. Cabinet dryers are frequently employed to lower the moisture level of delicate goods.

Specification

- Material: stainless steel
- Automation grade: automatic
- Heating media: electric
- No of tray: depends upon height

Principle of Operation

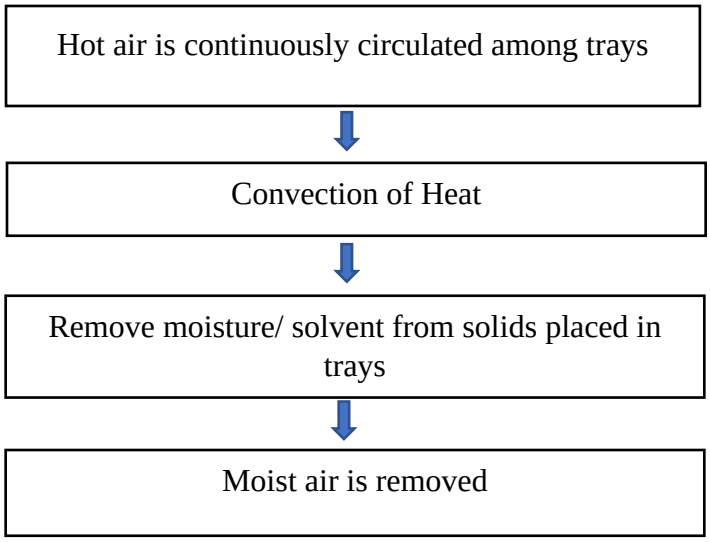


Figure 3.16: Working Principle of Liebig Cabinet Dryer

Figure 3.17: Cabinet dryer with sample

Procedure of drying

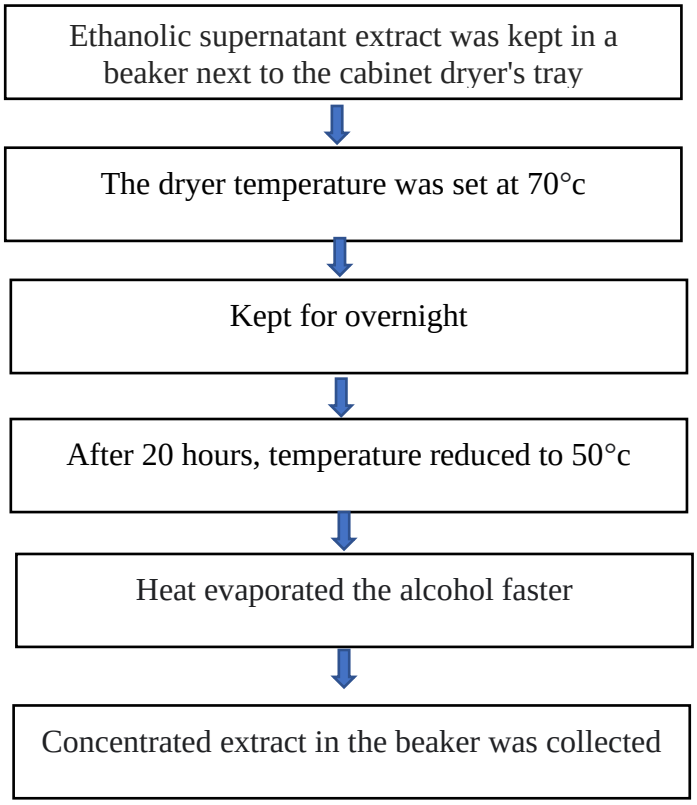


Figure 3.18: Working procedure of drying with cabinet dryer

3.11.4 All the extracted samples

By following three different method the extraction of flavor was done. The basic steps of preparation of ethanolic extract were same for all the three methods. All the extracted samples are named as below:

S_1= Extracted seed flavor by rotary evaporator

S_2= Extracted husk flavor by rotary evaporator

S_3= Extracted seed flavor by Liebig condenser

S_4= Extracted husk flavor by Liebig condenser

S_5= Extracted seed flavor by cabinet dryer

S_6= Extracted husk flavor by cabinet dryer

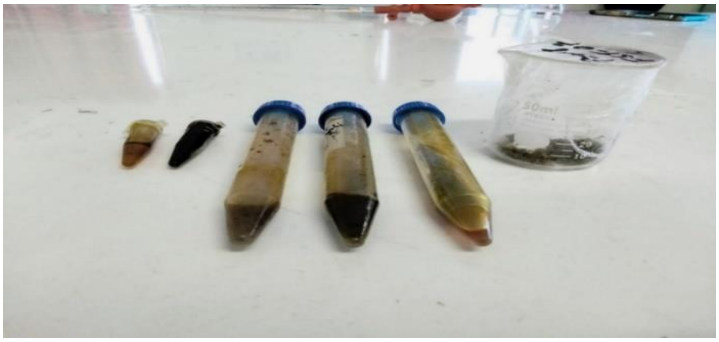


Figure 3.19: All the extracted flavor named from left to right as S_1, S_2, S_3, S_4, S_5, S_6

3.12 Total Flavonoid content (TFC)

Total flavonoids content (TFC) of the samples was determined by using the aluminum chloride colorimetric process reported by Chang *et al.*, (2002) with slight modifications.

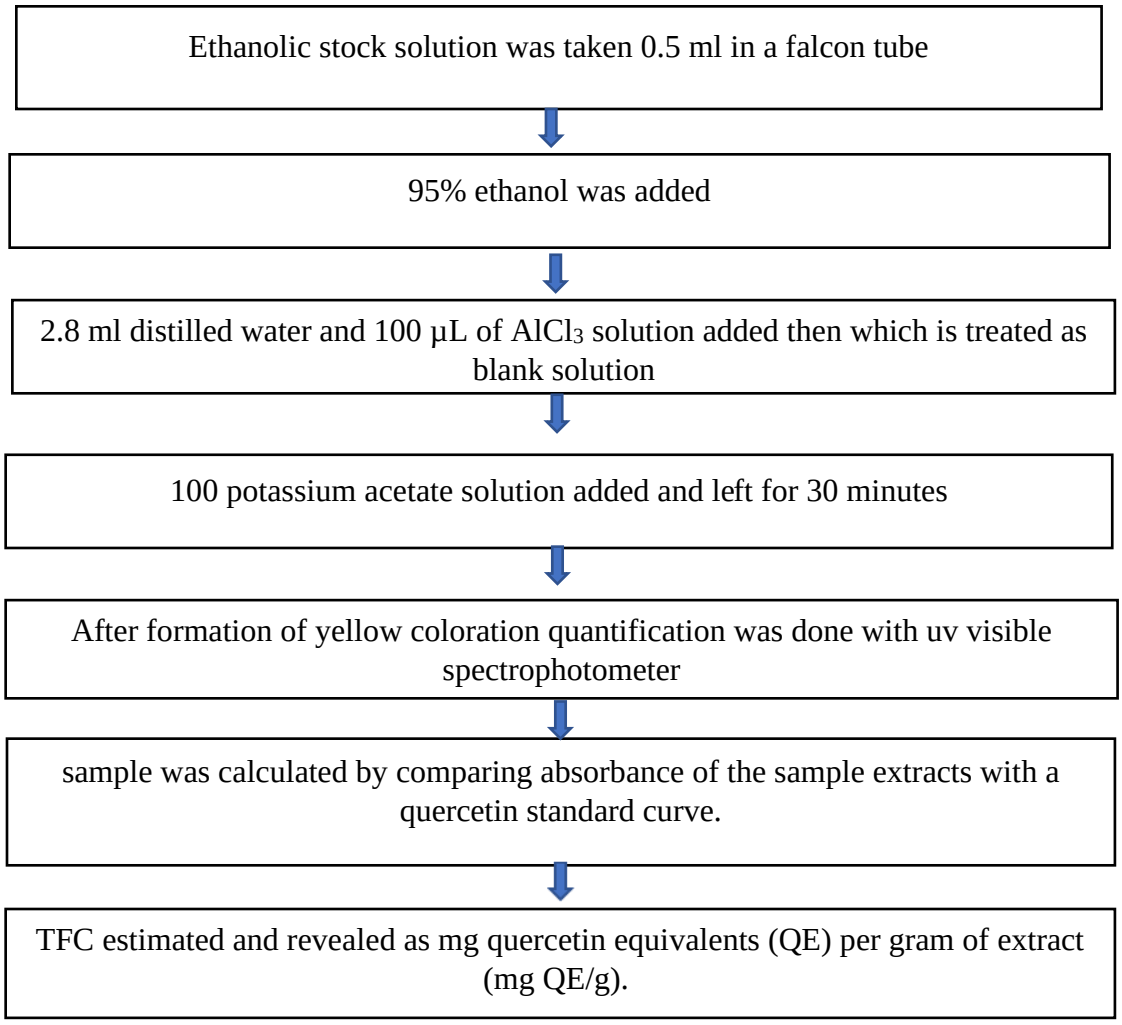


Figure 3.20: Total Flavonoid Content (TFC) determination flow diagram

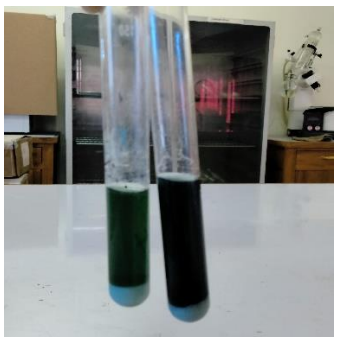


Figure 3.21: Standard preparation of quercetin

3.13 Total Phenolic Content (TPC)

TPC of the extracts were determined according to the Folin-Ciocalteu reagent method described with slight modifications (Al-Owaisi *et al.*, 2014). Total polyphenol content (TPC) of extracted flavor was determined according to the Folin-Ciocalteu method reported by Vergani *et al.* (2016) with slight modifications.

