Physiochemical Analysis, Nutritional Characterization and Antioxidant Activities of Black Cumin (*Nigella sativa*) Seeds and Extracted Oil Available in Bangladesh

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Roll No.: 0117/03

Registration No.: 00406

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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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June 2019

Dedicated

To

My Beloved

Mentors

Acknowledgements

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List of abbreviations

AACS	American Analytical Chemical Society
AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
AOAC	Association of official analytical chemists
AOCS	American Oil Chemical Society
AV	Acid Value
BCS	Black Cumin Seed
EFA	Essential Free Acid
FFA	Free Fatty Acid
FP	Flame Photometer
FT-IR	Fourier Transform Infrared
g	Gram
GC-MS	Gas Chromatography with Mass Spectroscopy
MM	Millimeter
ML	Milliliter
N	Normality
⁰C	Degree centigrade
P. S	Particle Size
SG	Specific Gravity
TIC	Total Ionic chromatography
T	Temperature
WHO	World Health Organization

Abstract

Black cumin seed oil has a long history of uses for food flavors, perfumes, medicinal value, cosmetics industry, sausages, cheese, cakes, candies and for preparation curries, bread, preservation of butter and flavored food. The core objective of this research was to characterize two indigenous variety of black cumin (*Nigella sativa* L.), locally known as " Kali jira" and "Kalonji" and its proximate and mineral analysis, extraction of essential oil and characterization, followed by determination of iodine value, saponification, acid value, pH, kinematics viscosity and specific gravity, investigation of the effects of extraction time and particle size, identification of the essential oil by GC-MS & FT-IR and antioxidant activity. The extraction was carried out using Soxhlet method, and determination of proximate compositions, physicochemical and functional properties was done. Soxhlet extraction using n-hexane was chosen to investigate the effect of time and particle size on yield and quality of the extracted oil. A general full factorial design was applied to both seed varieties, and 54 experimental runs were performed followed by optimization of the two varieties of essential oil. The value of moisture content, crude protein content, crude fat, crude fiber, total ash and carbohydrate of Kali jira seed were 5.29g/100g, 19.83g/100g, 41.3g/100g, 18.96g/100g, 5.10 g/100g and 9.52 g/100g whereas Kalonji registered 5.43g/100g, 19.95g/100g, 40.3 g/100g, 14.28g/100g, 4.35g/100g and 14.69g/100g, respectively. The mineral analysis indicated that potassium was the dominant one (829.11mg/100g, 746.27mg/100mg) followed by phosphorous, calcium, sodium, iron and zinc in Kali jira and Kalonji varieties. The results obtained from the experiment were analyzed using Design Expert software. The minimum oil yield was found to be 46.77% in Kalonji variety whereas Kali jira contained 49.57%, after the extraction time of 2 hours with a particle size of 1.4-2.5mm. A maximum oil yield of 91.08 and 94.767% was found for Kalonji seed and Kali jira seed, respectively at the extraction time of 6 hours and at a particle size of 0.25-0.5mm. Finally, GC-MS and FT-IR analysis has been carried out on the two seed oil varieties contains of linoleic, oleic and palmitic acids were the dominant fractions respectively and both seed varieties of the dominant functional component contain p-cymene but longifolene available only in Kalonji oil by 0.51%. Due to the above properties and characteristics Kali jira varieties have been found to possess higher yield variety. In "Kali jira", carotenoids and tocopherols were 450.66±16.21 mg/kg-oil, whereas thymoquinone

contents were observed to be 201.31±13.17 mg/kg of seeds. In comparison, analysis of essential oil revealed that it contains functional ingredients like thymoquinone, dihydrothymoquinone, p-cymene, carvacrol, α-thujene, thymol, α-pinene, β-pinene and t-anethole as major constituents. Furthermore, In vitro antioxidant capacity indicated that fixed and essential oils inhibited lipid peroxidation by 25.62 and 92.56% and 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity by 32.32 and 80.25%, respectively. The present findings showed that black cumin fixed, and essential oils are rich source of phytochemicals and can be utilized against lifestyle disorders like hyperglycemia and hypercholesterolemia.

Chapter I: Introduction

Natural essential oils are the volatile, odoriferous oils obtained from plants. The oils are usually present in very small amounts and comprise only a tiny fraction of the entire plant material. The oils are produced during some metabolic processes of the plant and are secreted or excreted as odoriferous by-products. The fragrant oils may not necessarily be present as such in the living plants but may occur as odorless compounds termed as glycosides. When the plant tissues are macerated an enzymatic reaction occurs which causes the glycosides to undergo a chemical change. This action in turn liberates the distinctive essential oil.

Black cumin (*Nigella sativa*) is an annual spicy, dicotyledon of the Ranunculaceae herbaceous plants growing in countries bordering the Mediterranean Sea. The plant belongs to the *Ranunculaceae* family of flowering plants and genus of about 14 species including Nigella *arvensis, Nigella ciliaris, Nigella damascene, Nigella hispanica, Nigella integrifolia, Nigella nigellastrum, Nigella orientalis and Nigella sativa, respectively* Among these, *Nigella sativa* is the species most exhaustively investigated for therapeutic purposes although other species have also been implicated for therapeutic uses.

Nigella sativa L. or the common English name, black cumin is known for its seeds that has special healing properties as being made known in Quran which states that black cumin seeds are used to treat all kinds of illnesses except death itself. Black cumin seeds have been used for centuries long in culinary due to the strong, hot peppery taste to it (Ramadan, 2007). The seed oil or extract is found to have therapeutic properties and is considered as one of the newer sources of edible oils (Cheikh-Rouhou et al., 2007). Both seeds and oils are often used as nutritional supplement due to its various health properties as they have been reported to possess antitumor activity (Worthen et al., 1998), antioxidant activity (Burits & Bucar, 2000), anti-inflammatory activity (Houghton et al., 1995), antibacterial activity (Morsi, 2000) and a stimulatory effect on the immune system (Salem & Hossain, 2000).

Scientific investigations have reported its proximate results for moisture, oil, protein, ash and total carbohydrate to be in the range of 3.8-8.65%, 24.48-40.35%, 20.8-26.7%, 3.7-4.86% and 24.9-40.0%, respectively (Atta 2003; Cheikh-Rouhou et al., 2007; Takruri & Dameh, 1998). The chemical properties of the oil reported were 0.9110- 0.9210 g/cm3 for specific gravity, 1.46-1.47 for refractive index, 192-218 mg/g for saponification value and 4.35-18.1 meq O_2/kg for peroxide value (Abdel-Aal et al., 1993; Atta. 2003; Cheikh-Rouhou et al., 2007). Minerals that are found dominantly in the seeds are potassium, calcium, phosphorus and magnesium (Sultan et al., 2009). Both seeds and oils are abundant in oleic, linoleic and linolenic acids which are unsaturated fatty acids (Atta 2003). The vastness of medicinal properties of the *N. sativa* can be attributed to its phenolic compounds that contain high levels of antioxidant activity. It has been reported that black cumin seeds have a phenolic content that is higher than most edible oils except for olive oil (Salvador et al., 2001).

The health enhancing potential of black cumin has been attributed to the active ingredients that are mainly concentrated in fixed and essential oil (Ramadan, 2007). Black cumin seed fixed oil contains appreciable quantities of unsaturated especially polyunsaturated fatty acids; constitute the bulk of oil ranging from 48-70%, while monounsaturated (18-29%) and saturated fatty acids (12-25%) are in lesser proportions (Nickavar *et al.,* 2003; Cheikh-Rouhou *et al.,* 2007). Besides better fatty acid profile, it also contains considerable quantities of tocopherols and allied bioactive compounds that are important in attenuating the overall antioxidant capabilities of the body (Valko *et al.,* 2007). Moreover, presence of phytosterols further strengthens its hypoglycemic and hypercholesterolemic perspectives (Cheikh-Rouhou *et al.,* 2007; Ramadan & Mörsel, 2003; Atta, 2003). Likewise, pharmacological investigations explored the effectiveness of essential oil and its active ingredient i.e., thymoquinone against various maladies like oxidative stress, cancer, immune dysfunction and diabetic complications (Gali-Muhtasib et al., 2004; Hussein et al., 2005).

Like most herbs, the composition of black cumin varies with the geographic distribution, time of harvest and agronomic practices. This project was designed to characterize indigenous variety of black cumin seeds and thus nutritional profile of fixed and essential oils could possibly be used for their potential applications against lifestyle disorders.

Even with the amount of work done on *N. sativa*, there is a lack of data on the nutrient composition of both seeds and oils, thus, preventing further comparisons to be done as to determine the effectiveness of either forms of *N. sativa*. Besides that, local varieties have not been studied and compared with those imported. It is of utmost importance to determine the composition of local varieties as black cumin seeds are often affected by geographical differences, climate, soil, harvest and storage. This study was carried out to determine and compare the physicochemical characteristics, nutrient profile and antioxidant activities found in both *Nigella sativa* seeds and oils that were obtained from local market of Bangladesh.

With the afore mentioned background the present investigation has been performed with the following objectives:

1.1. Objectives

- To study the physical parameters of Black cumin seed.

- To study proximate and mineral composition analysis of Black cumin seed.

- To extract essential oil from Black cumin seeds by Solvent extraction technique

- Investigation the effects of particle size and extraction time.

- To study the physical and chemical composition of fixed oil of Black cumin seed and

- To study antioxidant activities of fixed oil and essential oil of Black cumin seed.

1.2. Expected outcomes

- This study is contributing a significant method of identification of high-quality variety of black cumin essential oil.

- This work will show the possibility of extraction technology of black cumin seed essential oil extraction process.

- Black cumin essential oil production will get more recognition and black cumin grower's farmers will be beneficial from selling of black cumin seed.

- To establish local industries of essential oil production because of available raw material, land and cheap raw materials.

- To create jobs for those that will be engaged in planting/cultivating of the plant as well as establishing small scale extraction plants.

- To show economical, health importance of black cumin seed and the uses of black cumin seed essential oil in production process.

- Increase the foreign currency by reducing the raw seed export rather we will send essential oil form.

Chapter II: Review of Literature

2.1. Background of Black cumin (Nigella sativa)

Black cumin is an annual flowering plant, native to southwest Asian**.** It grows to 20–30 cm (7.9– 12 in) height, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually colored pale blue and white, with 5–10 petals. The fruit is a large and inflated capsule composed of 3–7 united follicles, each containing numerous seeds. The seed is used as a spice (Atta, 2003; Burits and Bucar, 2000; Yazachew, 2011).

Black cumin is one the most revered medicinal seeds in history. The best seeds come from Egypt where they grow under almost perfect conditions in cases where they are watered until the seed pods form. Black cumin seeds were found in the tomb of Egyptian boy-king Tutankhamen (King Tut). Though black cumin seeds are mentioned in the Bible as well as in the words of the Prophet Mohammed, they were not carefully researched until about 1550 many years ago. Since 1959, over 200 studies at international universities and articles published in various journals have shown remarkable results supporting its traditional uses recorded almost 1400 years ago (Atta,2003; Burits, 2000; Takruri and Dameh, 1998). Dioscorides, a Greek physician of the 1st century, recorded that black seeds were taken to treat headaches, nasal congestion, toothache, and intestinal worms. They were also used, he reported, as a diuretic to promote menstruation and increase milk production (Atta, 2003; Ostlund, 2002).

Figure: 2.1. Black cumin plant before maturation	**Figure: 2.2. Black cumin flower**	**Figure: 2.3. Black cumin seed**

The Muslim scholar al-Biruni (973-1048), who composed a treatise on the early origins of Indian and Chinese drugs, mentions that the black seed is a kind of grain called alwanak in the Sigzi dialect. Later, this was confirmed by Suhar Bakht who explained it to be habb-i-Sajzi (viz. Sigzi grains). This reference to black seed as "grains" points to the seed's possible nutritional use during the tenth and eleventh centuries.

In the Greco-Arab/Unani-Tibb system of medicine, which originated from Hippocrates, his contemporary Galen and Ibn Sina, black seed has been regarded as a valuable remedy in hepatic and digestive disorders and has been described as a stimulant in a variety of conditions, ascribed to an imbalance of cold humors. The many uses of black seed have earned the Arabic approbation Habbatui Barakah**,** meaning "the seed of blessing".

2.2. Bangladeshi black cumin varieties

2.3. The Nigella Sativa Family

The three most important varieties of black cumin are:

1. *Nigella Sativa* **(true black cumin):-** grows to a height of 6-12 inch; bears milky white apical blossoms that turn bluish-green near the tip, containing a coarse ball like fruit capsule that developed after the plant blossoms. It is a crown of five protruding beak- like spikes (Ostlund, 2002; Yazachew, 2011).

2. **N.damascenais an ornamental (Turkish black cumin, garden black cumin or damask funnel):-** is an ornamental that grows to height of 2.5 feet with upright stem and dark green, finely slit leaves with long, thin tips reminiscent of dill leaves. The blossoms are surrounded by five similar slit leaves. The plant's black, triangular horizontally wrinkled seeds resemble but taste milder and smell more pleasant. The seeds make a better seasoning for sweet baked goods, fruit salad or an ingredient in snuff (Ostlund, 2002; Yazachew, 2011).

3. **N.arvensis field black cumin (also known as wild black, oat or horse black cumin):-** grows just 8 inches tall. Its upright, hairless stem bush like branches with alternating lancinated leaves of seeds and apical blossoms bring a light blue five-leaved flower cup rimmed with greenish strips. Unlike the other two spices, the three of five leaves of the seed capsule reach only halfway up the stem and are neither coarse nor puffed into a ball, but are long with little horns (Ostlund, 2002; Yazachew, 2011).

2.4. Essential Oils

Essential oils can be defined as a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oil is volatile oil, ethereal oils, or simply as the "oil of" the plant from which they were extracted, usually having the characteristic odor or flavor of the plant from which it is obtained, used to make perfumes and flavorings. Oil is essential in the sense that it carries a distinct scent, or essence of the plant. Essential oils are used in the manufacture of high quality perfumes and lotions, food flavorings, medicines, cosmetics and as fragrant seed and antiseptic additives in many common products. Besides essential oils promote hormonal balance, improve digestion and cure respiratory problems and infections. (Kumar, 2010).

An essential oil is a liquid that is generally extracted from the leaves, stems, flowers, bark, roots, or other elements of a plant. Essential oils, contrary to the use of the word "oil" are not really oily feeling at all. Most essential oils are clear, but some oils such as patchouli, orange and lemongrass are amber or yellow in color.

Essential oils contain the true essence of the plant it was derived from and are highly concentrated. Essential oils are not the same as perfume or fragrance oils. Where essential oils are derived from the true plants, perfume oils are artificially created fragrances or contain artificial substances and do not offer the therapeutic benefits that essential oils offer. Formerly, essential oils are produced by tedious hand pressing and sponge pressing. They are now produced by high-speed machines. The yield of essential oils varies widely from species to species.

Essential oils are generally liquid at room temperature. Many components of essential oils are chemically active, thus could participate readily in metabolic reaction. They are sources of plant metabolic energy. The chemical composition and aroma of essential oils can provide valuable psychological and physical therapeutic benefits. These benefits are usually achieved through methods including inhalation and application of the diluted oil to the skin.

Essential oils basically are made up of carbon, hydrogen, oxygen and occasionally nitrogen, sulphur and other minerals and vitamins for example Western Red Cedar, Ravensara, Rosemary cineole, caraway seed and Eucalyptus species. The volatile components of essential oils usually contain 15 carbon atoms or less. Essential oils contain the DNA of the plant of herb they are extracted from. Essential oils or as they are sometimes called volatile oils are believed to be that small portion of the plant material, which imparts the characteristic odor and flavor most closely associated with the vegetative matter from which they are obtained. It is normally concentrated, having about 100 times the flavoring strength of the parent plant. Most essential oils are used at about a level of 0.01-0.1 percent in the finished product. They are often slightly colored and have a specific gravity of about 1. The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majorities of them are fairly stable (notable exception is the citrus oil) and contain a few natural antioxidants. Although most are soluble in high strength alcohol (greater than 90 percent), they have poor water solubility, and most contain terpenes that contribute to their poor water solubility (Ferrari, 2004). Most of the essential oils have used it medicinal purposes (Burits and Bucar, 2000). For example, some essential oils are adaptogenic. This implies that the essential oil increases resistance and resilience to stress, enabling the body to avoid reaching collapse. Adaptogenic essential oils aid the body in maintaining homeostasis throughout stressful periods.

2.5. Sources of Natural Essential Oil

Plant organs containing natural essential oils. Essential oils are generally derived from one or More plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavender), leaves (e.g. mint, Ocimumspp., lemongrass, jamrosa), leaves, stems (e.g. geranium, patchouli, petitgrain, verbena, cinnamon), bark (e.g. cinnamon,

cassia, canella), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, sassafras, vetiver, saussurea, valerian), seeds (e.g. fennel, coriander, caraway, dill, nutmeg), fruits (bergamot, orange, lemon, juniper), rhizomes (e.g. ginger, calamus, curcuma, orris) and gums or oleoresin exudations (e.g. balsam of Peru, balsam of Tolu, storax, myrrh, benzoin). Depending upon the plant family, essential oils may occur in specialized secretary structures such as glandular hairs (Labiatae, Verbenaceace, Geraniaceae), modified parenchymal cells (Piperaceae), resin canals (conifers), oil tubes called vittae (Umbelliferae), lysigenous cavities (Rutaceae), schizogenous passages (Myrtaceae, Graminae, Composite) or gum canals (Cistacae, Burseraceae).

It is well known that when a geranium leaf is lightly touched, an odor is emitted because the long-stalked oil glands are fragile. Similarly, the application of slight pressure on a peppermint leaf will rupture the oil gland and release oil. In contrast, pine needles and eucalyptus leaves do not release their oils until the epidermis of the leaf is broken. Hence, the types of structures in which oil is contained differ depending on the plant type and are plant family specific. Unfortunately, not enough is known even today about these oil secretary structures to carefully categorize them. From the practical standpoint, they can be categorized into superficial and subcutaneous oils. During handling, some flowers continue to produce aroma while other quickly lose their odor. Flowers collected at different times may also give different perfumery values. Regarding the rose, half-open flowers with plump anthers give higher oil yield than fully opened flowers with shriveled anthers. Humidity, wind, rain and surface temperature also affect the oil yield considerably. Harvesting schedule affects both quantity and quality of the oil.

2.6. Uses of Essential Oils

Essential oils are used in several healing systems, including aromatherapy, medicine and massage therapy. Essential oils are used for skin and scalp conditions including acne, burns, cuts, dandruff, insect bites, parasites, warts, and wrinkles. They are recommended for muscle, joint, and circulation problems such as high blood pressure, cellulite (fatty deposit causing a dimpled or uneven appearance, around the thighs and buttocks), aches and pains. For respiratory problems and infections, various essential oils are prescribed for allergies, asthma, earache, sinus infections,

colds and flu. Essential oils are also used to improve digestion, promote hormonal balance, and tone the nervous system in conditions including anxiety, depression, sexual dysfunction, and exhaustion.

Essential oils can be used as quick and effective mood enhancers, for increasing energy and alertness or reducing stress and promoting relaxation. Essential oils can be used as perfumes and lotions, and can be used as incense to improve the atmosphere in houses and offices. The industrial application of essential oils are as adhesives, automobile industry, food industry, insecticide industry, meat packing industry, paint industry, paper and printing, perfume industry, pharmaceuticals industry, rubber industry, soap industry, tobacco industry, textile processing industry, etc.

2.7. *Black cumin properties and Application*

Black cumin has a long history of uses as food flavors, perfumes, cosmetics and medicinal values. Oil has been used for bringing smell to some medicines, sterilization of surgical operation fiber, production of some veterinary and agricultural medicines and plastic components (Aminpour and Karimi, 2004).

Black cumin seeds have an aromatic odor and bitter taste. They are used as an essential ingredient in soup component, sausages, cheese, cakes and candies. The Bangladeshi variety of cumin seed Kali jira, Kalonji and Deribera accumulate up to 50% thymol, a monocyclic phenolic compound (Ermias Assefa, 2015). The presence of this compound makes cumin valuable source for health care industry (Black et.al., 2006) and medicinal purposes (Ashraf et al., 2006). Black cumin is used principally to flavor food, either as whole grain, in powdered form or as an oleoresin extract. It is also used in gripe water and other herbal medicines. Within Ethiopia its main use is as a spice, which is typically ground and mixed with other spices. There is also some use in traditional medicine.

Nigella sativa seeds have very little aroma but are carminative, meaning they tend to aid digestion and relieve gases in the stomach and intestines. They aid peristalsis and elimination. The essential oil of black cumin is antimicrobial, antioxidant and helps to rid the intestines of worms (Atta, 2003; Yazachew, 2011).

Black cumin is regarded by many as a panacea (universal remedy) and may therefore not be taken seriously by some, but for those inclined to dismiss folklore, it should be noted that these humble seeds have been found superior to almost every other natural remedy when used for autoimmune disorders, conditions in which patients suffer greatly because their own systems attack their bodies. Black cumin, especially when combined with garlic, is regarded as a harmonizer of the imbalance which allows immune cells to destroy healthy cells (Atta, 2003; Burit and Bucar, 2000; Ostlund, 2002).

2.7.1. Cosmetic application and properties

Nigella oil is amber oil which is a natural antioxidants source thanks to the presence of thymoquinone and several phytosterols, responsible also for the lenitive and emollient effects. It has been reported that black cumin oil has a higher radical scavenger activity than extra virgin olive oil, when they are compared using stable DPPH and galvinoxyl radicals.

Nigella oil is able to inhibit the production of 5-lipoxygenase, reducing the inflammation. Consequently, it can find topical application for cosmeceutical treatment of skin inflammatory conditions such as psoriasis and eczema. It is also active against several bacteria and yeast.

Nigella oil has a good amount of linoleic acid which is involved in skin barrier function and skin permeability. These features make the oil for cosmetic products aimed for sensitive skin.

2.8. Constituents of black cumin

N.Sativia seeds are aromatic and contain a disagreeable odor. The composition varies with the varieties, region and the age of the product. Black cumin seed is rich in nutritional values. Monosaccharide in the form of glucose, rhamnose and xylose are found in the black seed. The black cumin seed contains a non-starch polysaccharide component, which is a useful source of dietary fibers. It is rich in fatty acids, particularly the unsaturated and essential fatty acids (linoleic acid). Fifteen amino

acids make up the protein content of the black cumin (*Nigella sativa*) seed, including the nine amino acids. Black cumin seed contains arginine, which is essential for infant growth. Chemical analysis has further revealed that the black cumin seed contains carotene, which is converted by the liver into vitamin A. Black cumin seeds have the following properties: such as Stable Shelf life (up to 2 Years), Golden Brown Color, Moderate Viscosity, Quick Absorption, Mildly Herbaceous Aroma, Vitamin composition A, B1, B2, B6, C, Niacin and Folacin, Mineral composition includes Ca, Fe, Mg, Cu, P, and zinc, moisture, oil, proteins, ash and total carbohydrates contents were in range of 3.8-7.0, 22.0 to 40.35%, 20.85- 31.2, 3.7-4.7 and 24.9-40.0% respectively (Dandik and Aksoy, 1992; Abdel-Aal et al., 1993; El-Dhaw and Abdel-Munaem, 1996; Takruri and Dameh,1998; Haq et al., 1999; Atta, 2003; Salem, 2005) and contain up to 80% unsaturated essential fatty acids, Rich in anti-oxidants, High Linoleic (Omega-6) Essential fatty Acid Content < 55%, High Oleic (Omega-9) fatty Acid Content: > 24.7%, Supports and strengthens the immune system (Abdel-Aal et al., 1993; Dandik and Aksoy, 1992).

Besides the volatile oil and fatty oil, the black cumin seeds contain a bitter principle (nigellin), tannins, resins, protein reducing sugar and saponins. The free amino acids present in dormant seeds are cystine, lysine, aspartic acid, glutamic acid, alanine, tryptophan, vallne and Lucien (Atta, 2003; Burits, 2000; Dandik and Aksoy, 1992; Yazachew, 2011).

2.9. Black cumin essential oil

Black cumin is a good source of essential oils. Before discussing black cumin seed essential oil first discuss the uniqueness of essential oil, uses and industrial application and extraction technologies is explained below.

2.9.1. Uniqueness of essential oils

In early work, the term "essential oils" was defined as the volatile oils obtained by the steam distillation of plants. This definition was clearly intended to make a distinction between "fatty oils" and the oils which are easily volatile. Gradually with the advance of science came improvements in the methods of preparing the oils, and parallel with this development a better knowledge of the constituents of the oils was gained. It was

found that the oils contain many classes of organic substances with varying volatility. Although a list of all the known oil components would include a variety of chemically unrelated compounds, it is possible to classify these into four main groups of essential oils (Guenther, 1960): Terpenes, related to isoprene; Straight-chain compounds, not containing any side branches; Benzene derivatives and Miscellaneous.

Terpenes: The most characteristic group present in any essential oil contains hydrocarbons of molecular formula $C_{10}H_{16}$ and their oxygenated derivatives $C_{10}H_{16}O$ which are known as monoterpenes.

Straight chain compounds: These groups contain straight chain hydrocarbons and their oxygenated derivatives and range from n-heptane to hydrocarbons containing 15-35% carbon atoms.

Benzene Derivatives: Many benzenoid compounds are found distributed throughout the essential oil kingdom.

Miscellaneous: Many compounds obtained in this group are sulfur compounds and are rather specific for a limited species.

2.9.1.1. Essential oils are different from other oils by their properties:

A. **Essential oils are volatile:** essential oils are the volatile fragrant components from various indigenous and exotic plants which have been traded internationally for several centuries (Yesenofski, 2005).

B. **Essential oils are aromatic:** essential oils are highly aromatic and therefore, many of the benefits can be obtained by simply inhaling them. This can be done by breathing in the fragrance from the bottle, or they can be diffused into the room. Essential oils, when diffused, can be the best air filtration system in the world. They will:
   - ➤ Purify the air by removing metallic particles and toxins from the air;
   - ➤ Increase atmospheric oxygen;
   - ➤ Increase ozone and negative ions in the house, which inhibits bacterial growth destroy mold, cigarettes and animal odors; fill the air with a fresh, herbal aromatic scent (Becker, 2005).

C. **Essential oils have penetrating characteristics**: the penetrating characteristic of essential oils greatly enhances their ability to be effective. Essential oils will

penetrate into the body when applied to the skin. Essential oils rubbed into the feet will be distributed to every cell in the body in minutes. They will even penetrate a finger or toe nail to treat fungal infection underneath. Other vegetable oils do not have this propensity to penetrate (Becker, 2005).

D. Pure Essential oils have very high frequency: the effectiveness of essential oils is sometimes also described in terms of frequency. It has been reported that the human body has an electrical frequency and that much about a person's health can be determined by frequency.

In 1992, Bruce Tainio of Tanio Technology, an independent division of Eastern State University in Cheny, Washington, built the first frequency monitor in the world. Tainio has determined that the average frequency of the human body during the day time is 62-68 MHz. (a healthy body frequency is 62-72). When the frequency drops, the immune system is compromised. If the frequency drops to 58 MHz, cold and flu symptoms appear, at 55 MHz, diseases like Candida take hold, at 52 MHz, Epstein bar and at 42 MHz, cancer. According to researcher (Dr. Royal R. Rife), every disease has a frequency (Becker, 2005). He found that certain frequencies can prevent the development of disease and that others would destroy disease. Substances with higher frequency will destroy diseases of a lower frequency. The study of frequencies raises important questions, concerning the frequencies of substances we eat breath and absorb. Many pollutants lower healthy frequency. Processed canned food has a frequency of zero. Fresh produce has up to 27 MHz. Essential oil start at 52 MHz and go as high as 320 MHz, which is the frequency of Rose oil. Clinical research shows that essential oils have the highest frequency of any natural substance known to man, creating an environment in which disease, bacteria, virus, fungus, etc., cannot live (Becker, 2005).