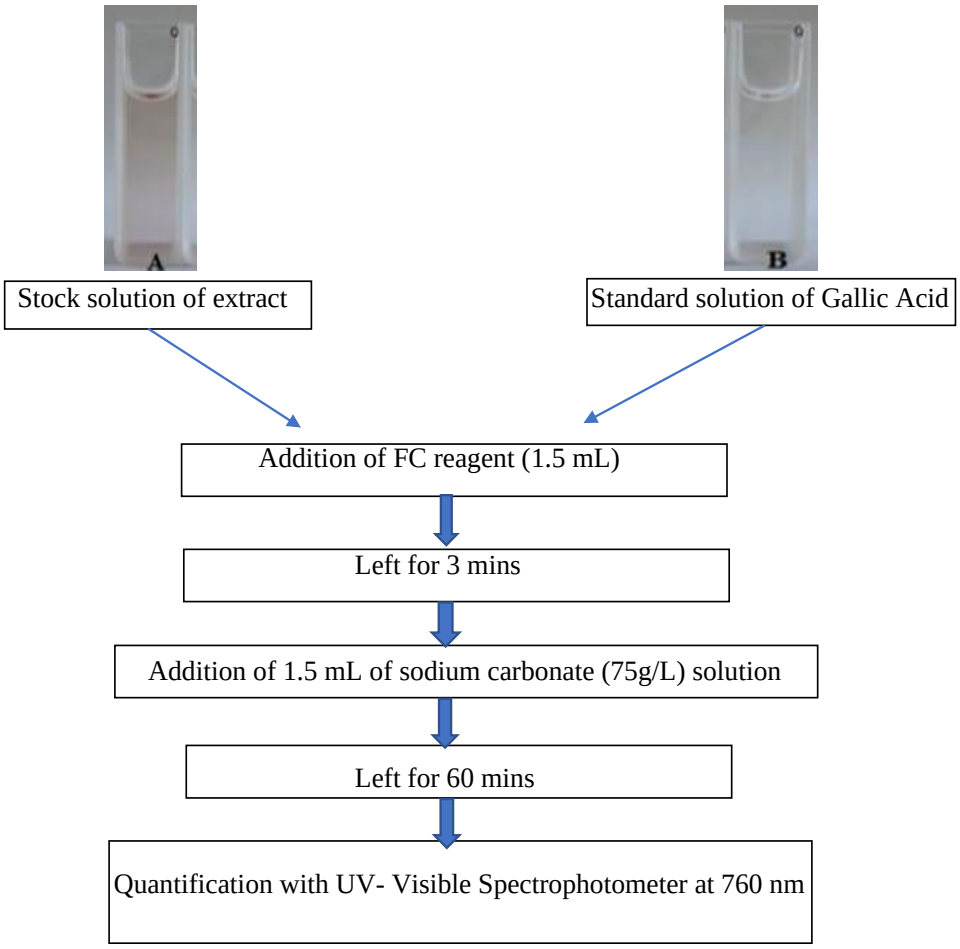


Figure 3.22: Total Phenolic Content (TPC) determination flow diagram

3.14 Determination of Antioxidant capacity by DPPH scavenging method Extract

The 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical test is commonly used to measure the antioxidant capacity of a substance. In this work, the DPPH test was utilized to assess the antioxidant capacity of the samples (Menezes *et al.* 2001; Mittal *et al*. 2013).

Required Chemical

1. DPPH
2. Trolox
3. Methanol (99%)
4. Text tube
5. Volumetric Flask

Preparation of DPPH Solution

To prepare 1mM DPPH solution, 0.004gm DPPH was dissolved in 100mL methanol. The solution was kept in a dark room (Tomczyk, 2021). DPPH reactions are very sensitive to reaction media e.g., water, pH, DO, light exposure, etc. The absorbance of DPPH at 517nm is decreased by light (Kamiloglu *et al*., 2014)

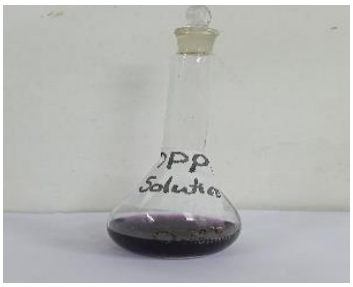


Fig.3.23: DPPH solution

Preparation of Trolox Standard Solution

0.001gm of Trolox was dissolved in 10mL methanol to prepare a 100 µg/mL stock solution. To prepare 10ug/ml to 60ug/ml solution, 50µL, 100 µL,150 µL, 200 µL, 250 µL, 300 µL stock solution was taken and add 450 µL, 400 µL, 350 µL, 300 µL, 250 µL, 200 µL methanol were respectively. 1.5 mL methanol and 1.5mL DPPH solution were used as a blank/control solution.

Preparation

- Taking 5gm of sample in falcon tube
- Adding 10ml absolute methanol and left for 72 hours
- Straining the solvent
- Collection of filtrates

- Evaporation at 60^0 c using rotary evaporator.
- Collect methanolic extract.

Methodology Applied for Antioxidant activity

The free radical scavenging activity was measured by the 1-1-diphenyl-2-picryl-hydrazyl (DPPH) following the method. 1.5mL of DPPH solution was mixed with 1.5mL of each concentration (Trolox) solution and the mixture was then vortexed (Bursal et al., 2019). Here, Trolox was used as standard. After vortexing the solution mixture was kept in a dark room for 30minutes. A blank solution containing all reagent (without Trolox or cardamom extract) solution was also taken.

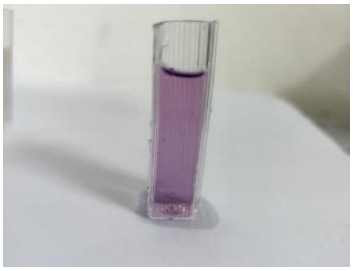


Figure 3.24: Control (Mixer of Methanol and DPPH Solution)

The absorbance of the solution was measured at 517nm against a blank (methanol) using a UV-Vis spectrophotometer. The percentage of inhibition capacity was calculated from the following formula:

Percentage of Radical Scavenging Activity (RSA) = $(A_o – A_1)/A_o \times 100$

Here, A_o - Absorbance of the control; A_1 - Absorbance of the Trolox solution. Percentage of scavenging was plotted against concentration and from this curve value of IC_{50} was calculated (Fafal et al., 2017; Nagaich et al., 2016).

Trolox was used as standard and TEAC composite (Trolox equivalent antioxidant mobility) was used for the calibration standard curve. The results were revealed in mg/ 100 g of Trolox equivalent per gram of powder on a dry weight (DW) base (Azlim Almey *et al.*, 2010**).**

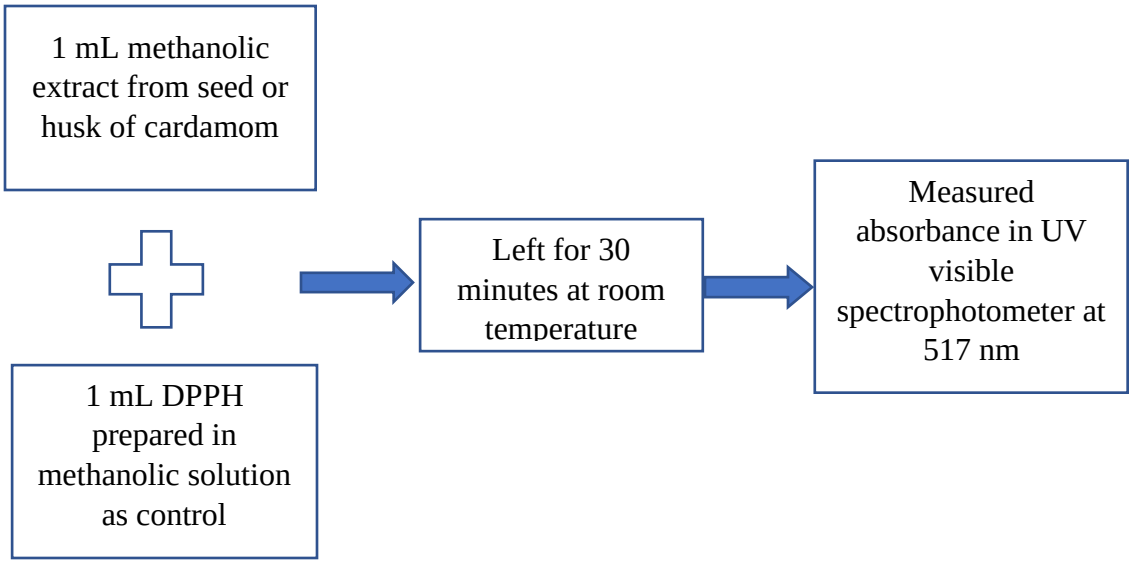


Figure 3.25: Flowsheet of antioxidant capacity determination

3.15 Microbiological analysis

To determine whether an antibiotic is efficient in treating a particular bacterium, the Kirby-Bauer test or the disc diffusion antibiotic sensitivity test are utilized.

Disc diffusion method

The disk diffusion method (DDM) is classified as an agar diffusion method (ADM) because the plant extract to be tested diffuses from its reservoir through the agar medium seeded with the test microorganism. Generally, the reservoir is a filter paper disk, which is placed on top of an agar surface.

Zone of inhibition (ZOI) is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The zone of inhibition can be used to measure the susceptibility of the bacteria towards the antibiotic.