2.9.2. Extraction technology of essential oils

The quality, flavor and nutritional value of essential oils are directly related to the way the oil is extracted and processed. The highest quality oils are exposed to the least amount of heat, light, pressure and chemicals in the extraction and refining process. The yield and composition of essential oils depends on geographic location and agricultural factors. The extraction of essential oils from plants may be processed

by several methods such as steam distillation, effleurage, alcoholic extract, maceration, solvent extraction and supercritical fluid extraction, etc.

2.9.2.1. Steam Distillation

Most essential oils are produced by steam distillation. There are, however, different processes that are used. In all of them, water is heated to produce steam, which is used to extract the most volatile aromatic chemicals. The steam is then cooled (in a condenser) and the resulting distillate is collected. The essential oils will normally float on top of the hydrosol (the distilled water component) and may be separated off. Steam distillation is the most commonly used method for extracting essential oils. Many traditional distillers favor this method for distilling most oils as they claim that none of the newer methods produce better quality oils (Boucard *et al*., 2005). Steam distillation, as described by (Boucard *et al*., 2005), is done in a steal in which fresh or sometimes dried plant material is placed in a chamber of the steal. Pressurized steam, generated in a separate chamber, is then circulated through the plant material. The heat of the steam forces opens the tiny intercellular pockets in which the essential oils are contained releasing the oils. During steam distillation, the temperature of the steam must be moderated so that it is high enough to open the oil pouches without destroying the plants, fracturing or burning the essential oils as has been recommended in the literature (Sheridan, 2000).

Some or most essential oils have been found to be heat sensitive and hence thermo-degradable. As the tiny droplets of essential oils are released, they evaporate and mingle with the steam, travelling through a pipe into a condenser. The steam and oil vapor are then condensed to a liquid mixture. As the oil-water mixture has been found to be nearly immiscible at a temperature lower than about 65°C (Sheridan, 2005), and the mixture can be separated using various gravity related techniques. Due to the immiscibility of the oil and water at low temperature, the essential oil can be separated from the water by either decanting off the water or skimming of the oil from the top as the oil is less dense than water at these conditions. The density of some essential oils such as lavender oil has been reported to average 0.89g /l, as opposed to l g/1 (Ndou, 1986) for water at room temperature and atmospheric pressure conditions. The water obtained as a byproduct of distillation is referred to as floral

water or distillate and retains many of the therapeutic properties of the plant. For this very reason, floral waters are valuable in skin care for making facial mists and toners and are also preferred to essential oils when treating a sensitive individual or child or when a more diluted treatment is required (Sheridan, 2000).

2.9.2.2. Solvent Extraction

Solvent extraction is adapted in producing essential oils generated by some flowers (Rose, Violetta and Geranium), gums and resins. The raw material is placed in a glass, vessel and soaked with a suitable solvent (petroleum, hexane, ether or benzene). After the extraction, the solids are separated from the liquid mixture. The latter is heated so that the more volatile essential oils can be evaporated to be subsequently condensed. Alternatively if the solvent is more volatile, such as ethanol, it could then be vaporized leaving behind the essential oils (Ndou, 1986).

As solvent extraction uses very little heat, it is found to be advantageous in producing essential oils with whole fragrances that would otherwise be destroyed or altered during steam distillation. Therefore this extraction technique can be used to extract essential oils from very delicate plants to produce higher amounts of essential oils at lower costs (Ndou, 1986). There are, however, some disadvantages associated with the solvent extraction technique. Solvent residues often contaminate the product causing side effects which make the use of essential oil undesirable for skin applications but could still be fine for fragrances or perfumes (Ndou, 1986).

Therefore, with solvent extraction effective separation of the extracted oil from the solvent is necessary to remove any solvent which may contaminate the essential oils. This process also sometimes yields an aromatic resinous product known as oleoresin which is more concentrated than essential oils with an even wider application in the food and other industries, as discussed by Heath (1981).

2.9.2.3. Supercritical fluid extraction

Supercritical fluid extraction (SFE) of essential oils is a modern technique, currently being applied in the process industry, which competes with conventional processes such as steam distillation and organic liquid (solvent) extraction. It has been widely accepted by many investigators that SFE provides a rapid and quantitative method for

extracting essential oils from aromatic plants that compares favorably with steam distillation (Kerrola, 1995). A single-component fluid is said to be supercritical when its temperature and pressure both exceed their critical values, without being far from the critical state (Gaspar, 2002). At these elevated conditions the properties of the fluid have both liquid and gas properties.

All materials have a critical point, but for some materials however this state is more easily reached than others. Carbon dioxide is the most commonly used fluid in extracting essential oils and its application and technique have been extensively researched. Supercritical extraction by carbon dioxide is isolation and separation process taking advantage of the fact that above critical conditions of 31.1°C and 78.8 bars, carbon dioxide cannot be liquefied by any further increases in pressure. Whilst the carbon dioxide is in this supercritical state, the dense gas gains considerable solvent power, dissolving the primary target such as the essential oil in the plant material. The pressure is then dropped in the separator, causing the carbon dioxide to lose its solvating power and hence releasing the extracted oil drops, leaving behind a high purity essential oil extract. This high purity of these products has recently attracted the attention of the pharmaceutical industry, due to the heavily regulated trade demands for lower quantities of solvent in the final product. This area of research is currently being pursued and analyzed by various researchers such as Gaspar (2002), in their work for various pharmaceutical companies worldwide, in developing new production techniques based on supercritical carbon dioxide. Using carbon dioxide is especially useful as it is cheap, clean and intrinsically safe with no harmful residues like solvents in the extracts that would be found for solvent extraction as discussed before. The operating temperatures are relatively low, which enables thermally labile compounds to be extracted. This method of extraction has also been reported to be ecologically harmless (Gaspar, 2002) as no harmful residues of toxic solvents result from it. Supercritical fluid extraction by carbon dioxide has therefore been described by (Simon et al., 1990) as a new method; potentially commercially viable but is less common and beyond the financial means of most small-scale processors (Singh et al., 2001).

2.9.2.4. Cold pressed expression method

Another method of extraction of essential oils is cold pressed expression, or scarification. It has been reported to be used mainly to obtain citrus fruit oils such as bergamot, grapefruit, lemon, lime, mandarin, orange, and tangerine oils (Worwood, 1990). In this process, the fruit is rolled over a tracer with sharp projections that penetrate their peels thereby piercing the tiny pouches containing the essential oil. The fruit is then pressed to squeeze out the juice from the pulp thereby releasing the essential oils from the pouches. The essential oils rise to the surface of the juice and are separated by centrifugation. As discussed in the literature (Stahl et al., 1988), cold pressing is more competitive for specific raw material than methods such as supercritical fluid extraction as it is extremely fast, cheap and does not pollute the extracts, although it does not provide a way of selectively extracting essential oils.

2.9.2.5. Effleurage method of extraction

Some flowers, such as jasmine or tuberose, have such low contents of essential oils and are so delicate that heating them would destroy their blossoms before releasing the essential oils. In such cases, an expensive and lengthy process called effleurage is sometimes used to extract the essential oils. As described in the literature (Stahl et al., 1988), flower petals are placed on trays of odorless vegetable or animal fat, which absorb the essential oils from them. At the end of every day or even after a few hours, when the vegetable or fat has removed as much of the essential oil as possible, the depleted petals are removed and replaced with fresh ones. Adding alcohol to this effleurage mixture separates the essential oils. This method employs a similar operating principle and technique to what was discussed for solvent extraction.

2.9.2.6. Microwave extraction

Microwave energy is a superior alternative to several thermal applications owing to its efficient volumetric heat production. The volumetric heating or heating of the bulk as opposed to transferring heat from the surface, inwards, is more efficient, uniform and less prone to overkill or supererogation. Controllability is by far the greatest advantage of microwaves over conventional thermal technologies. In processing applications, the ability to instantaneously shut the heat source makes enormous

difference to the product quality and hence the production economics. The raw material is heated directly by microwaves and this brings about quality consistency and minimizes the impact on the environment as opposed to using fossil fuels or less efficient, indirect electrical heating systems. Specifically, in the essential oil extraction, microwave mediated processes are highly desirable due to their small equipment size (portability) and controllability through mild increments of heating. However, so far, the microwave technology has found application in very few industrial bio-processing installations due to the lack of available data on microwave interaction with heterogeneous natural raw materials.

2.9.3. Physical properties

❖ **Specific gravity**

Specific gravity is an important criterion of the quality and purity of an essential oil. Values for essential oils vary between the limits of 0.696 and 1.188 at 15 °C, in general, specific gravity is less than 1.000 (Guenther, 1960). Hence essential oil can be collected over (floating on) water.

❖ **Refractive index**

When a ray of light passes from a less dense to a denser medium, it is bent or "refracted" toward the normal. If e represents the angle of refraction and i the angle of incidence, according to the law of fraction,

$$\text{Sin } i \,/\sin e = N \,/\, \text{n}$$

Where n is the index of refraction of the less dense, and N, the index of refraction of the denser medium. Refractometers offer a rapid and convenient method for the determination of this physical constant.

❖ **Boiling range**

In the case of isolates and synthetics, the boiling range is an important criterion of purity.

❖ **Evaporation residue**

An important criterion of purity is the evaporation residue; i.e., the percentage of the oil which is not volatile at 100°C. It is important to study the odor of oil as it volatilizes during the heating.

❖ **Flash point**

The flash point may prove useful in the valuation of an essential oil. The flash point has value as an indication of adulteration: additions of adulterants such as alcohol and low boiling mineral spirits will greatly lower the flash point.

2.9.4. Chemical properties

In general, essential oils consist of chemical compounds that have hydrogen, carbon, and oxygen as their building blocks. These can be subdivided into two groups: hydrocarbons, which are made up almost exclusively of terpenes (monoterpenes, sesquiterpenes, and diterpenes); and the oxygenated compounds, mainly esters, aldehydes, ketones, alcohols, phenols, and oxides; acids, lactones, sulphur and nitrogen compounds are sometimes also present.

- ✓ **Aldehydes:** citral, citronellal, and neural are important aldehydes found notably in lemon scented oils such as Melissa, lemongrass, lemon verbena, citronella, etc. Aldehydes in general have a sedative effect; citral has antiseptic properties.
- ✓ **Phenols:** These tend to have a bactericidal and strongly stimulating effect but can be skin irritants. Common phenols include eugenol (found in clove and West India bay), thymol (found in thyme), carvacrol (found in oregano and savoury).
- ✓ **Terpenes:** common terpene hydrocarbons include limonene (antiviral, found in 90 per cent of citrus oils) and pinene (antiseptic, found in high proportions in pine and turpentine oils). Sesquiterpenes have outstanding anti-inflammatory and bactericidal properties.
- ✓ **Ketones:** some of the most common toxic constituents are ketones, such as thujone found in mugwort, tansy, sage and wormwood; and pulegone found in pennyroyal and buchu. Non-toxic ketones include jasmine (in Jasmine) and fenchone (in fennel oil).
- ✓ **Oxides:** by far the most important oxide is cineol (or eucalyptol). It has an expectorant effect and is well known as the principal constituent of eucalyptus oil. It is also found in a wide range of other oils, especially those of a camphoraceous nature such as rosemary, bay laurel, tea tree, and cajuput.
- ✓ **Esters:** probably the most widespread group found in essential oils, which includes linalyl acetate (found in bergamot, clary sage, and lavender) and geranyl

acetate (found in sweet marjoram). They are characteristically fungicidal and sedative, often having a fruity aroma.

✓ **Alcohols:** these compounds have good antiseptic and antiviral properties with an uplifting quality; they are also generally non-toxic. Among the most common terpene alcohols are linalool (in rosewood, linaloe, and lavender), citronellol (in rose, lemon, eucalyptus and geranium) and geraniol (found in palmarosa); also borneol, methol, terpineol, nerol, farnesol, vetiverol, benzyl alcohol, and cedrol (Lawless, 1998).

2.9.5. Factors affecting the yield and quality of the essential oils

The yield and quality of essential oils have been known to vary due to several factors.

➢ **Particle size**

To increase the rate of solvent extraction, it is desirable that the range of particle size to be small. This is due to the greater interfacial areas between the solid and liquid and therefore the higher is the rate of transfer of material.

➢ **Choice of solvent**

The liquid chosen should be good selective solvent, less hazardous for mass Production, low viscosity and economical. The organic solvents more frequently used are:

   a) Aliphatic hydrocarbons: propane, butane, hexane

   b) Alcohols: methanol, ethanol, 2-propanol

   c) Hydrocarbons with a carbonyl group: acetone, methyl acetate

   d) Halogen derivation, dichloromethane, Freon.

➢ **The fluid agitation**

Agitation of the solvent is important because it increases the eddy diffusion and therefore increases the transfer of material from the surface of the particles to the bulk of the solution.

➢ **The temperature**

The solubility of the material which is being extracted will increase with temperature to give a higher rate of extraction.

> **Condition of raw material**
>
> Condition of raw material is important because some materials like roots and seeds will not yield essential oil easily if distilled in their natural state. These materials must be crushed, powdered or soaked in water to expose their oil cells.

2.9.6. Composition of essential oils

The chemical composition and aroma of essential oils can provide valuable psychological and physical therapeutic benefits. These benefits are usually achieved through methods including inhalation and application of the diluted oil to the skin.