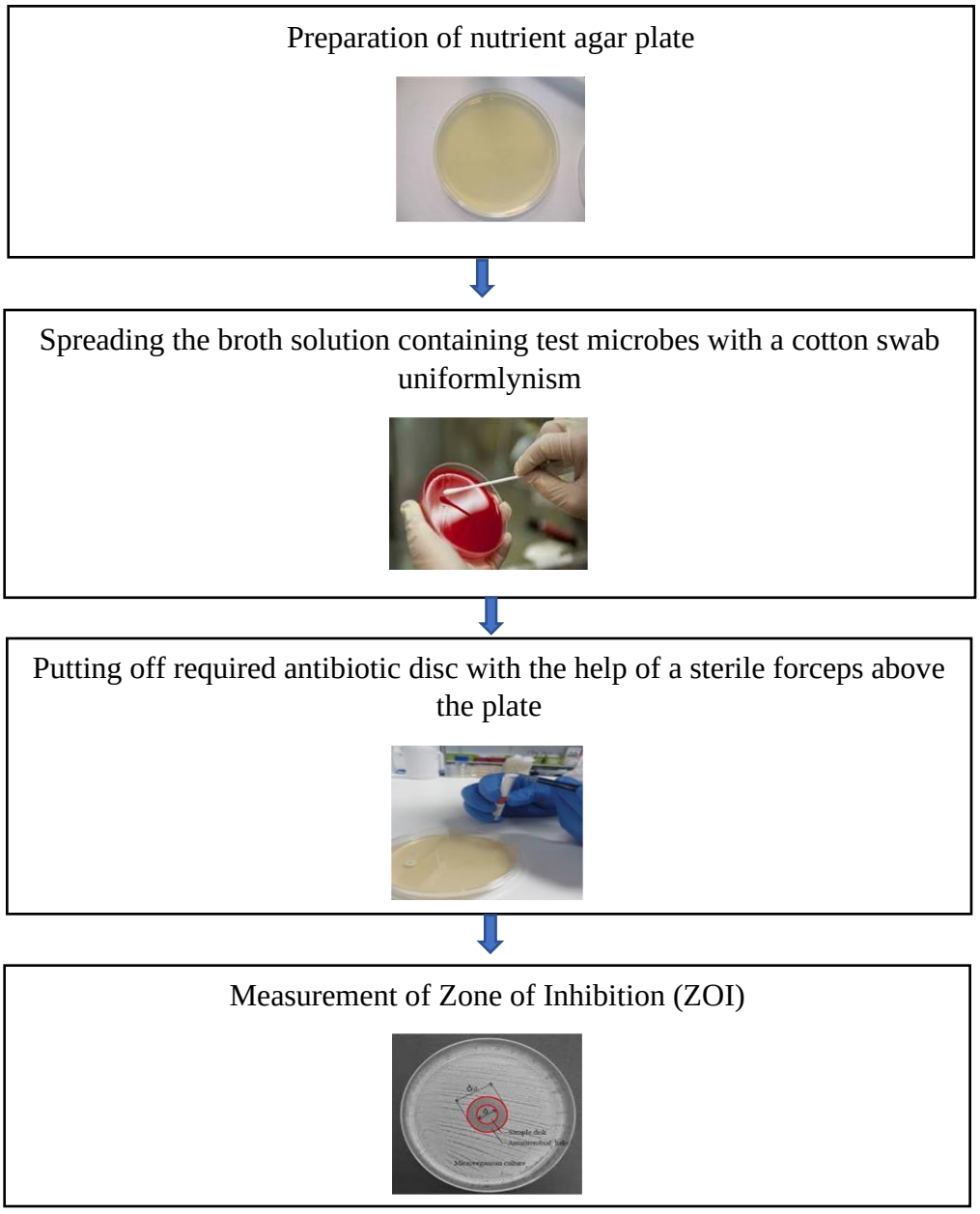


Figure 3.26: Stepwise procedure of measurement of antimicrobial activity

Selective agar for E. coli

The description of selective agar for an Escherichia coli and coliform organism is selectively enhanced lauryl sulfate-aniline blue agar media. The medium produced more colonies that produced acid from lactose in fecal samples than media containing bile salts. Th (Banwart, 2012). (Andrews, 1992).

Agar preparation

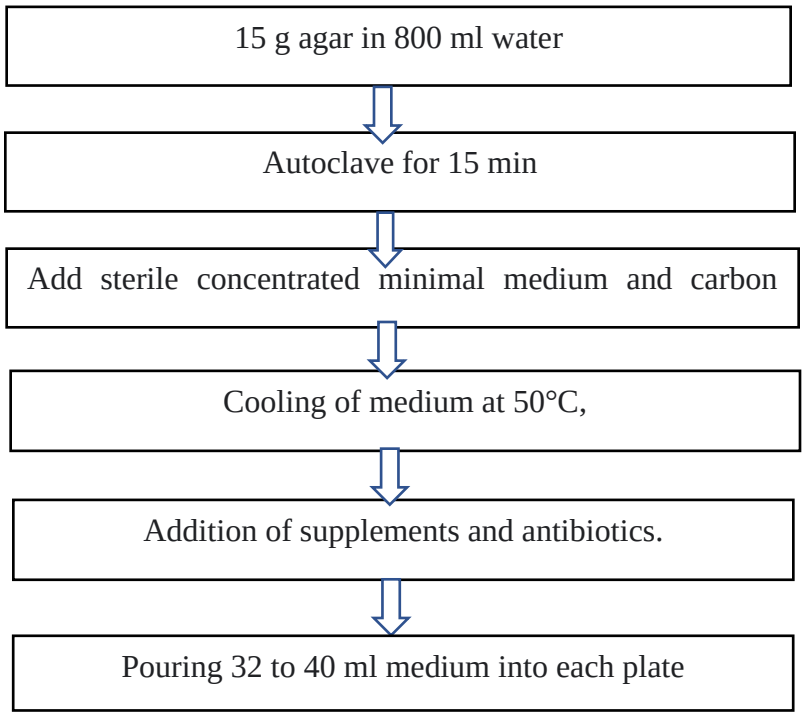


Figure 3.27: Lauryl sulfate-aniline blue agar media preparation for *E. coli*

Selective agar for s. aureus

Staphylococcus aureus is isolated and identified from clinical and non-clinical materials using Mannitol Salt Agar (MSA), a selective and differentiating medium. By suppressing the development of additional bacteria, it encourages the development of a particular bacterial family.

Preparation of Mannitol Salt Agar (MSA)

1. Suspend 111 grams of Mannitol Salt Agar in 1000 ml of distilled water.
2. Boil to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs.

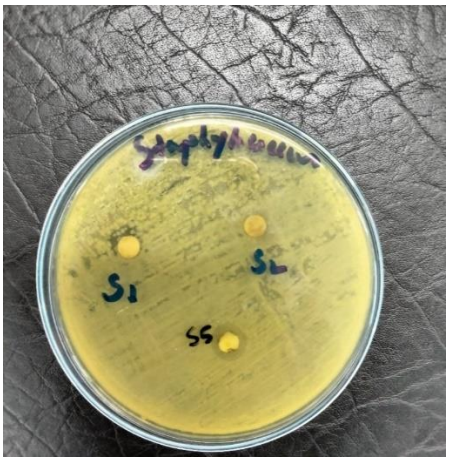


Figure 3.28: Sensibility against *staphylococcus aureus* (Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample)

Chapter 4: Results

4.1 Nutritional attributes

Nutritional characteristics of whole cardamom as separated husk and seed was assessed by determining moisture, protein, fat, crude fiber, ash and carbohydrate content.

Table 4.1: Proximate analysis report showing nutritional composition

Sample	Dry matter (%)	Moisture (%)	Crude fiber (%)	Ash (%)	Ether extract/ fat (%)	Crude protein (%)	Carbohydrate (%)
Seed	85.1±0.10	14.9±0.10	11.8±0.12	4.6±0.02	3.6±0.10	10.5±0.10	56.04±0.052
Husk	88.2±0.12	11.6±0.10	31.8±0.65	15.4±0.13	2.2±0.07	5.2±0.10	32.21±0.05

All values are Means ± Standard Deviation.

Here, Number of replications, n = 3

4.2 Vitamin & minerals analysis

Water-soluble vitamin C was subjected to vitamin analysis. Calcium, magnesium, phosphorus, and potassium were studied as minerals. Although the calcium, magnesium, and phosphorus values were calculated in mg/dL units, they were converted to mg/gm. Aside from that, potassium was measured in mmol/L and converted to mg%.

Table 4.2: vitamin and minerals composition of cardamom seed and husk

sample	**Vitamin C (mg/gm)**	**Calcium (mg/gm)**	**Magnesium (mg/gm)**	**Phosphorus (mg/gm)**	**Potassium (mg%)**
Seed	0.2 ± 0.005^{a}	2.46 ± 0.072^{ab}	2.48 ± 0.015^{b}	13.1 ± 0.053^{b}	13.1 ± 0.055^{a}
Husk	0.1 ± 0.051^{a}	13.11 ± 0.095^{b}	4.84 ± 0.026^{ab}	0.91 ± 0.049^{b}	24.03 ± 0.049^{ab}

All values are Means ± Standard Deviation for n=3. The presence of different superscripts along a row indicates significant difference and the same superscript shows not significant difference at ($p<0.05$).

4.3 Yield percentage, Energy content and water activity analysis

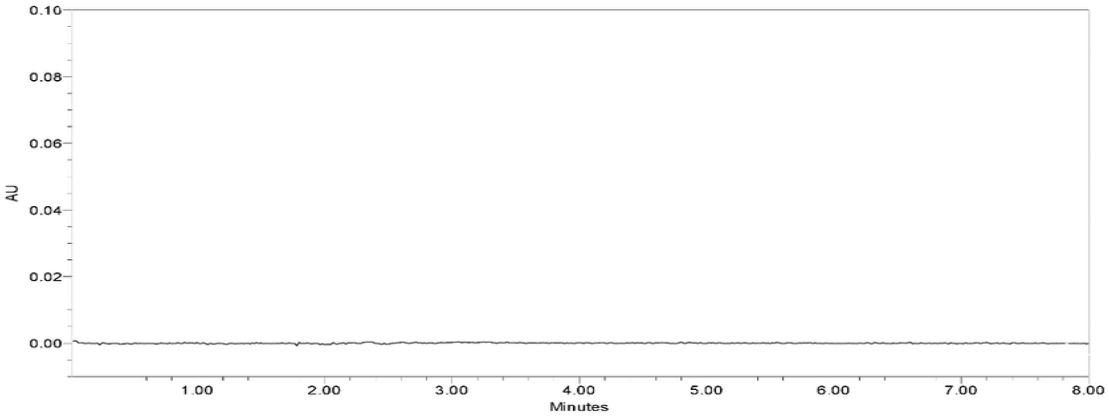
The cardamom sample with which the study was carried out had a seed-to-husk ratio of (1.7:1). Energy content was calculated highest for cardamom seed is 292.5 94 kcal/ 100gm and for cardamom husk is 187.145 kcal/ 100gm. The water activity of cardamom seed was measured 0.51 by water activity meter. Where husk of cardamom showed the value 0.48.