Essential oils basically are made up of carbon, hydrogen, oxygen and occasionally nitrogen, sulfur and other minerals and vitamins. The volatile components of essential oils usually contain 15 carbon atoms or less. The largest class of component is terpenes which are 10 carbon atoms.

Chemical constituents of essential oils: an essential oil contains more than 200 chemical components, but some are many times more complex. Essential oils consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks.

They can be essentially classified into two groups:

- ✓ **Volatile fraction:** essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.
- ✓ **Nonvolatile residue:** this comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

2.9.7. Clarification and storage of essential oils

Usually, the spoilage of an essential oil is attributed to some general reactions like oxidation, polymerization, hydrolysis of esters, and to interaction of functional groups. These processes seem to be activated by heat, by the presence of air (oxygen), of moisture and catalyzed by exposure to light. There is no doubt that oils with a high content of terpenes are particularly prone to spoilage, to oxidation. Being unsaturated hydrocarbons, the terpenes absorb oxygen from the air (Handa et al., 2008).

Light seems to be of lesser importance as a factor causing deterioration, then is moisture. Essential oils containing a high percentage of oil turn acid after improper storage, due to partial hydrolysis of esters (Handa et al., 2008).

Fatty oils, with a few exceptions, are very prone to oxidation, but such spoilage can be prevented altogether by the addition of suitable antioxidants, such as hydroquinone or its mono ethyl ether. Certain types of essential oils, especially those containing alcohols, are quite stable and stand prolonged storage.

As a general rule, any essential oil should first be treated to remove metallic impurities, freed from moisture and clarified, and then be stored in well filled, tightly closed containers, at low temperature and protected from light.

The small lot can be dehydrated quite readily by the addition of anhydrous sodium sulphate, by thoroughly shaking, standing overnight to 6-8 hours and filtration. Calcium chloride must never be used for dehydration of essential oils, as this chemical is adaptive to form complex salts with certain alcohols. Larger commercial lots of oil are always easy to clarify (Handa, 2008).

Some oils give a great deal of trouble. The simplest procedure is to add a sufficient amount of common salt to the lot, to stir the mixture for a while, and to let it stand until the supernatant oil has become clear and can be drawn off from the tank. The lower layer will be cloudy and needs to be filtered clear. If filtration through plain paper does not give clear oil, specially prepared filtering clay should be placed into the filter. Care must be exercised in the selection of filtering medium as some media; activated carbon for example, may react chemically with certain constituents of the oil and affect its quality. Large quantities of oil should be filtered through filter presses, which are readily available. Centrifuging in high-speed centrifuges is an excellent means of clarifying essential oils.

Not only moisture but also waxy material depositing after a certain period of storage, if possible at low temperature in a freezing room, can thus eliminated. Some lots of essential oils, especially those with a high content of phenols arrive from the producing fields often in a crude form and dark colored, due to the presence of metallic impurities. Such lot must be decolorized before they can be placed at the

disposal of the consumer. This dark color may be removed by the formation of complex slats with certain organic acids. For this purpose sufficient powdered tartaric acid is added to the oil, the mixture stirred for some time and permitted to settle. The supernatant clear oil can finally be drawn off, while the lower layer has to be filtered until clear. If the treatment with solid tartaric acid does not give satisfactory results, a concentrated aqueous solution of the acid is added to the oil. After thoroughly stirring the mixture, is allowed to stand until the two liquid layers separate clearly. The supper part of the oil layer should then be sufficiently clear to be drawn off, while the lower layer and especially the intermediary layer need further treatment by clarification and filtration. Here again high-speed centrifuge are of great help. In case where the color cannot be eliminated by treatment with organic acids, the oil will have to be clarified by reinstallation or rectification. If the oil quantity is less they can be easily stored in bottles of hard and dark colored glass. For larger quantities of oil stainless steel or aluminum containers should be preferred. During storage oil should be filled up to the brim and containers should be kept in shaded/cool areas away from direct heat and sunlight. A layer of carbon dioxide or nitrogen gas blown into container before it is sealed will replace the layer of air above the oil and there by assured added protection against oxidation.

2.9.8. Essential oil extraction from black cumin

Black cumin (*Nigella sativa*) locally known as - Kali jira is good source of nutritionally essential components. The oil we get from the extraction of black cumin is used as essential oil. Extraction of black cumin using solvent extraction method is discussed as follow.

2.9.8.1 Solvent extraction of black cumin

❖ **Selection of Solvent**

The most important factor for the success of the extraction process is the quality of the solvent employed. The ideal solvent should possess the following properties:

- It should completely and quickly dissolve all the materials being extracted.
- It should possess a sufficiently low boiling point to permit its being easily removed without resorting to higher temperature; yet the boiling point

should not be too low, as this would involve considerable solvent loss by evaporation in the warm climate.

- ✓ The solvent must not dissolve in water.
- ✓ The solvent must be chemically inert.
- ✓ The solvent should have a uniform boiling point when evaporated and does not leave any residue.
- ✓ The solvent should be low priced, non- flammable.

But the ideal solvent, which fulfills these entire requirements, does not exist. Considering every feature, highly purified petroleum ether appears to be the most suitable. There are different solvents used for the extraction of black cumin; benzene, hexane, diethyl ether and petroleum ether are some of the solvents.

Benzene has a high boiling point (80.1⁰C) resulting high amount of benzene in the last product. Besides, benzene is highly flammable. Therefore, benzene is not preferable solvent for the extraction of black cumin.

Diethyl ether on the other hand has a low boiling point resulting in a lot of solvent lose and is highly flammable as well as costly solvent; hence diethyl ether is not preferable solvent option for the extraction of back cumin oil.

Petroleum ether is the best solvent, unfortunately it is very costly and is not found easily as a result it will not be an option as solvent for the extraction of black cumin.

Hexane which has a moderate boiling point (69⁰C) is relatively cheap and safe compared to diethyl ether and benzene; hence hexane is a preferred solvent for the extraction of black cumin oils.

2.9.8.2 Soxhlet Extraction

Soxhlet extraction is a more efficient way to extract solids. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then connected to the use a condenser.

The solvent is heated to reflux, the solvent vapor travels up and floods into the chamber housing, the thimble neck solid. The condenser ensures that any solvent vapor cools and drops back down into the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compounds will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the round bottom flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the round bottom flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction, the non-soluble portion of the extracted solid remains in the thimble and is usually discarded. The mixture of solvent and essential oil has been separated by means of a rotary evaporator, and then the essential oil was used for further characterization of analysis and other applications, but solvent has been used other extraction process (recycling) operation.

2.9.9. Quality assessment of essential oils

Every essential oil is basically a mixture of different components or compounds. The percentage of these constituents in the oil plays an important role in determining its quality. The apparatus by which the fractions and their percentages are determined gas liquid chromatography unit, GC- MS and HPLC. Most of the oils being sold in the market today are based on gas liquid chromatography analysis, GC-MS and other equipment's report. For medicinal extracts thin layer chromatography and high-pressure liquid chromatography techniques are being used.

➤ **Types of test and detection of essential oils**

The quality of essential oil is determined by physical, chemical and special tests. Physical characterization is the assessment of the physical properties of crude oils focuses on such as viscosity, density and pour point, etc. and whereas chemical characterizations can be used to quantify the presence of toxic hydrocarbons and other

compounds present in crude oils that could contribute to the overall toxicity, and to fingerprint the oil for future monitoring purposes. Some of the physical tests are determination of specific gravity, optical rotation test, refractive index, color, melting point, boiling point, evaporation residue and determination of flash point.

Chemical tests include determination of acids, determination of esters by saponification with heat, determination by acetylation, determination of tertiary terpene alcohol and determination of aldehydes and ketones. Special tests include flavor test, test for halogens, test for heavy metal and determination of water content.

2.9.10. Characterization of black cumin seed oil

Black cumin essential oil is important in many aspects as it is rich in unsaturated fatty acids; linoleic and oleic acids predominate with amounts found in the ranges of 47.0- 60.0 and 18.9- 25.69%, respectively. Additionally, it also contains some minor quantities of linolenic, arachidic and eicosanoid acids (Atta, 2003; Ashraf et al., 2006; Cheikh- Rouhou et al., 2007). Polyunsaturated fatty acids constitute the bulk of the oil with quantities ranging from 48-70%, while monounsaturated and saturated fatty acids are comparatively in lesser proportions (Ustun et al., 1998; Nergiz and Otlesx, 1993; Nickavar et al., 2003; Ramadan and Morsel, 2003; Atta, 2003). Recently, Cheikh-Rouhou et al. (2007) reported that it contains 49.8-50.7% of polyunsaturated fatty acids, 25.0-26.6% of monounsaturated fatty acids, while saturated fatty acids account for 22.7-25.5% of its oil contents (Nickavar et al., 2003; Ashraf et al., 2006). The World Health Organization (WHO) enumerated standards that dietary fat should be rich in polyunsaturated fatty acids (more than 33%) and with reduced contents of saturated fatty acids (less than 33%) to boost human health. Fatty acid composition of N. sativa L. fulfill the WHO standards as it contains around 80-84% unsaturated fatty acids and 14- 20% saturated fatty acids (Al-Jassir 1992; El-Tahir et al., 1993; Nickavar et al., 2003; Ashraf et al., 2006). Black cumin has been probed as a source of polyphenols and selenium (Weinreb et al., 2004; Ustu et al.1998; Dandik and Aksoy, 1996). Fat-soluble vitamins (FSV) comprised more than 0.2% of total oil content (Ramadan and Morsel, 2002). Similarly, Al-Saleh et al. (2006) reported the concentrations of selenium, DL-tocopherol, DL- γ-tocopherol and all-trans- retinol in N. sativa seeds as 0.177, 9.027, 5.427, 0.277mg/kg seed, respectively. Formerly, Ramadan and Morsel (2002) reported that one gram of black cumin fixed oil contains

284, 40, and 225, 48µg of -Tocopherol, β-Tocopherol, γ-Tocopherol and Tocopherol, respectively. They also reported the presence of β-carotene in amounts of 593µg/g oil. Selenium, tocopherols and carotenoids are nutritionally essential components required for proper sustainability of the life as they play a key role in improving human health (Gylling et al., 1999; Ramadan et al., 2003; Thomson, 2004; Goldhaber, 2003; Sen et al., 2006). Moreover, it contains phytosterols ranged from 0.33-0.36%; the β-sitosterol (1135–1182mg/kg), Δ5-avenasterol (925–1025mg/kg), and Δ7- avenasterol (615–809mg/kg) are the major phytosterols, while stigmasterol, campesterol and lanosterol are present in small amounts (Ramadan and Morsel, 2002). Latterly, Cheikh-Rouhou et al. (2008) determined total sterol content in Tunisian and Iranian cultivars i.e. 18.03% and 13-17.41% of the unsaponifiable matter, respectively. B-Sitosterol was the major sterol with 44% and 54% of the total sterols, respectively. The next major sterol was stigmasterol in both oils (16.57–20.92% of total sterols). D7-avenasterol and cholesterol were detected at lower levels. The utilization or addition of black cumin seed oil in diet, mixed dishes and desserts could have positive impact on the amount of fat-soluble vitamins and phytosterols in the diet (Ramadan and Morsel, 2002). Essential oil extracted from black cumin is also of functional importance as it is naturally bestowed with antioxidant rich volatiles (0.40-1.50%); contain 18.4-24% thymoquinone and 46% monoterpenes (Al-Jassir 1992; El-Tahir et al., 1993; Ashraf et al., 2006).

Likewise, Burits and Bucar (2000) analyzed essential oil using GC-MS and characterized many components like thymoquinone (27.8–57.0%), ρ-cymene (7.1–15.5%), carvacrol (5.8–11.6%), tanethole (0.25–2.3%), 4-terpineol (2.0–6.6%) and 1.0–8.0% of longifoline (Nickavar et al., 2003; Mozzafari et al., 2000; Ashraf et al., 2006). Recently, determined ρ-cymene as a major component of black cumin seed essential oil. Several scientists explored the antioxidant potential of black cumin oil and its fractions containing active ingredients. Burits and Bucar (2000) observed IC50 value in DPPH (2,2-diphenyl-1- picrylhydrazyl) assay for black cumin essential oil, thymoquinone and carvacrol i.e. 460.0, 211.0 and 28.8mg/mL, respectively. Later, (Singh et al., 2005) indicted that DPPH radical scavenging effect of black cumin fixed oil in the range of 82 to 95%, comparable to butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). Black cumin seeds inhibited DPPH radical formation and mean IC50 (µM) was found to be 515 ±

20.1mg/mL (Erkan et al., 2008). Lately, Khattak et al. (2008) reported DPPH scavenging activity remained in the range of 70-90% and 60-80% for 5mg/mL of black cumin methanolic and water extract, respectively. Aqueous and 80% methanolic extract of black cumin resulted in marked inhibition of 14 DPPH radicals with IC50 (mg dry wt.) of 2.80±0.10 and 1.24±0.10, respectively (Thippeswamy and Naidu, 2005; Sultan, 2009).

The types of volatile isolates that are obtained commercially from aromatic plants are essential oils, concretes, absolutes, pomades and retinoid. Essential oils are isolated from plant material by distillation whereas other volatile isolates are obtained by solvent extraction.

Chapter III: Materials and Methods

3.1. Study Area

The experimental work has been done in the Quality Assurance Laboratory of department of Applied Chemistry and Chemical Technology under Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University; Bangladesh Standards and Testing Institution (BSTI) Laboratory, Chattogram Divisional Office and Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratory, Chattogram. In this research, black cumin seeds of 'Kali jira' and 'Kalonji' varieties of local origin were characterized for their seed property, essential oils, and explored further for their yield potential by using solvent extraction method. Materials used, and protocols followed are described below.

3.2. Study period

The study was conducted during January 2018- June 2019.