Table 4.3: Yield, Energy content, water activity analysis

Parts of cardamom	% yield of whole cardamom	Kcal energy/ 100gm	Water activity (a_w)
Seed	62.5	292.5 94	0.51
Husk	37.5	187.145	0.48

4.4 Analysis of amino acid

Though cardamom seed and husk contain certain amounts of protein, during amino acid analysis no chromatogram was shown for amino acid sequencing by Gas Chromatography.



4.5 Analysis of fatty acids

For analysis of fatty acids fat extraction was done for individual seed and husk sample. The higher magnitude of peak shows the higher retention time of respective fatty acid in GC machine. The x-axis of a gas chromatogram typically shows the time it takes for analytes to pass through the column and reach the mass spectrometer detector. The peaks shown correspond to the times when each of the components arrived at the detector.

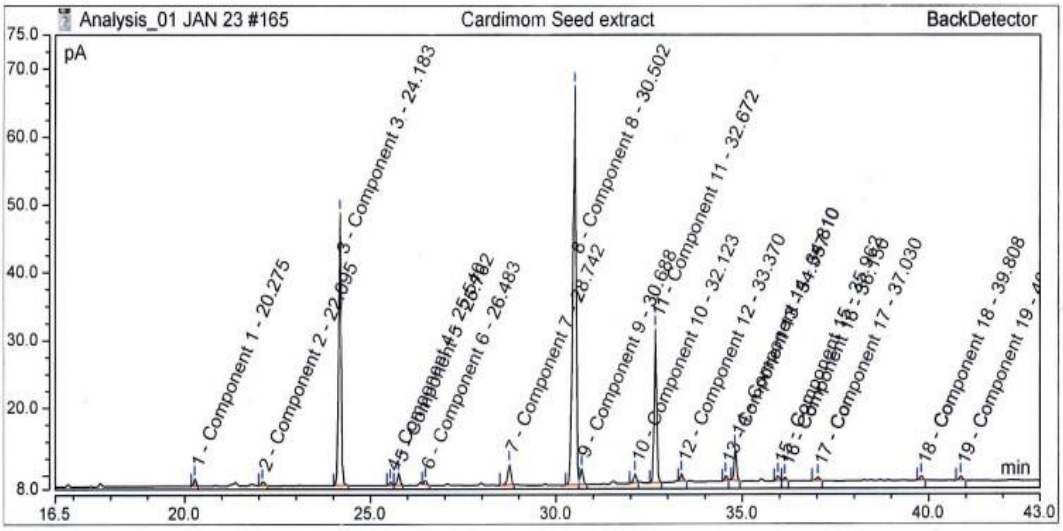


Figure 4.1: Chromatogram peak of fatty acids shown by cardamom seed sample

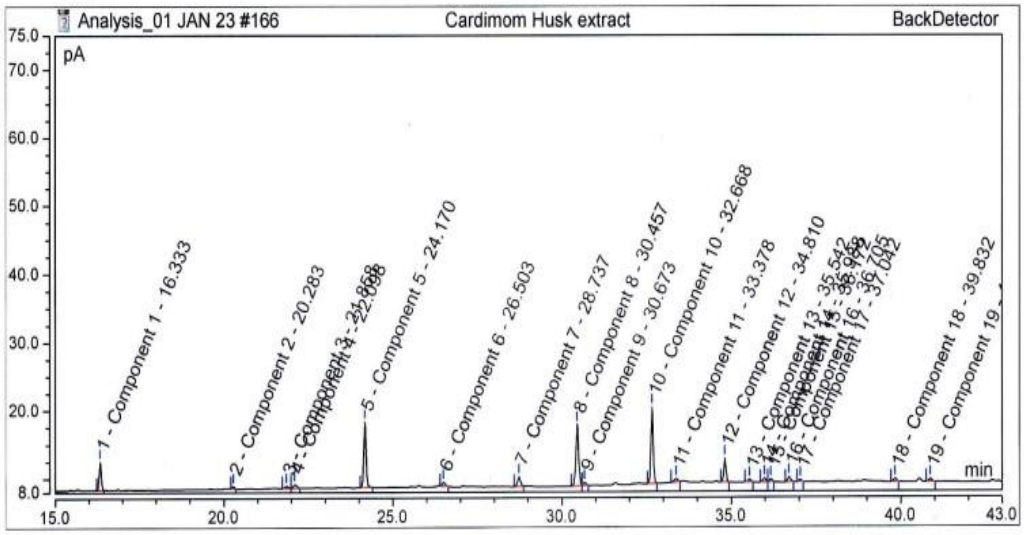


Figure 4.2: Chromatogram peak of fatty acids shown by cardamom Husk sample

Table 4.4 Analysis of Fatty Acids

Fatty acid	Cardamom seed (%)	Cardamom husk (%)
Lauric acid (C 12:0)	----	7.92
Myristic acid (C 14:0)	0.59	0.59
Myristoleic acid (C 14:1)	0.47	1.04
Pentadecylic acid (C 15:0)	----	1.66
Palmitic acid (C 16:0)	25.06	19.72
Palmitoleic acid (C 16:1)	1.23	----
Margaric acid (C 17: 0)	0.39	1.59
Stearic acid (C 18: 0)	2.49	3.77
Cis-Oleic acid (C 18:1)	46.87	22.79
Trans-Oleic acid	1.59	1.18
Linoleic acid (C 18: 2)	13.76	22.65
Gamma Linoleic acid (C 18 :3)	0.46	-----
Alpha Linolenic acid (C18:3)	2.77	6.91
Arachidonic acid (C 20:0)	0.67	1.42
Eicosadienoic acid (C 20:2)	----	1.97
Docosadienoic acid (C 22:0)	0.45	0.74

4.6 Physicochemical properties of extracted flavor from different methods

To justify each individual parameter of physicochemical characteristics, flavor was extracted using three different methods.

Table 4.5: Physicochemical properties of extracts by different methods

Extraction	Yield (%)	Color	Odor	Physical state	Transparency
Evaporation of seed (S1)	7.5 ± 0.01528^{c}	Brown	Pleasant	Liquid	Transparent
Evaporation of husk (S2)	6.66 ± 0.351^{c}	Black	Pleasant	Semi solid	opaque
Condensation of seed (S3)	38 ± 0.1528^{a}	Brown	squishy	Semi liquid	opaque
Condensation of husk (S4)	40 ± 0.1528^{a}	Black	squishy	Semi solid	opaque
Drying of seed (S5)	12.90 ± 0.1102^{b}	Brown	Pleasant	liquid	Transparent
Drying of husk (S6)	12.18 ± 0.220^{b}	Black	Slightly squishy	solid	opaque

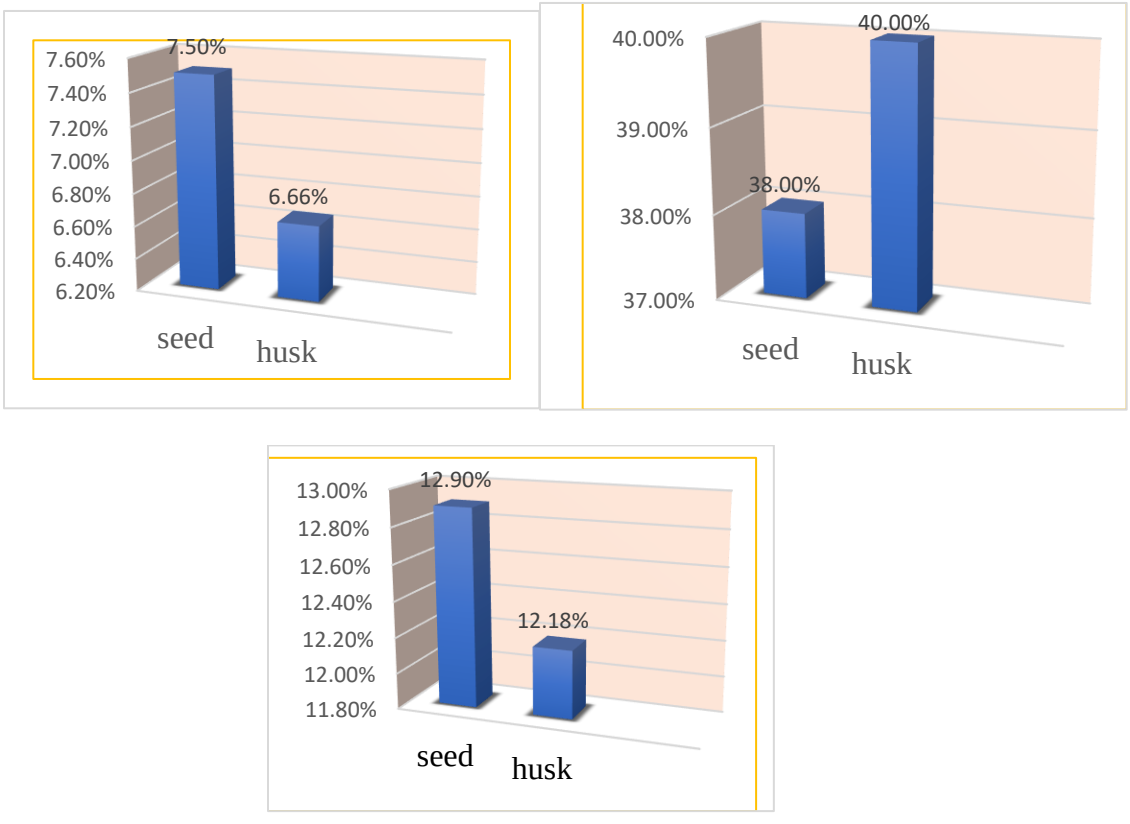


Figure 4.3: Comparison of yield % of extract by 3 methods

4.7: Bioactive compounds of seed and husk extract

As bioactive components Total flavonoid content, total phenolic content, and antioxidant capacity were all determined. Tannic acid was used as the standard for calculating antioxidant capacity in this case.