3.3. Materials and Equipment

Materials used during the experiment were black cumin seeds, local varieties: Kali jira and Kalonji, hexane (99.9%), sodium hydroxide (99%), potassium hydroxide (85%), hydrochloric acid, Gallic acid, saturated sodium carbonate, acetone, phenolphthalein, filter paper, distilled water. All the chemical and reagents were obtained from of department of Applied Chemistry and Chemical Technology under Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University.

The equipment used were Soxhlet extractor, chiller, rotary evaporator, centrifuge, condenser, oven, viscometer, flask, beaker, Distiller, balance, dissector, test tube, FTIR, AAS, GC-MS, muffle furnace, Kejaldhal, sieve, density bottle and centrifugal mille. This equipment was used from Bangladesh Standards and Testing Institution (BSTI) Laboratory, Chattogram Divisional Office and Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratory, Chattogram.

3.4. Experimental Methods

3.4.1. Raw Material Preparation

Black cumin seeds indigenous varieties were purchased from different local markets of Bangladesh. The most available 2 varieties 'Kali jira' (Bangladeshi variety, produced in Sirajgonj, Bangladesh) and 'Kalonji' (Indian variety) were collected from Khatungonj spices market, Chattogram, Bangladesh. The dust, stone and other foreign materials were removed by hand to obtain the pure seed. The seeds were dried for 1 day by sun and by oven at 50°C for 18 hr.

3.4.2. Size reduction and sieve analysis

After the moisture was removed by placing in an oven at 50°C for 18 hours, the dried black cumin seed was milled in Cross Beater Miller with a size of 3mm. Then the sample was shaken using vibrating shaker for 8 minutes with amplitude of 10mm. The sieve size was arranged in descending order of mesh size 3mm, 2.5mm, 2mm, 1.8mm, 1.4mm, 1mm, 0.85mm, 0.5mm and 0.25mm to obtain particular size of 2.5-1.4mm, 1.4-0.5mm and 0.5-0.25mm. This particular size range was selected because literature reveled that to have a higher yield of oil particle size should be less than 5mm and higher than 0.2mm (Henry, 1983).

Figure: 3.1. Cross beater mill with hopper   **Figure: 3.2. Cross beater mill**

3.5. Solvent extraction and Soxhlet extraction

Experimental work was conducted using soxhlet extractions. In solvent extraction process, hexane was used as a solvent.

Black cumin two varieties of sample were placed in the thimble and was inserted in the center of the extractor. The Soxhlet was heated to 69.9⁰C. This was allowed to continue for two, four and six hours. The experiment was repeated by placing the same amount of the sample into the thimble again by varying particle sizes (2.5-1.4mm, 1.4-0.5mm and 0.5-0.25mm). The weight of oil extracted was determined for each run hours. At the end of the extraction, the resulting mixture (essential oil and hexane) containing the oil was heated to recover solvent from the oil, separation of oil from hexane was carried out using rotary evaporator (Lawson et al., 2007).

The extraction was conducted in triplicate for the two varieties: Kali jira and Kalonji. 40 gm. of black cumin seed of different particle size: 2.5-1.4mm, 1.4-0.5mm and 0.5-0.25mm were fed to soxhlet extractor with 250ml of normal hexane was poured into round bottom flask for three different times: 2 hours; 4 hours and 6 hours, these values were selected based on previous research on similar seeds (Lawson et al., 2007). Extraction temperature of 70⁰C was chosen to avoid thermal degradation of bioactive compounds like phenolic compounds in the extract and also the temperature is in the range of boiling point of the solvents (Kittiphoom and Sustasinee, 2013). The resulting extracts obtained under different operating conditions were separated by evaporating the solvents using rotary evaporator in which the setup was established in the laboratory under specific temperature of 700C of boiling points of the solvent of hexane. The products were weighed, and the oil physicochemical properties were determined (Kittiphoom and Sustasinee, 2013).

Figure: 3.3. Laboratory set up of Soxhlet extraction unit

Figure: 3.4. Rotary evaporator

3.5.1. Determination of percentage of oil extracted

The percentage yield was calculated in two forms i.e. oil yield and extraction yield using the formula below,

$$\text{Percentage oil yields} = \frac{mass\ of\ oil}{mass\ of\ oil\ percent\ in\ the\ seed} \times 100\% \qquad ....3.2$$

Since black cumin seeds have an oil content of 20-40%, an average 30% oil content was taken for calculating the yield therefore:

$$\text{Percentage of oil yield} = = \frac{mass\ of\ oil}{0.30 \times mass\ of\ oil\ percent\ in\ the\ seed} \times 100\% \qquad ....3.3$$

The second form that the yield calculated was

$$\text{Percentage extraction yield} = = \frac{mass\ of\ oil}{mass\ of\ the\ sample} \times 100\% \qquad ....3.4$$

3.6. Characterization of the seed and extracted oil

3.6.1. Characterization of black cumin seed

➢ **Weight seed**

100 seed weight (g): The seeds obtained from each of the ten-tagged plants were dried in the sun and by the oven at 50°c for 18hr., 5.3% Kali jira seed whereas Kalonji variety contained 5.43 % moisture content, weighed and counted with a seed counter. Their weight measured by an Analytical balance and the average weight 100 seed weight was 75gm Kalonji seed whereas Kali jira seed 78gm.

➢ **Color seed**

The two varieties of Bangladeshi black cumin seed color are shiny black color.

➢ **Density seed**

The two varieties of Bangladeshi black cumin seed density are calculated the following method:

Density of seed Kalonji = Mass of black cumin seed / Volume of the container

$$= 75\text{gm} / 0.083\text{cm}^3 = 907 \text{ kg} / \text{m}^3 \text{ for Kalonji}$$

Whereas Kali jira density $= 78\text{gm} / 0.085\text{cm}^3 = 917 \text{ kg} / \text{m}^3$

Black cumin seeds were analyzed for various quality attributes including proximate analysis and mineral composition. The procedures followed are given below:

3.6.1.1 Proximate analysis

Black cumin seeds were analyzed for moisture content, total ash, crude protein, crude fat, crude fiber and carbohydrate.

i) **Moisture content**

It was worked out by weighing 5g sample accurately and subjected to oven drying at 110°C for 4-6hrs. Oven dried samples were cooled in desiccators and weighed. The

drying was repeated until the constant weights were obtained. The resultant loss in weight was calculated as per cent moisture content.

Moisture = $(W_1-W_2)/W_2$ ……………………………3.1

Where: W_1 = original weight of the sample before drying; W_2 = weight of the sample after drying.

ii) Total ash

Total ash was determined according to A.O.A.C. (2000) Sample (5g) was weighed into a crucible and ignited at low flame till all the material was completely become smokeless. Then it was kept in muffle furnace for 6 hrs at 600ºC and further cooled in desiccators and weighed. This was repeated till two consecutive weights were constant and percentage ash was calculated.

iii) Crude protein

Crude protein content was estimated by Kjeldhal method using 0.5g of moisture and fat free sample by digestion with conc. Sulphuric acid at 130-140˚C. Then it was distilled with 40% sodium hydroxide and liberated ammonia was trapped in 4% boric acid, using mixed indicator (methyl red : Bromoceresol green 1: 5). It was then titrated with 0.1N hydrochloric acid, the percent nitrogen was estimated, and protein content was calculated by multiplying percent nitrogen by 6.25.

iv) Crude fat

Sample (5g) was weighed accurately in thimble and defatted with petroleum ether in soxhlet apparatus for 2hrs. The resultant ether extract was evaporated, and crude fat content was calculated as per A.O.A.C. 2000 method.

v) Crude fiber

Crude fiber was estimated in fat free samples by treating with 1.25% H2SO4; left over material was subjected to further treatment with 1.25% NaOH solutions. Crude fiber of the samples was determined through Labconco Fibertech (Labconco Corporation Kansas, USA) as per procedure in A.A.C.C. (2000) Method No. 32-10.

vi) Carbohydrate (CBH)

CBH was calculated according to the following expression:

CBH % = 100 – (Moisture contents % + crude protein % + crude fat % +

      crude fiber % + ash %)   ………………………………3.5

3.6.1.1 Mineral contents

The black cumin seed was analyzed for its mineral profile following AOAC (Anon., 2003). Concentrations of calcium (Method 968.08), zinc (Method 991.11), iron (Method 985.01) and phosphorous (Method 965.17) were determined by Atomic Absorption Spectrophotometer (Varian AA240, Australia), while sodium (Method 968.08) and potassium (Method 968.08) were measured through Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge).

3.7. The Extraction process, parameter determination and comparison

The extraction process was performed using Soxhlet extraction apparatus. It has been possible to extract essential oils successfully using solvent extraction system both the two varieties. Therefore, parameter determination, product quantities and quality comparisons were done on the two varieties of Kali jira and Kalonji black cumin seed varieties.

Optimum parameters for the two varieties in extraction processes were determined. The solvent extraction methods were compared for the two seed varieties product quality and quantity as well as for their cosmetic application.

3.8. Characterization of the physical properties of oil

3.8.1. Determination of moisture and volatile matter of oil

5 gm of oil was weighted and putted in a dish and then dried in an oven at 105°C for 1 hour. The dish was removed from the oven and cooled in a dissector and weighed. The process was repeated until a constant weight was observed and the moisture and volatile matter of the oil was determined (Singh et al., 1981).

Moisture and volatile matter of Kali jira = $W_1/W_0 * 100\%$ 3.5

3.8.2. Determination of specific gravity

The density of oil was determined using density bottle method. A clean and dry density bottle of 25ml capacity at 30°C was weighed in gram (W_0). Then the bottle was filled with water and reweighed at 30°C (W_1). Melted oil was brought to 300C and the water was substituted with this oil after drying the density bottle and weighted again (W_2) and the specific gravity was determined (A.O.A.C Official Method 920.212, 2000).

$$Sp.gr = (W_1 - W_0) / (W_2 - W_0) = \frac{mass\ of\ the\ substance}{mass\ of\ an\ equal\ volume\ of\ water}$$ 3.7

3.8.3. Determination of kinematic viscosity of oil

A kinematic viscosity of oil was measured indirectly using Viscometer model. Initially, a sample was heated at a temperature of 30°C. A sample of 35 ml oil was measured and fed to a sample holder of the Vibro Viscometer. A sensor of the viscometer was immersed the oil and the dynamic Viscosity of oil was displayed on the Vibro Viscometer screen at a temperature of 30°C. Then the Kinematic Viscosity was calculated.

Kinematic viscosity of the two seeds (V) = $\frac{\mu}{\rho}$ 3.8

μ = Dynamic Viscosity

ρ = Density of oil

3.8.4. Determination of pH

2 gm of the sample was taken and placed in a clean dry 25 ml beaker and 13 ml of hot distilled water was added to the sample in the beaker and stirred slowly. Then it was cooled in a cold-water bath to 25^0C. The pH electrode was standardized with a buffer solution first and then the electrode immersed in to the sample and the pH was read and recorded (A.O.A.C Official Method of Analysis 960.19, 2000).

3.9. Characterization of the chemical property of oil

3.9.1. Determination of saponification value

2 gm of the sample was taken and placed in to 250 ml flask. 25 ml of alcoholic potassium hydroxide solution was added in to the flask and connected to reflux condenser and kept on the water bath and boiled gently for 1 hour. After the flask and the condenser were cooled, the inside of the condenser was washed with 10 ml of hot ethyl alcohol. Then few drops of phenolphthalein indicator were added and the excess potassium hydroxide was titrated wit 0.5 N hydrochloric acid to the end point, until the pink color of the indicator just disappears. The same procedure was conducted for the blank and the saponification value (SV) expressed as the number of milligrams of KOH required saponifying 1 gm of fat and calculated (A.O.A.C official method of analysis 920.160, 2000).

The required solutions were prepared with the required concentration.

- ✓ Preparation of 0.5 N alcoholic potassium hydroxide solutions: to prepare 0.5N of Ethanolic KOH solution, 14.027gm of KOH dissolved in 500ml of ethanol.
- ✓ 5.6gm/0.85gm = 6.59gm of 85 percentage pure KOH was dissolved in 200ml 80 percentage ethanol.
- ✓ Preparation of 0.5N hydrochloric acid solution: to prepare this solution, 43.7ml of HCl poured on 1000ml of distilled water.
- ✓ A stock solution of 13.7 ml of hydrochloric acid was poured in 500 ml distilled water.

After 2 gm of fat was dissolved in alcoholic KOH and heated gently for 1 hour. It was titrated with HCL to the end point. Similar titration was done for the blank. In both case the value of HCL was recorded.

Saponification value (S.V) = 56.1*N*(B-S)/W   ……………..…3.9

Where: B = the volume of the solution used for blank test; S = the volume of the solution used for determination S.V; N= Actual normality of the HCl used; W = Weight of oil taken in gram.

3.9.2. Determination of Iodine value

The iodine value of the sample was determined by A.O.A.C Official Method 993.20, for iodine values of oil. The method specified by ISO 3961 (1989) was used. 0.4gm of the sample was weighed into a conical flask and 20ml of carbon tetra chloride was added to dissolve the oil. Then 25ml of iodine monochloride solution in glacial acetic (Wijs solution) was added to the flask using a safety pipette in fume chamber. Stopper was then inserted, and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours and 30 minutes. At the end of this period, 20ml of 10% aqueous potassium iodide and 125ml of water were added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow color almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples. The iodine value (I.V) is given by the expression:

Iodine value (I.V) = 12.96*C*(V$_1$-V$_2$) / W   ……………..…3.10

Where: C = Concentration of sodium thiosulphate used; V$_1$ = Volume of sodium thiosulphate used for blank; V$_2$ = Volume of sodium thiosulphate used for determination, W = Mass of the sample.

3.9.3. Determination of acid value

25ml of Toluene and 25ml of ethanol was mixed in a 250ml beaker. The resulting mixture was added to 2g of oil in a 250ml conical flask and few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1M KOH to the end point with consistent shaking for which a dark pink color was observed and the volume of 0.1M KOH (V_0) was noted.

The required solutions were prepared with the required concentration as follows.

> ➢ Preparation of 80 percent ethyl alcohol; 19.6 ml distilled water was added in to 80.4ml 99.5 percent absolute ethanol.
> ➢ Preparation of 0.5N sodium hydroxide solution: 10.1 gm of 99 percent NaOH was dissolved in 500ml distilled water.

The acid value was calculated as:

Acid value (AC) = 56.1*N*V / W   ………………………………….3.11

Where V = volume of potassium hydroxide (ml), N = concentration of ethanolic KOH, W= sample weight

3.10. Fourier Transform Infrared (FTIR)

The FT-IR spectrum of the essential oil was obtained using Perkins Elmer Spectrum 65 FT-IR spectrometer in BCSIR and functional groups were determined with the help of IR correlation charts. The IR spectra were reported in % transmittance. The wave number region for the analysis was 4000-400 cm-1(in the mid-infrared range).

The Fourier Transform-Infrared spectroscopy was carried out on a Perkin Elmer FT-IR using Spectrum software version 10.3.2 using a liquid sampler and show the functional groups and provides structural information obtained from the different varieties solvent extraction methods and the commercial black cumin in the wavelengths between 400 and 4,000 cm-1(Perkin Elmer 1600 Spectrometer, USA). The major functional groups in black cumin are usually in the region between 1,000 and 2,000 cm-1 of the FTIR spectra (Kittiphoom and Sustasinee, 2013). The carbonyl

bands at 1,630-1,650 and 1,740-1,760 cm-1 indicate the free and esterified carboxyl groups, respectively. The increase in DE values will also increase the intensities and band area of the esterified carboxyl groups. This could be used to compare the different types of black cumin seed oil. The absorption bands between 1,100 and 1,200 cm-1 were from ether (R-O-R) and cyclic C-C bonds in the ring structure of black cumin molecules. No major structural difference in the FTIR spectra of the black cumin samples produced by various extraction conditions were observed, and the indigenous black cumin seed oil structures were similar to those of the commercial black cumin sample. The broad band, from 2,400 to 3,000 cm-1, was due to absorbed moisture in the black cumin samples.

3.11. GC-MS

3.11.1. Material & Method

Samples of seeds of *Nigella sativa* were purchased in BCSIR (samples of Kali jira and Kalonji varieties), from local markets of Bangladesh. The essential oils were isolated from 1 g of black cumin seeds by the solvent extraction method using laboratory glass apparatus. Just before the analysis, aliphatic and aromatic fractions are to be dissolved in hexane and DCM respectively, in separate vials. From each vial, 1 µl of solution is to be injected GC- MS machine. Agilent Technology 5890 gas chromatograph with a split detector and Mass Spectrometer Detector (MSD). Compounds were separated Agilent Technology MSD DB-5ms (60m x 250um, 1.4um thickness). The GC is usually coupled to a Mass Spectrometer (detector), 5973 Mass Selective Detector (MSD) which has the onus function of recording the mass spectrum of the chemical compounds as they come out of the GC and after fragmentation processes by a stream of electrons in the mass spectrum. Helium was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 1 µl was employed, injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 50 °C (isothermal for 4min.), with an increase of 3 0C/min, to 280 0C and held for 10min at 30C/min, isothermal at 280 °C and Mass scan range from 29-800amu. Total GC running time was 90.67 min. and the total length of time for running the analysis determined and programmed by the analytical geochemist (GC-MS analyst). Peaks in the chromatograms produced by these analyses were identified by a combination of

references to their mass spectra and the NIST08 mass spectral database, and by compare and elution orders with those of known standards.

3.11.2. Identification of components

The components of essential oil was identified on the basis of comparison of their retention time and mass spectra with published data (Analytical Methods Committee, 1984; Josiah, Y. and RP, 1976) and computer library matching or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat"s indices (Adam, 2001) were determined by conjunction of the sample with a solution containing a homologous series of n-hydrocarbons, in a temperature run identical to that described above. Provided with computer controlling the GC-MS system, in BCSIR, Chattogram. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

3.11.3. Qualitative analysis

The qualitative analysis was identified by comparison of their retention times and mass spectra with those of authentic standards. The identification was also performed by comparing the obtained mass spectra of relevant chromatographic peaks with corresponding spectra from the Wiley MS library. Multivariate data analysis was performed by using Simca v.13.0.3.

3.11.4. Quantitative analysis

The internal standard method was used for the quantitative analysis. Calibration samples from stock standard solutions, calibration solutions were prepared in methanol at individual concentrations of 0.1, 0.075, 0.05, 0.025, 0.0125, 0.005, 0.0025 and 0.0005 mg/mL of each compound. To each of them 0.25 mg/ml internal standard was added.

3.12. Antioxidant potential of fixed and essential oil

Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was evaluated using the method described by Taga et al. (1984). The degradation rate of the extracts was calculated according to first order kinetics (Al-Saikhan et al., 1995). DPPH radical scavenging activity of black cumin fixed oil was measured according to the method of Brand-Williams et al. (1995).

3.13. Design of the experiment

> **Full factorial design**

Factorial design is used to investigate the effect of each factor. In a factorial experiment all the possible combination factor level would be tested, and it would be possible to determine the effect of individual factors and to assess the effect of change of two or more variable at a time (Zivorad, 2004).

The analysis was performed by utilizing design expert software using general full factorial design method. This method of experiment design helps to differentiate the significance of the main and the interaction factors. The software also used to develop the mathematical model that will describe the effect of the main and interaction factors on the response.

Factor: 2 factors were investigated as mentioned earlier, these are: particle size and time with the 2 black cumin varieties of Kali jira and Kalonji.

Factors levels: for each factor, three levels were considered. Replicates: Each independent experiment was repeated three times. Black cumin varieties: 2. Kali jira and Kalonji.

Number of runs: For "m" level, "n" factors , "l" varieties of black cumin seed and "k" replicate, the number of experimental runs that need to be performed is equal to k*l*mn (Zivorad, 2004). In this research m=3, n=2, l=2 and k=3 thus, 3*2*32 = 54 experimental runs were performed.

The factors and their levels

The levels that were selected for each factor are:

1. Particle size (mm)**:** 0.25-0.5, 0.5-1.4 and 1.4-2.5.
2. Extraction time(hour): 2, 4, and 6.

And black cumin varieties of Kali jira and Kalonji seed.

2. Model equation

Finally, regression models were established for the dependent variables to fit the experimental data for the response using design expert 7.0.0 software.

Chapter IV: Results

4.1. Characterization of seed of black cumin

Black cumin seeds were characterized for proximate composition and minerals profile. Afterwards, black cumin essential oil was extracted and characterized, and for their functional components as well as optimized the black cumin seed oil varieties. Proximate composition is important in determining the quality of raw materials. Black cumin seeds varieties of Bangladesh were analysed for different attributes:

4.1.1. Moisture content of the black cumin seed

The moisture content of the sample was determined using equation (Moisture content (%) of the black cumin seed $= \frac{W_1 - W_2}{W_1} \times 100\%$) and summarized in Table 4.1.

Table: 4.1. Moisture content of Kali jira and Kalonji black cumin seed variety

Mass	Sample weight in gram						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Initial	11.8	13	8	12.1	9.4	5.7	3.4
After 2hrs	11.5	12.7	7.8	11.8	9.2	5.6	3.3
After 4hrs	11.4	12.5	7.7	11.7	9.15	5.5	3.27
After 6hrs	11.26	12.4	7.74	11.4	9.1	5.45	3.23
After 8hrs	11.2	12.3	7.6	11.2	9	5.4	3.2
After 10hrs	11.2	12.3	7.6	11.3	9.0	5.4	3.2
Final content Kali jira	5.0%	5.5%	4.9%	6.6%	4.6%	5.9%	4.8%
Initial	11.2	16.4	9.3	10.5	14.7	5.1	8.2
After 2hrs	10.9	16.0	7.6	10.3	14.6	5.0	8.1
After 4hrs	10.8	15.8	6.4	10.2	14.5	4.9	8.0
After 6hrs	10.7	15.6	5.8	10.1	14.2	4.89	7.9
After 8hrs	10.6	15.4	4.7	9.8	14.0	4.8	7.8
After 10hrs	10.6	15.4	4.7	9.8	14.0	4.8	7.8
Final content Kalonji	5.4%	6.1%	4.9%	6.7%	4.76%	5.8%	4.9%

The moisture content of the seven Kali Jira black cumin seed samples having mass of 11.8, 13, 8, 12.1, 9.4, 5.7, and 3.4 gram were 5.0, 5.5, 4.9, 6.6, 4.6, 5.9 and 4.8 percent respectively. Mean plus the standard deviation of the seven samples gives 5.29±0.82 of Kali jira whereas Kalonji variety 11.2, 16.4, 9.3, 10.5, 14.7, 5.1, and 8.2 gram was 5.36, 6.1, 4.95, 6.67, 4.76, 5.8 and 4.87 percent respectively. Mean plus the standard deviation of the seven samples gives 5.43± 0.11. The result obtained is both varieties agreement with those reported in literature (Dandik and Aksoy, 1992; Abdel-Aal et al., 1993; El-Dhaw and Abdel Munaem, 1996; Salem, 2005).

4.1.2. Proximate and mineral analysis

Table: 4.2. General proximate analyses of black cumin seed varieties

Code	Parameter tested and result (%)					
	Crude Protein (Mean±SD)	Crude Fiber (Mean±SD)	Moisture (Mean ±SD)	Crude Fat (Mean ±SD)	Ash (Mean ±SD)	Carbohydrate (Mean±SD)
Kali jira	19.83±0.10	18.96±0.21	5.29±0.12	41.3±0.11	5.10±0.11	9.52 ± 2.31
Kalonji	19.95±0.14	14.28±0.22	5.43±0.13	41.1±0.11	4.35±0.12	14.69 ± 1.45

Proximate composition is important in determining the quality of raw materials. Black cumin seeds were analyzed for different quality attributes; contains 5.29±0.12% and 5.43±0.13%, 19.83±0.10% and 19.95±0.14%, 41.3±0.11% and 40.3±0.08%, 18.96±0.21% and 14.28±0.22%, 5.10±0.11% and 4.35±0.12 % of moisture, crude proteins, crude fat, crude fiber, ash contents, Kali jira and Kalonji respectively, while carbohydrate was found to be 9.52±2.31% and 14.69± 1.45% Kali jira and Kalonji respectively.

Findings of present research regarding characterization were in close conformity with the values described in the literature (Dandik and Aksoy, 1992; Takruri and Dameh, 1998; Atta, 2003; Salem, 2005) slight differences ash content and carbohydrate; environmental factors like climate and location might be a possible reason for these variations. Moreover, difference in genetic makeup could also be a contributing factor as indigenous variety was tested in the trial. The composition of black cumin seeds and moisture, fat, protein, ash and total carbohydrates contents reported in literature were in the range of 3.8-7.0, 22.0-40.35, 20.85-31.2, 3.7-4.7 and 24.9- 40.0%

respectively (Dandik and Aksoy, 1992; Abdel-Aal et al., 1993; El-Dhaw and Abdel Munaem, 1996; Salem, 2005). In another study, Cheikh-Rouhou *et al.* (2007) compared Tunisian and Iranian varieties for various quality characteristics. They observed that Tunisian variety contains 8.65, 28.48, 26.7, 4.86 and 40.0% of moisture, oil, proteins, ash and carbohydrates as compared to 4.08, 40.35, 22.6, 4.41, and 32.7% for respective traits in Iranian variety.

Generally Kali jira varieties has larger value than Kalonji varieties in crude fat, crude fiber, and ash content but moisture, crude protein and carbohydrate is higher in Kalonji.

Table: 4.3. Mineral analysis results of black cumin seed varieties of Bangladesh

Variety	Parameter tested (mg/100g)					
	Fe (Mean ±SD)	Zn (Mean ±SD)	Ca (Mean ±SD)	K (Mean ±SD)	Na (Mean ±SD)	P (Mean ±SD)
Kali jira	76.48±4.3	5.39±0.12	498.45±16.3	829.11±7.6	110.55±3.5	698.89±5.4
Kalonji	56.45±3.2	5.40±0.14	481.57±14.9	746.27±5.8	37.31±2.8	710.67±8.6

Mineral composition indicated that potassium is dominant (829.11mg/100g, 746.27mg/100mg) followed by phosphorous (698.89mg/100g, 710.67mg/100g), calcium (498.45mg/100g, 481.57mg/100g), sodium (110.55mg/100g, 37.31), iron (76.48mg/100g, 56.45mg/100g) and finally zinc (5.39mg/100g, 5.40mg/100g) for Kali jira and Kalonji variety respectively.

Some studies supported the present results regarding mineral contents such as potassium, phosphorus, calcium, sodium, iron and zinc i.e. 830-725, 690-715, 480-500, 122-40, 80-55mg/kg respectively like Ashraf et al. (2006); Nickavar et al. (2003) and Atta (2003). However, findings of Cheikh-Rouhou et al. (2007) showed slight differences, while comparing the composition of minerals but they also reported that potassium is dominant mineral in both varieties of Kali jira and Kalonji black cumin seed. Kali jira varieties have higher value than Kalonji varieties in all minerals content

except phosphorous and iron. Possible causes of these differences could be climatic variations and genotype.

4.1.3. Sensory evaluation

➢ **Samples and tasting environment**

All assessments were conducted in a well-ventilated daylight-illuminated room. After initial discussions all participants assessed samples independently in separate booths at room temperature, under normal fluorescent illumination. Two different types of black cumin oil.