Table 4.6: Bioactive compounds of seed and husk extract

Samples	Total flavonoid content / (TFC) (mg QE/100gm)	Total phenolic content / (TPC) (mg GAE/100gm)	Antioxidant capacity (mg TA/ 100g)
Evaporation of seed, S_1	15.415 ± 0.0427^a	0.758 ± 0.0272^b	3.185 ± 0.0589^a
Evaporation of husk, S_2	144.085 ± 0.235^b	0.944 ± 0.0454^a	2.571 ± 0.0265^b
Condensation of seed, S_3	15.712 ± 0.214^a	0.697 ± 0.0280^b	3.115 ± 0.0539^a
Condensation of husk, S_4	145.011 ± 0.247^b	0.912 ± 0.032^a	2.442 ± 0.0205^b
Drying of seed, S_5	16.151 ± 0.0759^a	0.851 ± 0.0215^b	3.335 ± 0.0584^a
Drying of husk, S_6	142.231 ± 0.301^b	0.881 ± 415	2.115 ± 0.019^b

All values are Means ± Standard Deviation for n=3. The presence of different superscripts along a column indicates significant difference and the same superscript shows not significant difference at ($p<0.05$).

4.8: Radical Scavenging Activity (RSA)

The antioxidant capacity was measured in the previous section as mg TA/100 g. The radical scavenging activity was calculated using a UV-visible spectrophotometer and the IC50 value. In this case, the absorbance of the sample and standard trolox solution were taken into account.

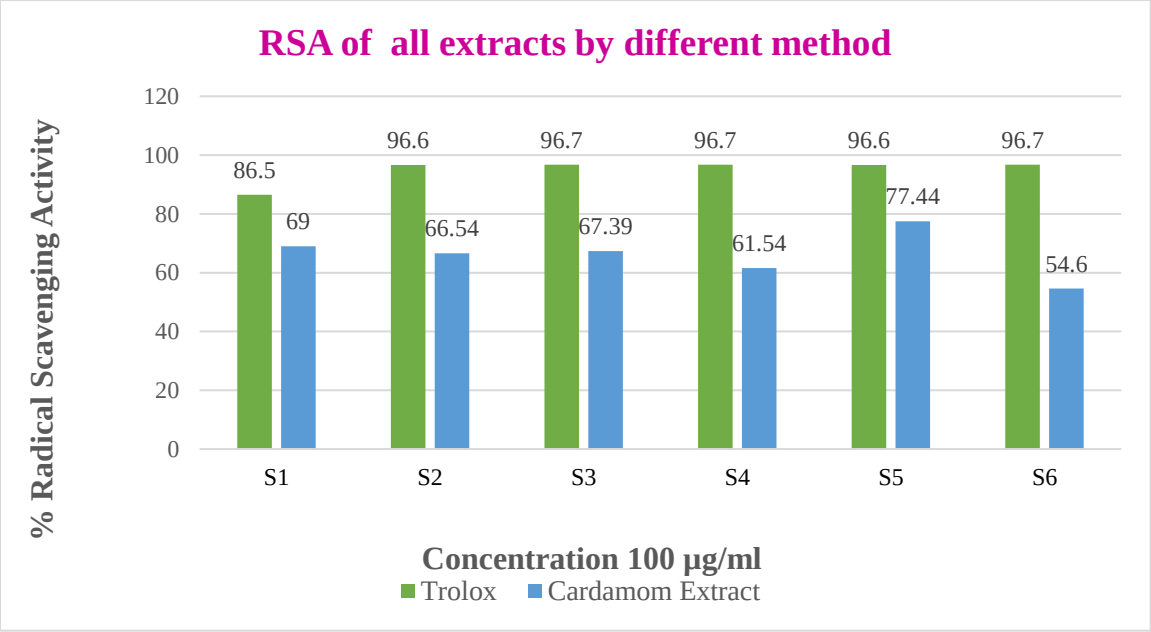


Figure 4.4: Comparison of the RSA value of all extracts to the standard Trolox

4.9 Antimicrobial properties

The disc diffusion method provided sensitivity against microbes. Sample names were previously described for individual extracts from different methods.

Table 4.7: Measurement of Zone of Inhibition as antimicrobial property

Extracted sample	Zone Of Inhibition (ZOI) in mm	
	Against *Escherichia coli*	**Against *staphylococcus aureus***
S_1	7±0.50	7±0.48
S_2	--	7±0.45
S_3	8±0.42	8±0.42
S_4	--	7±0.52
S_5	7±0.49	12±0.61
S_6	--	--

The values represent mean of sample ± SD for n = 3. Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample.

Chapter 05 - Discussion

5.1 Nutritional attributes

Cardamom presents a fantastic chance to flavor foods and beverages while consuming fewer calories and fats. (Juber *et. al* 2022). According to the data of proximate analysis the husk of cardamom contains more dry matter (88.25%) than seed (85.1%).

Data showed in table 4.1makes it clear that seed of cardamom contains more moisture (14.9%) in comparison to husk (11.75%) of it. The moisture interval of 8.41-24.87% (w.b.), the physical traits of the cardamom capsule were identified. (Balakrishnan *et al*., 2010)

The moisture level of food ingredients prior to grinding is a crucial component because it affects both the physical characteristics of the ingredients and the attributes of the powders, such as flowability, after processing. (Jung *et al*., 2018). Moisture content and dry matter content are inversely related. So, the seed sample containing higher moisture (14.9%) shows lower amount of DM (85.1%) in comparison to husk sample.

The amount of crude fiber found in the husk sample is almost three times higher (31.79 %) than the seed sample. (11.78 %). From figure 2.2 it is seen that the outer layer of seed known as husk contain more fibrous part than seed.

The bran, germ, and endosperm are the three components that make up each whole grain kernel. Health-improving nutrients are contained in each segment. The outer, fiber-rich layer known as the bran contains antioxidants, phytochemicals, B vitamins, iron, copper, zinc, and magnesium. (Harvard, TH Chan)

During the measurement of ash content, it was found that the husk of cardamom contains more ash percentage (15.42%) than the seed sample (4.63%). That is almost four times the seed of cardamom. A similar concept was described by T. John Zachariah (2002). On the other hand, the solid that is left behind when a substance has been extensively burned or oxidized chemically is known as ash. When husk is burned, it contains more substance because it contains more fiber.

The fat content of cardamom seed (3.67%) is higher than that of cardamom husk (2.15%). It is caused by the containment of an oil cellular layer at the outer membrane of cardamom seed. (Figure 2.2)

The protein percentage of cardamom seed is almost twice (10.5%) that of cardamom husk (5.20%). The majority of flowering plants create endosperm, a tissue, in their seeds just after fertilization. the structure is made up of proteins, it envelopes the embryo and supplies nutrients (peter *et. al*, 2012)

Cardamom husk has a lower value for carbohydrate content (32.21%) than cardamom seed (56.04%). This is so because the husk has a higher concentration of the majority of the nutrients than the seed does. It is evident from the CHO calculation equation in Section 3.5.7.

5.2 Analysis of Vitamin and Minerals

According to data analysis, cardamom contains a lower amount of water-soluble vitamin C. Cardamom seed contains 0.2 mg per gram, while the husk contains 0.1 mg per gram. The findings are consistent with a study conducted by Sharma and Mohon in 2012, which stated that cardamom contains 21 mg per 100 g.

During the calculation of minerals, it was shown that cardamom husks contain more calcium, magnesium, and potassium than phosphorus. Cardamom seeds contain 2.46 mg of calcium, 2.48 mg of magnesium, 13.1 mg of phosphorus, and 13.1 mg of potassium per gram. Cardamom husks contain 13.11mg calcium, 4.84 mg magnesium, 0.91 mg phosphorus per gram, and 24.03 mg potassium.

A study conducted by Roopan and Madhumita in 2018 revealed a similar concept for cardamom seed. Cardamom contains 3.5 mg calcium, 2 mg magnesium, 12 mg phosphorus, and 13 mg potassium per gram, according to the study. There is no research on cardamom husk minerals. The higher mineral content of cardamom husk may be explained by the husk's higher ash content. Ashes are primarily mineral byproducts of incomplete

combustion, but they may also contain combustible organic or other oxidizable residues. Spackman and Moses (1960)

5.3 Yield, Energy content, Water activity analysis

Table 4.3 shows the seed-husk ratio as 62.5:37.5, which is in line with the study of V.K. Joshi (2011), where the yield of seeds and husk from cardamom pods is in the ratio of 70:30, respectively. The seed-to-husk ratio changes depending on the type of cardamom, according to a different study by Padmakumari *et al*., (2015). The ratio varies from origin to origin as Mysore 62:38; Vazhukka 70:30; Malabar 76:24; Guatemala 66:34. So, based on this information, it may be concluded that the cardamom sample originated in Mysore.

According to the calculations, each 100 g of cardamom contains 187.145 kcal of husk and 292.594 kcal of seed. The equation provided by 3.8 is used to determine this. The nutritional value of cardamom per 100 g of ground spice is 311 Kcal, according to a related study (Elizabeth, 2021).

Water activity of cardamom seed was 0.51, while cardamom husk water activity was 0.48. Husk has a lower AW value because it contains a higher percentage of dry matter. The ratio of the vapor pressure in a food (P) to the vapor pressure of pure water (P0) is used to represent water activity (a_w) (P_0). It foretells if water would likely transfer from the food product into any potential microorganisms' cells. aw= P/P_0. (Gustavo *et al*., 2020). The majority of foods have a water activity above 0.95, which provides enough moisture for the development of bacteria, yeasts, and mold. It is possible to lower the available moisture to a level where the organisms' growth is inhibited. (Osman *et al*., 2016). The findings indicated a safe zone for food microbe safety. It also improved the shelf life of cardamom powder, which has a variety of culinary uses. This phenomenon was discussed by FDA as, more microorganisms are typically found in environments with higher water activity; bacteria typically need water activity values of at least 0.91, while fungi need at least 0.6. Each microbe has a water activity threshold below which it cannot proliferate. mechanical drying to lower the water activity to 0.51-0.56.

5.4 Amino acid analysis

An "incomplete protein" is defined as protein that does not contain all nine types of amino acids that must be obtained from diet. Examples of incomplete proteins include: seeds and nuts. Complete grains (like brown rice or whole-wheat bread) Vegetables. Several legumes, including lentils, peas, and beans (Carol, 2010). Leucine (n/d), isoleucine (n/d), and valine (n/d) are three necessary amino acids that make up the complex known as branched-chain amino acids (BCAA). Every 100 grams of cardamom does not contain BCAAs. It might be the reason behind not having any chromatogram of amino acid sequencing.

5.5 Fatty acid analysis

Analysis of fatty acid was done by using gas chromatography (GC). A larger peak of retention area in the seed sample indicates that it contains more fatty acids. This is because during fat extraction it was seen that husk sample showed lesser amount of fat percentage.

According to reports, cold-pressed cardamom seeds had 40.6%–49% of oleic acid (Hamdan *et. al*, 2007), which is consistent with the results of the current investigation, which were drawn from the whole cardamom pods. In this study cardamom showed good number of fatty acids. Around 14 fatty acids are detected from cardamom seed and husk by GC-MS. Seed of cardamom contains Mristic acid (0.59%), Myristoleic acid (0.47%), Palmitic acid (25.06%), Palmitoleic acid (1.23%), Margaric acid (0.39%), Stearic acid (2.49%), cis Oleic acid (46.87%), trans Oleic acid (1.59%), Linoleic acid (13.76%), gamma Linolenic acid (0.46%), alpha Linolenic acid (2.77%), Arachidonic acid (0.67%), Eicosadienoic acid, Docosadienoic acid (0.45%).

Besides cardamom husk contains Lauric acid (7.92%), Mristic acid (0.59%), Myristoleic acid (1.04%), Palmitic acid (19.72%), Margaric acid (1.59%), Stearic acid (3.77%), cis Oleic acid (22.79%), trans Oleic acid (1.18%), Linoleic acid (22.65%), alpha Linolenic acid (6.91%), Arachidonic acid (1.42%), Eicosadienoic acid (1.97%), Docosadienoic acid (0.74%).

Although lauric acid, pentadecyclic acid, and eicosadienoic acid were only found in the husk sample, the fatty acid chromatogram for the seed sample revealed a greater peak than the husk sample. Palmitic acid and cis-oleic acid were the two fatty acids that were in abundance. This result was consistent with the research on It was claimed that linoleic acid (2–16%), oleic acid (43–44% of fatty oils), and palmitic acid (28–38%) were the three major fatty acids present in these oils. (V. K. Joshi · 2011)

This occurred because inside the cells just below the epidermis of the cardamom seeds, the essential oil is found in a single layer. The component parts of the favor may be recovered. The color of the fruit has no bearing on the inherent organoleptic qualities of cardamom. (O. P. Chauhan 2022)

5.6 Physicochemical properties of all extracted flavor

Three different techniques were used to extract the flavor from the seed and husk of cardamom, two of the spice's component parts. They include drying, condensation, and evaporation. The physicochemical characteristics which were measured are yield percentage of flavor, color, odor, physical state and transparency.

According to a study, the essential oil of cardamom was extracted using a Clevenger apparatus (hydro distillation) from 22 accessions with an optimal time of 3 hours and a yield of 4.5–9.5%. (Three replicates, 20 g sample, 500 mL distillation flask) (Martinez *et al.*, 2007). In this investigation, the rotary evaporator produced a seed extract yield of 7.5 percent, whereas the husk produced an extract yield of 6.6 percent. The was taken almost 30 minutes to evaporate all ethanol.

The outcome demonstrated a discernible conclusion when the flavor is extracted using a Liebig condenser. It revealed that condensation produced a yield of 40% ground husk and 38% seed. The value displays a result that is 5–6 times that of the evaporation approach. Due to the Liebig condenser's use of liquid for cooling, it is far more effective than a standard retort. The steady water flow through to the water jacket maintains the condenser's temperature because water can capture so much more heat than the same amount of air can. (Armarego *et al.*, 2012)

The yield from the seed in the cabinet dryer experiment to obtain flavor extract was 12.90%, and the yield from the husk sample was 12.18%.

So, based on yield %, we can conclude that the Liebig condenser provided the maximum amount of yield because its refluxing mechanism brought back all the liquids and reduced the likelihood that other constituents would evaporate.

Table 4.4, in addition to displaying the yield %, also included some organoleptic information. Inspection of color showed almost all the seed extract are brown in color. According to Olesya Y. Shoeva, a scientist at the Institute of Cytology and Genetics in Novosibirsk, Russia, the blue anthocyanins that are beginning to build up in the macrosclereids are what give the blue coloration of the spice husk its dark hue (epidermal cells). This concept is also explained by Krishnarao *et al.*, (2001) that, husk ash is grayish-black in color due to unburned carbon. Analysis of the extract's odor reveals that the rotary evaporator produced a pleasing aroma. Condensation also imparted a mushy odor to both the seed and the husk. Nonetheless, the cabinet dryer produced seed extract with a lovely flavor that was a little mushy for the husk. A study on cardamom essential oil showed that, Cardamom oil has a warm, spicy, and aromatic scent. (Steffen, 2017).

The physical condition of the extract ranges from liquid to semi-solid. An exception happened during the drying of the ethanolic husk extract. The last time drying took place this way, the extract came in powder form. That is because the contents of the lower trays are prone to overdying. (Zeki Berk, 2008)

The final metric examined was the transparency of the extract. The husk sample displayed opacity in nature in contrast to all of the seed extract's transparency. A study by Oliveira et al. can be used to explain this characteristic. According to the study, rice and oat husks have higher levels of cellulose fiber, which aids in the production of hydrogel during the separation of its constituents. This demonstrated a dark color and an opaque nature as a result.

5.7 Bioactive components

In this section, Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Tannic Acid (TA) as an antioxidant were measured.

Between seed and husk extract, there was a significant (p 0.05) difference in the total flavonoid concentration. All of the seed samples (s_1, s_3, and s_5) contain 15.41, 15.712, and 16.151 mg QE/100g; however, the husk samples (s_2, s_4, and s_6) exhibited a significant difference with 144.045, 145.011, and 142.231 mg QE/100g. This data indicates a larger value than the study's results, which were determined using the superficial extraction method and showed total flavonoids ranging from 11.33 0.03-4.63 0.12 g/100 g. In 2013 (Prakash *et al*.)

Compared to seed extract, husk extract displayed a wider range of differences. Despite the fact that no relevant reference data were located. According to AN Panche (2016), flavonoids, which are mostly found in fruit peels or outer shells and are responsible for the bitter flavor of citrus fruits' juice and peel, are a likely reason for this phenomenon.

Total phenolic content (TPC) of seed extract was 0.758, 0.697, and 0.451 GAE/100gm by s_1, s_3, and s_5, respectively, whereas husk extract was 0.944, 0.912, and 0.881 GAE/100gm by s_2, s_4, and s_6. These figures are more significant than the study's findings, which indicated that green cardamom had total phenolic levels ranging from 0.317.0 to 1.660.05 g/100 g. (Prakash *et al*., 2013). Table 4.5 also showed the result for antioxidant capacity expressed as mg TA/ 100g. Hence, it was observed that the bioactive component content did not significantly alter as a result of the extraction method.

5.8 Radical Scavenging Activity

Figure 4.6 compares the RSA value to the standard Trolox value for an individual extract at 100 mg/mL concentration. It can be seen that all of the seed extracts had a slightly higher value than the husk extracts. The seed extract has RSA values of 69%, 67.39%, and 77.44% for s_1, s_3, and s_5, respectively, and the husk extract has values of 66.54%, 61.54%, and 54.6% for s_2, s_2, s_6. Seed extracts have a higher value due to a higher essential oil content in seed layers, which has antioxidant capacity. (Bhadra *et al*., 2021)

The antioxidant potential of cardamom seeds and pods extracts (0.001-5 mg mL^{-1} of each extract) in terms of DPPH° radical scavenging ranged between 69.89-79.27 and 76.04-

91.67%, respectively, indicating considerable ($p<0.05$) variations as function of extraction solvent and plant's tested parts. (Bhatti *et al*.,2020)

5.9 Antimicrobial property

There are many fluctuations in the interpretation of antimicrobial activity in all extracts. Cardamom seed demonstrated inhibitory activity on *M. smegmatis, K. pneumoniae, S. aureus, E. coli,* and *E. faecalis*, according to the results of the antimicrobial activity testing. (Seema *et al*., 2006). E. coli and Staphylococcus aureus, two of the most significant food-borne disease-causing bacteria, were found to be sensitive to cardamom extract in this investigation. During the evaluation of the zone of inhibition against E. coli, seed extract from a rotary evaporator exhibited the least amount of inhibition, while husk extract revealed nothing noteworthy. Similar results were obtained when ZOI against E. coli was measured using extracts from the Liebig condenser, where seed extract demonstrated moderate inhibition but husk extract did not. In the case of the cabinet dryer's extract, it produced results that were comparable to those of the rotary evaporator's extract.

Though the extracts did not show higher inhibition against E. coli, they showed significant inhibition against Staphylococcus aureus. Seed and husk Extracts from the rotary evaporator showed a minimum ZOI. Whereas Liebig condenser seed extract showed moderate ZOI, husk extract showed marginal. The higher ZOI was noticed by the seed extract of the cabinet dryer. It showed the most inhibition result among all. But the husk extract from this method showed nothing significant due to its dried texture, so the antimicrobial test could not be done. According to a similar study conducted by Gaurav *et al*., (2016), it was seen that cardamom extract having concentration of 33.3mg/ml showed ZOI which was15.33±0.50 mm against *E coli* and against *s aureus* it was 20.10±0.47 mm. The value in this study was lower than in those studies. It can be the freshness of the extract. The test was conducted after the extract had been kept in the freezer for about five months. It might be the cause of a temporary loss of antibacterial properties. Also, the concentration of the extract is crucial to understanding the value of the ZOI measurement. Another study showed the value as minimum inhibitory concentration of ethanolic cardamom extract was 8.33 ± 1.61 mg/ml. (MOULAI-HACENE *et al.,* 2020).