➢ **Sensory Panel, Panel Selection and Training**

Panel consisting of 12 (4 men and 8 women), 18-29 years old, was used in these experiments. The panelists have worked for Chattogram Veterinary and Animal Sciences University staffs and students. Panelists selections were availability and willingness to cooperate and commit the time to the thesis. Panelists were screened for ability to discriminate aroma, color and for ability to express themselves verbally and Panelists also practiced describing aroma characteristics of experimental and commercial black cumin essential oils. After a training period of about 3 days, ten (4 men and 6 women) final panelists were selected based on consistent performance. Each panelist had been involved in black cumin seed essential oil evaluation for 3 days at the time of these studies. Panelists required a maximum of five minutes to register the two measurements on each sample of the two varieties; the average time was normally about two minutes. At each session assessors were presented with three randomly-coded samples of Kali jira and Kalonji (5ml) in a black cumin oil sample glass (B.5) covered with a watch glass, and they assessed each for the intensity of the attributes using a nine-point continuous line scale.

➢ **Sample Preparation**

Experimental black cumin essential oils are processed using of thesis processing practices. Black cumin essential oil placed into room temperature. Often a major shortcoming of sensory evaluation of black cumin seed essential oil is the aroma variation found both between Kali jira as well as within a Kalonji. Samples are placed

at room temperature until presentation to the panel. This pooled sample technique assures that replicates are true and that subsamples taken for analysis are properly representative of the source sample.

In addition to the necessary major appliances, the controlled preparation of products requires adequate supplies and equipment such as scales, for weighing products and ingredients of black cumin seed, glassware, for measurement and storage of products and timers, for monitoring of preparation procedures, etc.

➢ **Preparation Procedures**

The controlled preparation of products requires careful regulation and monitoring of procedures used, with attention given to: amount of black cumin seed oil to be used, measured by weight or volume using precise equipment (volumetric cylinders)

➢ **Sample Presentation**

The equipment and procedures used for product presentation during the test must be carefully selected to reduce introduction of biases and new variables. Attention should be given to control of the following: Serving containers and Serving size. All samples were presented at room temperature in clear black cumin seed essential oil glasses (B.S.5 and B.S.6). During initial vocabulary development all samples were available, and assessors could select and assess in any order they chose. For the actual assessment samples were presented and order was randomized and different between individuals.

The panelists were asked to give their individual ratings on all the characteristics including color, aroma and overall acceptability of the black cumin essential oil. A 9-point structured hedonic scale (1-extremely disliked to 9-extremely liked) was used to conduct the preference test.

Table: 4.4. Sensory scores of essential oil for acceptability, aroma and color

variety	attributes	Weight in percent for each attribute								
		1	2	3	4	5	6	7	8	9
Kali jira seed oil	Color								60	40
	Aroma							30	50	20
	acceptability							15	80	5
Kalonji seed oil	Color							10	60	30
	Aroma							30	45	25
	acceptability							15	80	5

Hedonic scales: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike Slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.

The sensory score for the samples implied higher performance. In terms of aroma and color, there was no significant difference between black cumin seed essential oil. Regarding to black cumin seed essential oil was most preferred with no significant difference between the varieties. However, there exists a significant difference in Kali jira variety aroma as was rated higher than Kalonji, while color and acceptability have no significant difference between them.

According to the sensory analysis results in Table 4.4, about 80 % of the panelists preferred like very much of aroma sample at concentration of 20% essential oil. These two samples were also similar, according to sensory data.

4.2. Oil extraction

❖ **Percentage yield of extraction**

The percentage oil yield was calculated by using equation 3.2 and 3.3 respectively and the result is shown in the table 4.5 below,

Table: 4.5. Percentage oil yield of black cumin seed oil for two varieties of black cumin seed (Kali jira and Kalonji).

No of run	Factors		Oil yield (%)				Extraction yield (%)
	Particle size(mm)	time	Rep. 1	Rep.2	Rep.3	Mean± SD	$\frac{mass\ of\ oil\ extracted}{mass\ of\ cumin\ seed}$
Kalonji black cumin							
1	2.5-1.4	2	47.22	45.00	48.10	46.80±1.60	1.89±0.05
2	2.5-1.4	4	52.90	51.78	53.00	52.56±0.68	2.13± 0.03
3	2.5-1.4	6	74.00	75.00	72.00	73.67±1.53	2.51±0.02
4	1.4-0.5	2	50.38	52.56	53.90	51.60±1.26	2.04±0.11
5	1.4-0.5	4	71.00	70.00	76.80	72.60±3.67	2.29±0.04
6	1.4-0.5	6	79.00	80.50	84.60	81.37±2.90	2.65±0.05
7	0.5-0.25	2	53.78	60.00	61.00	59.77±1.36	2.15±0.03
8	0.5-0.25	4	79.00	78.00	80.00	79.00±1.00	2.5±0.10
9	0.5-0.25	6	90.84	92.80	89.60	91.11±1.31	2.82±0.01
Kali jira black cumin							
1	2.5-1.4	2	49.32	48.10	51.30	50.17±1.61	2.22±0.08
2	2.5-1.4	4	65.00	67.00	68.00	66.67± 1.52	2.35±0.05
3	2.5-1.4	6	76.00	77.00	75.00	76.00± 1.00	2.6±0.05
4	1.4-0. 5	2	53.41	55.80	56.50	55.23±1.62	2.28±0.06
5	1.4-0.5	4	74.00	72.00	79.40	75.13± 3.82	2.44±0.04
6	1.4-0.5	6	80.24	83.30	87.30	84.29± 2.67	2.7±0.10
7	0.5-0.25	2	61.40	62.60	63.00	62.33± 0.69	2.54±0.03
8	0.5-0.25	4	82.00	81.00	83.00	82.00 ± 1.00	2.73±0.02
9	0.5-0.25	6	94.20	94.40	95.70	94.77±0.81	2.91±0.01

Rep= Replication number 1, 2 and 3.

From Table 4.5 the maximum percentage oil yield obtained was 91.11 ± 1.31 and 94.77 ± 0.81 at particle size range of 0.25-0.5mm, extraction time of 6 hour for Kalonji and Kali jira respectively, whereas the minimum percentage oil yield was 46.80 ±1.60 and 50.17±1.61 obtained at particle size range of 1.4-2.5mm, extraction time 2 hour for Kalonji and Kali jira respectively.

A percentage extraction yield of 8.46±0.1 (which is equivalent to 70.5±0.83 percentage oil yield) was reported by (Kittiphoom and Sustasinee, 2013; Aksoy, 1992; Abdel-Aal et al., 1993) using hexane as a solvent with extraction time of 6 hour. (Saipraha et al., 2011) and (Aksoy, 1992) reported as yield of 10.2% (85 % oil yield) using hexane as a solvent and with extraction time of 5 hour. Additionally, (Nizikou et al., 2010) and (Abdel-Aal et al., 1993) reported a percentage extraction yield of 13.0 with hexane as solvent and extraction time of 8 hour.

4.2.1. Optimization

Using optimization functional in design expert software 7.0.0, it was predicted that at the following operating condition; 0.25-0.5 mm particle size, 6-hour extraction time Kali jira and Kalonji varieties respectively, a maximum oil yield of 94.7667% Kali jira whereas Kalonji variety of 91.08 %. A minimum yield of 49.5733% and 46.7733 % was predicted at particle size 1.4-2.5 mm, 2-hour extraction time of Kali jira and Kalonji respectively, which was also in agreement with the experimental value because the value of maximum and minimum in oil extraction value subtract from the value of optimized value is less than 0.05.

The optimization solutions for maximum and minimum yield are shown in Table 4.6-4.9 below by using categorical factor due to the above reason.

Table: 4.6. Solution output from categorical optimization for maximum oil yield of Kalonji

Number	particle size	Extraction time	Yield	Desirability	Remark
1	0.25-0.5	6	91.08	0.964	Selected
2	0.5-1.4	6	81.3667	0.761	
3	0.25-0.5	4	79	0.711	
4	1.4-2.5	6	73.6667	0.600	
5	0.5-1.4	4	72.6	0.577	
6	1.4-2.5	4	66.56	0.451	
7	0.25-0.5	2	52.28	0.309	
8	0.5-1.4	2	55.3407	0.152	
9	1.4-2.5	2	46.7733	0.037	

Table: 4.7. Solution output from categorical optimization for maximum oil yield of Kali jira

Number	particle size	Extraction time	Yield	Desirability	Remark
1	0.25-0.5	6	94.7667	0.980	Selected
2	0.5-1.4	6	84.48	0.764	
3	0.25-0.5	4	82.00	0.712	
4	1.4-2.5	4	76.00	0.586	
5	0.5-1.4	6	74.4667	0.554	
6	1.4-2.5	2	66.6667	0.390	
7	0.25-0.5	4	62.3333	0.299	
8	0.5-1.4	2	55.2367	0.150	
9	1.4-2.5	2	49.5733	0.031	

Table: 4.8. Solution output from categorical optimization of design expert software for minimum oil yield of Kalonji

Number	particle size	Extraction time	Yield	Desirability	Remark
1	1.4-2.5	2	46.7733	0.963	Selected
2	0.5-1.4	2	52.28	0.848	
3	0.25-0.5	2	59.7667	0.691	
4	1.4-2.5	4	66.56	0.549	
5	0.5-1.4	4	72.60	0.423	
6	1.4-2.5	6	73.6667	0.400	
7	0.25-0.5	4	79.00	0.289	
8	0.5-1.4	6	81.3667	0.239	
9	0.25-0.5	6	91.08	0.036	

Table: 4.9. Solution output from categorical optimization of design expert software for minimum oil yield of Kali jira

Number	particle size	Extraction time	Yield	Desirability	Remark
1	1.4-2.5	2	49.5733	0.969	Selected
2	0.5-1.4	2	55.2367	0.850	
3	0.25-0.5	2	62.3333	0.701	
4	1.4-2.5	4	66.6667	0.610	
5	0.5-1.4	4	74.4667	0.446	
6	1.4-2.5	6	76.00	0.414	
7	0.25-0.5	4	82.00	0.288	
8	0.5-1.4	6	84.48	0.236	
9	0.25-0.5	6	94.7667	0.020	

4.2.2. Effect of process parameters in percentage oil yield

4.2.2.1 Effect of particle size on percentage oil yield

The effect of particle size on oil yield for Kalonji variety is shown in Figure 4.1.
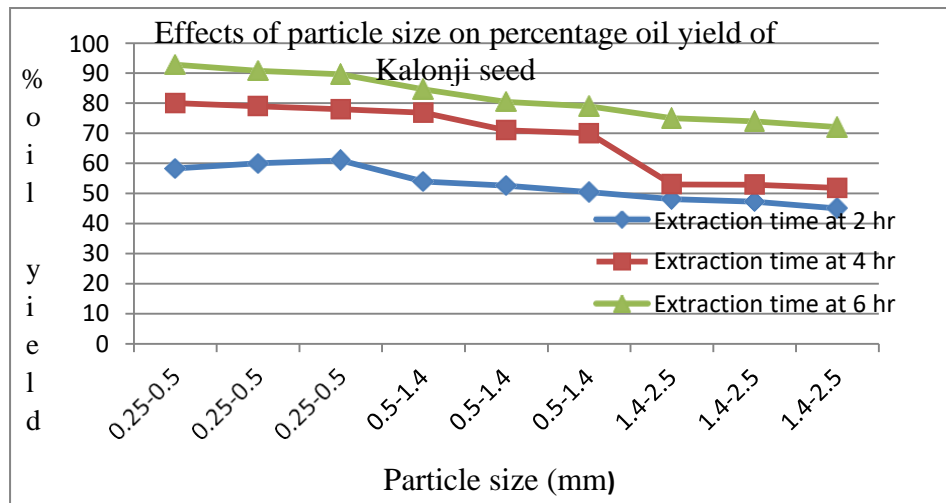
Figure: 4.1. Effect of particle size on percentage oil yield

The effect of particle size on oil yield for Kali jira variety is shown in Figure 4.2.
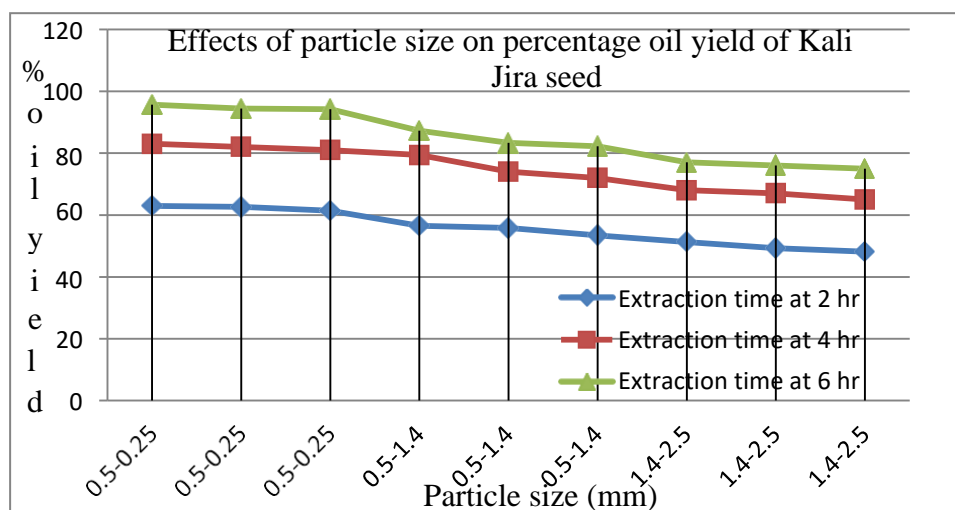
Figure: 4.2. Effect of particle size on percentage oil yield.

The particle size plays the biggest role on yield of black cumin seed essential oil (Fig 4.1 and 4.2). It is quite clear that there is an increase in the oil yield as the particle size decreased and an increase in the particle size results in a drop in oil yield. Thus, the percentage essential oil yield was inversely related to the particle size i.e. smaller size gives high yield while larger particle size results a lower yield. The reason is that larger particles have smaller surface area of contact and larger distance to solvent entrance and oil diffusion in comparison to smaller particle using n-hexane solvent. The results of n-hexane extracts are comparable with the previous studies (Nickavar et al. 2003) and (Atta et al. 2003). However, when the particle size is too small or very fine, the oil yield decreases the reason for this may be attributed to agglomeration of the particles which reduces the contact surface area.