Chapter 6: Conclusion

Nature creates the storehouse of remedies to cure diseases. Cardamom is a plant-based herb which has nutritional values as well as medicinal properties. The current study sought to investigate the nutritional properties of cardamom spice. Proximate analysis showed the report that cardamom seed contains moisture (14.9%), crude fiber (11.8%), ash (4.6%), fat (3.6%), protein (10.5%), carbohydrate (56.04%) where husk contains 11.6% moisture, 31.8% crude fiber, 15.4% ash, 2.2% fat, 5.2% protein, 32.21% carbohydrate. Determination of water-soluble vitamins and minerals provided the information that, carbohydrate seed contains 0.2% vitamin C, 2.46% calcium, 2.48% magnesium, 13.1% phosphorus and 13.1% potassium. Besides cardamom husk showed this result as 0.1% vitamin C, 13.11% calcium, 4.84% magnesium, 0.91% phosphorus and 24.03%. Seed contributes 62.5% of whole cardamom where this value for husk is 37.5%. having water activity of 0.51, cardamom seed provides 292.594 kcal energy where the value for husk showed 0.48 and 187.145 kcal respectively. 16 different fatty acids were identified from cardamom seed and husk remarkably cis- oleic acid (46.87%) for seed and 22.79% for husk and palmitic acid 25.06% for seed and 19.72% for husk. During the analysis of amino acid, it showed no chromatogram in spite of showing protein content from proximate analysis. Extraction of flavor from cardamom seed and husk were performed using three different methods. Comparison data showed the highest yield percentage from Liebig condensation was 38% for seed and 40% for husk. The bioactive compound showed highest value as 142.23% TFC for cardamom husk and 77.44% RSA value for seed extract of drying method. Antimicrobial assessment also showed highest value of ZOI against *staphylococcus aureus* for extraction of drying method. So, it may be concluded that, despite having lowest amount of seed extract yield (12.90%), cabinet drying method of cardamom seed showed higher efficacy.

Chapter 7: Recommendations and Future Perspectives

Based on the current investigation, the following suggestions and prospects for future research work are made.

a) The current studies could be repeated to confirm the experimental findings.

b) Essential oil can also be extracted from various parts of the cardamom plant, such as the leaf and flower.

c) The extraction technique may be altered for further investigation. Extraction may also be done with other solvent rather than ethanol.

d) Similar research should be conducted on other spices such as cinnamon and clove so that a comparison can be made.

e) The findings will be beneficial from a therapeutic standpoint because medicinal value of cardamom extract was seen.

f) Because cardamom has antimicrobial properties against food-borne disease microorganisms, its extract, both seed and husk, can be used as an alternative to chemical preservatives in meat preservation where cardamom can be blended with that food during food preparation.

g) Despite the fact that the sample size was adequate for statistical comparisons between analytical data, Because of the small number of analyzed samples, our conclusion should be viewed with caution, and the results should be confirmed in a larger study.

References

Bursal, E., Aras, A., Kılıc, O., Taslimi, P., Goren, A. C., & Gulcin, I. 2019. Phytochemical content, antioxidant activity, and enzyme inhibition effect of Salvia eriophora Boiss. & Kotschy against acetylcholinesterase, α-amylase, butyrylcholinesterase, and α-glycosidase enzymes. *Journal of Food Biochemistry*, *43*(3).

Fafal, T., Ta, P., Tuzun, B. S., Ozyazici, M., & Kivcak, B. 2017. *South African Journal of Botany Synthesis , characterization and studies on antioxidant activity of silver nanoparticles using Asphodelus aestivus Brot . aerial part extract*. *112*, 346–353.

Joshi, R., Sharma, P., Sharma, V., Prasad, R., Sud, R. K., & Gulati, A. 2013. Analysis of the essential oil of large cardamom (Amomum subulatum Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. *Journal of the Science of Food and Agriculture*, *93*(6), 1303–1309.

Kamiloglu, S., Pasli, A. A., Ozcelik, B., & Capanoglu, E. 2014. Evaluating the invitro bioaccessibility of phenolics and antioxidant activity during consumption of dried fruits with nuts. *Lwt*, *56*(2), 284–289.

Menezes, S., Leita, G. G., Reis, A. S., Tereza, C., & Mensor, L. L. 2001. *Screening of Brazilian Plant Extracts for Antioxidant Activity by the Use of DPPH Free Radical Method*. *130*, 127–130.

Mittal, A. K., Chisti, Y., & Banerjee, U. C. 2013. Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*.

Nagaich, U., Gulati, N., & Chauhan, S. 2016. *Antioxidant and Antibacterial Potential of Silver Nanoparticles : Biogenic Synthesis Utilizing Apple Extract*. *2016*.

Tomczyk, M. O. 2021. How to express the antioxidant properties of substances properly ? *Chemical Papers*, *0123456789*.

Bhaswant, M., Poudyal, H., Mathai, M. L., Ward, L. C., Mouatt, P., & Brown, L. 2015. Green and Black Cardamom in a Diet-Induced Rat Model of Metabolic Syndrome. *Nutrients*, *7*(9), 7691–7707.

Hamdan, S., Daood, H. G., Toth-Markus, M., & Illés, V. 2008. Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane. *Journal of Supercritical Fluids*, *44*(1), 25–30.

Parry, J., Hao, Z., Luther, M., Su, L., Zhou, K., & Yu, L. 2006. Characterization of cold-pressed onion, parsley, cardamom, mullein, roasted pumpkin, and milk thistle seed oils. *JAOCS, Journal of the American Oil Chemists' Society*, *83*(10), 847–854.

Rahman, T., Rahman, K. A., Rajia, S., Alamgir, M., Khan, M. T. H., & Choudhuri, M. S. K. 2008. Evaluation of antidiarrhoeal activity of Cardamom (Elettaria cardamomum) on mice models. *Oriental Pharmacy and Experimental Medicine*, *8*(2), 130–134.

Lepcha, R., Ray, R., Chandra, B., & Viswavidyalaya, K. 2023. *Effect of Microsprinkler Irrigation and Nutrient Management on Growth , Yield , Water Use E ciency and Macronutrient Accumulation of Large Cardamom in Sub-Himalayan Region of India*.

PATEL, S. M., PATEL, R. P., & MEHTA, B. 2023. Evaluating the effect of cardamom on lipolysis in heat desiccated milk product (khoa). *International Journal of Agriculture, Environment and Food Sciences*, *7*(1), 1–10.

Amma, K. P. A. P., Rani, M. P., Sasidharan, I., & Nisha, V. N. P. 2010. Chemical composition, flavonoid - phenolic contents and radical scavenging activity of four

major varieties of cardamom.

David, V. (2015). University of Huddersfield Repository University of Huddersfield Repository. *Electronics and Computing in Textiles*, 45–59.

Bhattacharjee, B., & Chatterjee, J. 2013. Identification of proapoptopic, anti-inflammatory, anti- proliferative, anti-invasive and anti-angiogenic targets of essential oils in cardamom by dual reverse virtual screening and binding pose analysis. *Asian Pacific Journal of Cancer Prevention*, *14*(6), 3735–3742.

PEARCE, E. L., SAUBERLICH, H. E., & BAUMANN, C. A. 1947. Amino acids excreted by mice fed incomplete proteins. *The Journal of Biological Chemistry*, *168*(1), 271–282.

Agaoglu, S., Dostbil, N., & Alemdar, S. 2006. Antimicrobial Effect of Seed Extract of Cardamom (Elettarıa cardamomum Maton). *YU Vet Fak Derg*, *16*(2), 99–101.

Castillo, N. E. T., Teresa-Martínez, G. D., Alonzo-Macías, M., Tellez-Perez, C., Rodríguez-Rodríguez, J., Sosa-Hernández, J. E., Parra-Saldívar, R., Melchor-Martínez, E. M., & Cardador-Martínez, A. 2023. Antioxidant Activity and GC-MS Profile of Cardamom (Elettaria cardamomum) Essential Oil Obtained by a Combined Extraction Method—Instant Controlled Pressure Drop Technology Coupled with Sonication. *Molecules*, *28*(3), 1093.

Hameedu, M. S. 2014. Supply Chain Analysis of Cardamom in Kerala. *International Journal of Scientific and Research Publications*, *4*(3), 1–7.

He, M., Qin, C.-X., Wang, X., & Ding, N.-Z. 2020. Plant Unsaturated Fatty Acids:

Biosynthesis and Regulation. *Frontiers in Plant Science*, *11*, 390.

Balakrishnan, M., Manikantan, M. R., Viswanathan, R., & Sreenarayanan, V. V. 2011. Moisture dependent physical properties of cardamom. *International Agrophysics*, *25*(4), 399–402.

Fatemeh, Y., Siassi, F., Rahimi, A., Koohdani, F., Doostan, F., Qorbani, M., & Sotoudeh, G. 2017. The effect of cardamom supplementation on serum lipids, glycemic indices and blood pressure in overweight and obese pre-diabetic women: A randomized controlled trial. *Journal of Diabetes and Metabolic Disorders*, *16*(1), 1–9.

Kumar, A., Tandon, S., Ahmad, J., Yadav, A., & Kahol, A. 2013. Essential Oil Composition of Seed and Fruit Coat of Elettaria cardamomum from South India. *Journal of Essential Oil Bearing Plants*, *8*, 204–207.

Akrayi, H. F. S. 2012. Antibacterial Effect of Seed Extracts of Cardamom (Elettaria cardamomum) against Staphylococcus aureus and Proteus mirabilis. *Tikrit Journal of Pure Science 17 (2)*, *17*(2), 14–18.

Rajpur, A., & Samratha, K. 2018. Cardamom: A Multipurpose Species in Food and Commercial Needs. In *Bioorganic Phase in Natural Food: An Overview* (pp. 89–102).

Ashokkumar, K., Vellaikumar, S., Murugan, M., Dhanya, M. K., Ariharasutharsan, G., Aiswarya, S., Akilan, M., Warkentin, T. D., & Karthikeyan, A. 2021. Essential Oil Profile Diversity in Cardamom Accessions From Southern India. *Frontiers in Sustainable Food Systems*, *5*(April), 1–8.

Tongnuanchan, P., & Benjakul, S. 2014. Essential Oils: Extraction, Bioactivities, and Their

Uses for Food Preservation. *Journal of Food Science*, *79*(7).

Jung, H., Lee, Y. J., & Yoon, W. B. 2018. Effect of moisture content on the grinding process and powder properties in food: A review. *Processes*, *6*(6), 6–10.

Mukhtar, S., Arshad, M., Basu, S., ul-Hassan, F., Ahmed, M., & Asif, M. 2012. Influence of capsule position on seed traits and oil content of linseed (Linum usitatissimum L.). *Plant Knowledge Journal*, *1*(2), 52–56.

Gebreyes, B. G. 2020. *NUTRITIONAL COMPOSITION OF CARDAMOM (ELETTARIA CARDAMOUM) International Pharmaceutical Research of Modern*. *March*, 23–26.

Małgorzata Brodowska, K. 2017. European Journal of Biological Research Natural flavonoids: classification, potential role, and application of flavonoid analogues. *European Journal of Biological Research*, *7*(2), 108–123.

Souissi, M., Azelmat, J., Chaieb, K., & Grenier, D. 2020. Antibacterial and anti-inflammatory activities of cardamom (Elettaria cardamomum) extracts: Potential therapeutic benefits for periodontal infections. *Anaerobe*, *61*, 102089.

Appendices

Appendix A: Preparation of ethanolic extract of cardamom husk

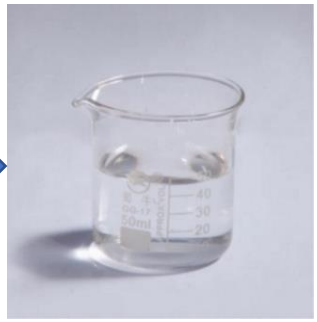
Separation of husk

weighing of husk

Blending of husk

sieving

weighing



Addition of ethanol

Stirring

Layer formation

Separation of supernatant

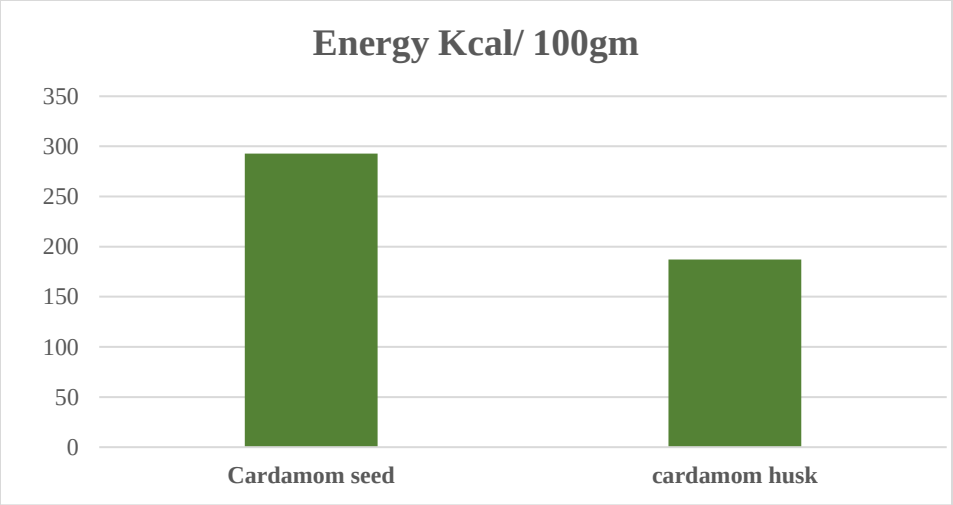
Appendix B: Additional data of analysis

No.	Peak Name	Retention Time min	Area pA*min	Rel.Area %
1	Component 1	20.275	0.080	0.59
2	Component 2	22.095	0.064	0.47
3	Component 3	24.183	3.384	25.06
4	Component 4	25.540	0.051	0.38
5	Component 5	25.762	0.167	1.23
6	Component 6	26.483	0.053	0.39
7	Component 7	28.742	0.336	2.49
8	Component 8	30.502	6.329	46.87
9	Component 9	30.688	0.215	1.59
10	Component 10	32.123	0.122	0.90
11	Component 11	32.672	1.858	13.76
12	Component 12	33.370	0.091	0.67
13	Component 13	34.557	0.063	0.46
14	Component 14	34.810	0.374	2.77
15	Component 15	35.962	0.075	0.56
16	Component 16	36.150	0.050	0.37
17	Component 17	37.030	0.060	0.45
18	Component 18	39.808	0.065	0.48
19	Component 19	40.862	0.067	0.49
Total:			13.504	100.000

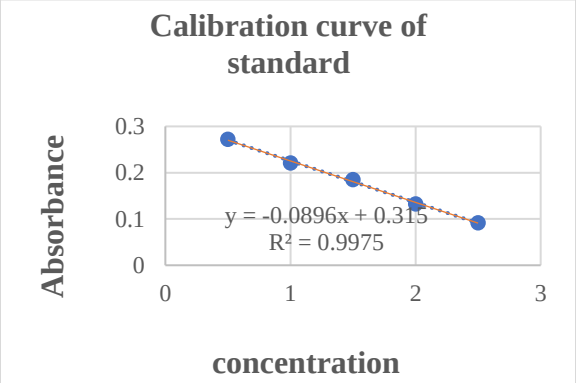
Determination of fatty acid cardamom seed by carbon chain no.

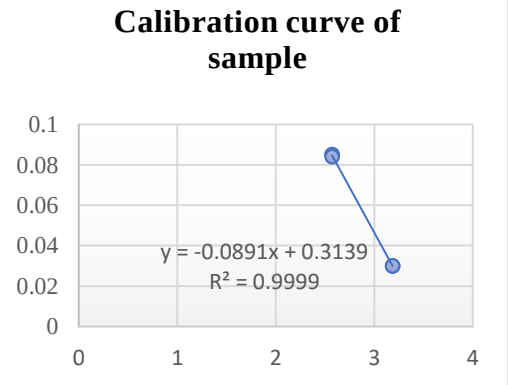
No.	Peak Name	Retention Time min	Area pA*min	Rel.Area %
1	Component 1	16.333	0.320	7.92
2	Component 2	20.283	0.024	0.59
3	Component 3	21.858	0.042	1.04
4	Component 4	22.098	0.067	1.66
5	Component 5	24.170	0.796	19.72
6	Component 6	26.503	0.064	1.59
7	Component 7	28.737	0.152	3.77
8	Component 8	30.457	0.920	22.79
9	Component 9	30.673	0.048	1.18
10	Component 10	32.668	0.914	22.65
11	Component 11	33.378	0.057	1.42
12	Component 12	34.810	0.279	6.91
13	Component 13	35.542	0.040	1.00
14	Component 14	35.988	0.060	1.48
15	Component 15	36.172	0.044	1.08
16	Component 16	36.705	0.079	1.97
17	Component 17	37.042	0.030	0.74
18	Component 18	39.832	0.052	1.28
19	Component 19	40.870	0.049	1.21
Total:			4.036	100.000

Determination of fatty acid cardamom husk by carbon chain no.

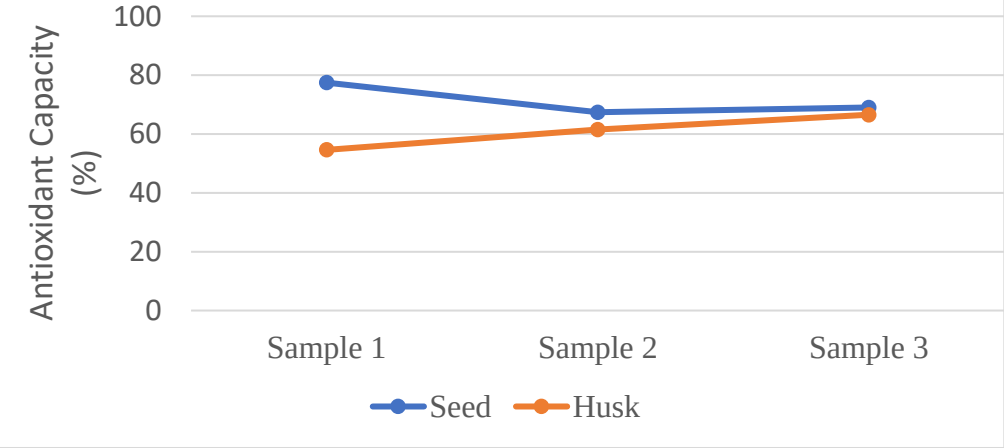


Comparison of energy provided by seed and husk of cardamom





Calibration curve for Antioxidant capacity



Comparison of Antioxidant capacity of seed and husk

Measurement of ZOI for all extracts against microbes

Appendix C: Photo Gallery

Determination of Dry matter

Ash determination by hot air oven

Fiber analysis by fiber analyzer

Estimation of crude protein by kjeldahl apparatus

Fat extraction by soxhlet apparatus

Titration for vit- C determination

Calcium determination by colorimeter

Operation of UV Visible Spectrophotometer

Brief Biography

This is Sultana Jannat Pomy, daughter of Md. Nuruzzaman and Zerin Afrose from Mirsarai upazila in the Chattogram district of Bangladesh. Sultana Jannat Pomy received her Secondary School Certificate Examination from Aparnacharan City Corporation Girls' High School in Chattogram in 2012 and her Higher Secondary Certificate Examination from Chittagong Govt. Women College in 2014. She obtained her B.Sc. (Hons.) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition at Chattogram Veterinary and Animal Sciences University (CVASU). She is very interested in working to improve people's health through proper guidance and suggestions, as well as raising public awareness about food safety and nutrition.