(Nickavar et al., 2003) and (Atta, 2003) and (Akram, 2014) reported has the result of extraction of oil from seed for three different particle sizes range < 0.5 mm, 0.5-0.75 mm and > 0.75mm. The highest percentage of oil yield was obtained with the intermediate particle size (0.5-0.75mm) which indicated that decreasing the particle size below a certain particle size doesn't increase the percentage of oil yield and may even decrease the yield.

4.2.2.2 Effect of extraction time on percentage oil yield

The effect of extraction time on oil yield is shown in figure 4.3 and 4.4.
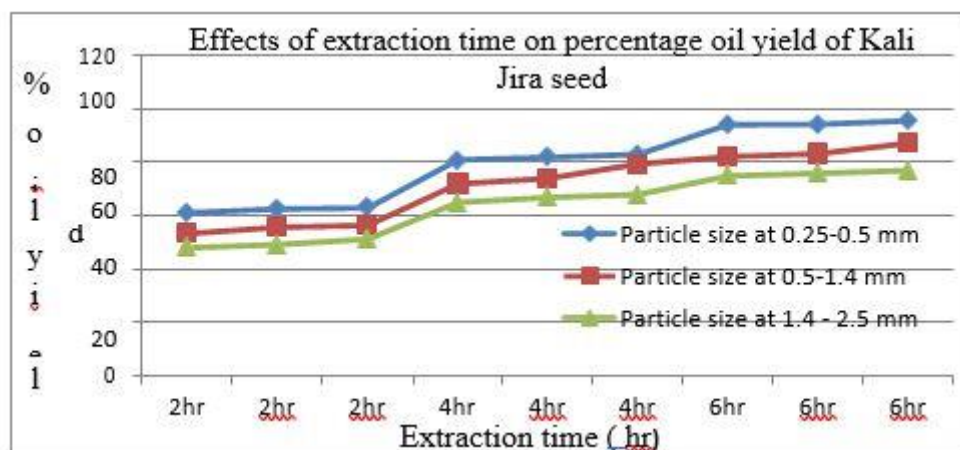
Figure: 4.3. Effect of the extraction time on oil yield of Kali jira
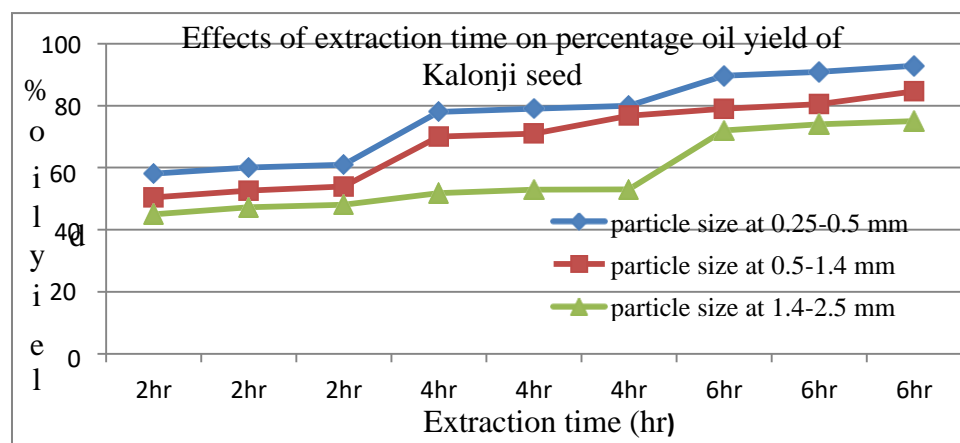
Figure: 4.4. Effect of the extraction time on oil yield of Kalonji

The percentage oil yield was directly related to extraction time i.e. the yield increases as extraction time increases (Figure 4.3 and 4.4 above). The same trend was reported by (Nickavar et al., 2003) and (Atta, 2003).

For smaller particle size range 0.25-0.5mm the yield rose rapidly with time up to 4 hour and then after the yield of oil wasn't varying (it was constant). At 4 hours, the oil in the seed was almost exhausted hence negligible oil yields. The oil yield increased by 24.04 % and 23.32 % of Kalonji and Kali jira respectively, as the extraction time increased from 2 hour to 4 hour and it increased by only 3.61% of Kalonji and 3.31 %

of Kali jira as the time increased from 4 hour to 6 hour. However, for larger particle size i.e. 1.4-2.5 mm the yield was lower at the beginning of the extraction and increased gradually as the extraction time increased. The yield increased by only 24.37% and 23.4 % for Kalonji and Kali jira respectively as the time increased from 2 hour to 4 hours while by 9.07% and 11.65% for Kalonji and Kali jira respectively as the extraction time increased from 4 hour to 6 hours.

The result obtained in this research indicates that smaller particle size needs small extraction time to obtain maximum yield in comparison to large particle size. According to this study the maximum oil yield is obtained at 6 hours extraction time and at lower particle size and since at 4-6 hours extraction time 97 % of the maximum yield was obtained, so extraction time above 6 hours is wastage of time and cost.

4.2.2.3 Interaction effect among the two factors and the two varieties

From design expert software 7.0.0 output, interaction effect between;

➢ Particle size and extraction time for Kalonji varieties
➢ Particle size and extraction time for Kali jira varieties

On percentage oil yield are shown in Figure Kalonji varieties below:
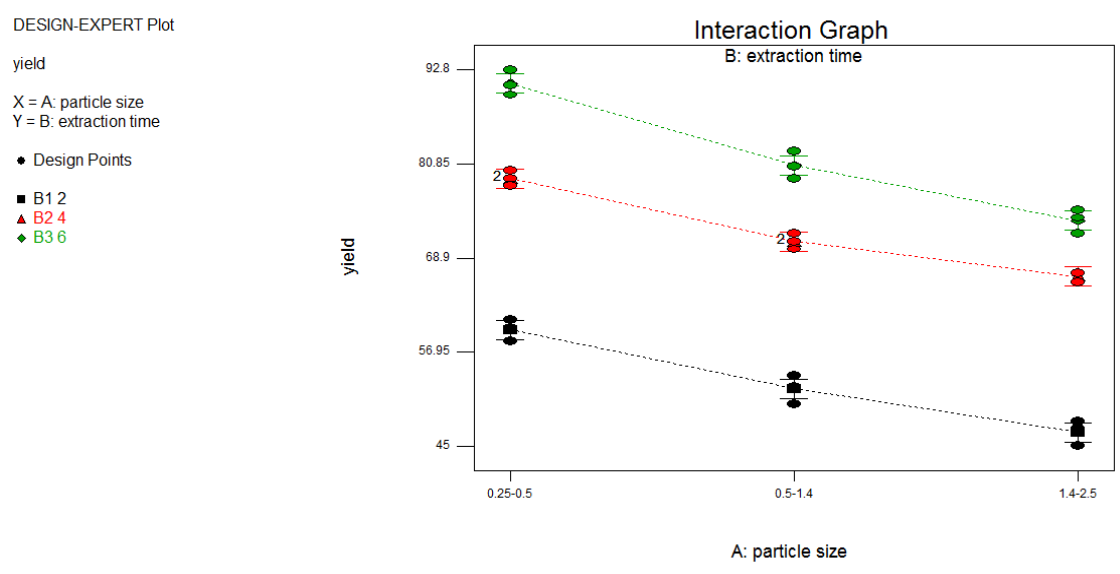
Figure: 4.5. Interaction effects of extraction time and particle size of Kalonji
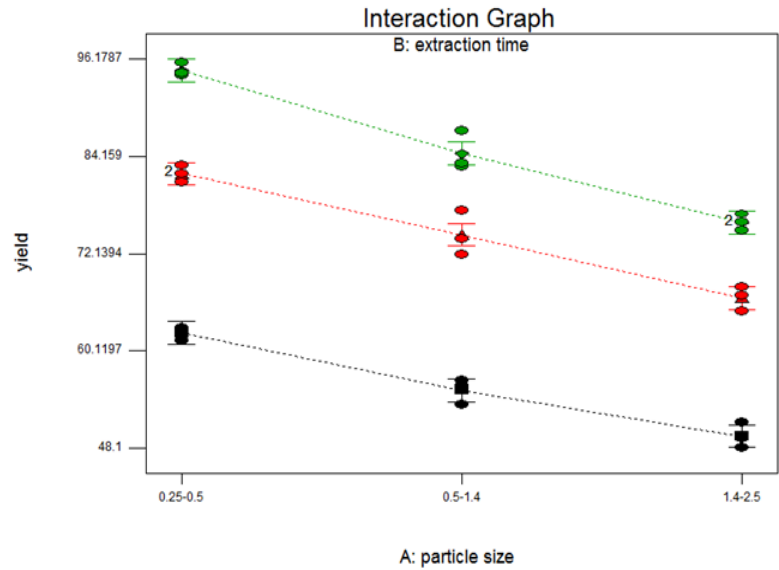
Figure: 4.6. Interaction effects of extraction time and particle size of Kali jira

There was no interaction between particle size and time as depicted by similar shape of the curves in the Figure 4.5 Kalonji and 4.6 Kali jira seed. This shows that irrespective of extraction time, lower particle sizes give a higher yield and irrespective of particle size, higher extraction time can give higher yield. Similarly, as can be noticed from Figure 4.5 and Figure 4.6, there was no interaction effect is between particle size and extraction time.

Where: A1, A2 and A3 are codes for particle sizes ranges from 2.5-1.4mm, 1.4-0.5mm, and 0.25-0.5mm, respectively. B1, B2 and B3 are codes for extraction time two, four, and six hours, respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response based on these points.

4.3. Regression model equation

The following table shows analysis of variance (ANOVA) obtained from design expert software, which tells as the significance of different factors.

Table: 4.10. Analysis of variance (ANOVA) table for a response of percentage oil yield black cumin seed of Kalonji

Source	Sum of squares	Degree of freedom	Mean square	F value	P value Prob > F
Model	4842.61	8	605.33	301.82	< 0.0001
A-Particle Size	931.28	2	465.64	232.17	< 0.0001
B-Extraction Time	3888.28	2	1944.14	969.35	< 0.0001
AB	23.05	4	5.76	2.87	0.0530

Table: 4.11. Analysis of variance (ANOVA) table for a response of parentage oil yield black cumin seed of Kali jira

Source	Sum of squares	Degree of freedom	Mean square	F value	P value Prob > F
Model	5066.60	8	633.32	208.89	< 0.0001
A-Particle Size	1099.40	2	549.70	181.31	< 0.0001
B-Extraction Time	3934.19	2	1967.09	648.80	< 0.0001
AB	33.01	4	8.25	2.72	0.0622

The model F-value of Kalonji and Kali jira 301.82and 208.89 implies the model is significant. Value of "prob >F" less than 0.05 indicate model terms are significant. In this case A-particle size and B-Extraction time are significant model terms of the two varieties. Values greater than 0.1000 indicates the model terms are not significant. The P-value of the two varieties AB (interaction factor) is 0.0530 > P-value and 0.0622 > P-value thus; the interactions of particle size and extraction time are not significant in the model terms.

Design-expert was applied to analyze results on the extraction process and a first order regression equation, with the interaction terms, of the form, the final model equation in terms of coded factor was presented by equations representing the variation of percentage oil yield of black cumin seed with independent factors:

Yield $_{Kali\ jira}$ (%) = +71.72+7.98* A[1]-0.33 * A[2]-16.01* B[1]+2.88* B[2]-1.36*A[1]B[1]-0.1*A[2]B[1]-0.58*A[1]B[2]+0.86*A[2]B[2]   …………………..4.1

Yield $_{Kalonji}$ (%) = +68.98+7.64*A[1]-0.99*A[2]-16.04 *B[1]+3.21*B[2]0.81*A[1]B[1]+0.33*A[2]B[1]-0.83*A[1]B[2]-0.19*A[2]B[2] ………………….4.2

Considering ANOVA table (4.10 and 4.11) the model terms A and B the extraction time were significant model terms whereas interaction model term AB are not significant model terms. Often, we think about removing non-significant model terms or factors from a model but in this case removing only AB will result in a model that is not hierarchal. The hierarchy principle indicates that if a model contains a high order term, it should contain all lower-order terms that compose it. Hierarchy promotes a type of internal consistency in a model, and many statistical model builders rigorously follow the principle.

Therefore, the final equation in terms of coded factor without the interaction effect is given by a first order regression equation:

Final Equation in Terms of Coded Factors:

Yield $_{Kali\,jira}$ (%) = +71.72+7.98 * A[1]-0.33 * A[2]-16.01 * B[1]+2.88 * B[2]

..................4.3

Yield $_{Kalonji}$ (%) = +68.98+7.64 * A[1]-0.99 * A[2]-16.04 * B[1]+3.21 * B[2]

……………4.4

Final Equation in Terms of Actual Factors: Not available, because this model contains more than 4 categorical equations.

Where: A [1] = the difference of particle size level-1 from the overall average. A [2] = the difference of particle size level-2 from the overall average.

B [1] = the difference of extraction time level-1 from the overall average and B [2] = the difference of extraction time level-2 from the overall average

It is evident from equation (4.3 and 4.4) that the coefficient of B [1] was negative but that of A[1], A[2] and B[2] were positive in Kali jira but Kalonji B [1] and A[2] was negative but that of A [1] and B [2] were positive. Therefore increasing the particle size, B [1] extraction time will decrease the percentage oil yield but in Kalonji not

only B [1] also A[2] will decrease extraction time. Whereas increasing the extraction time, percentage oil yield of black cumin seed will increase.

Diagnostics

The following Figure 4.7 shows the relation between the actual predicted values of the experiment by the model equation developed by the design expert software 7.0.0
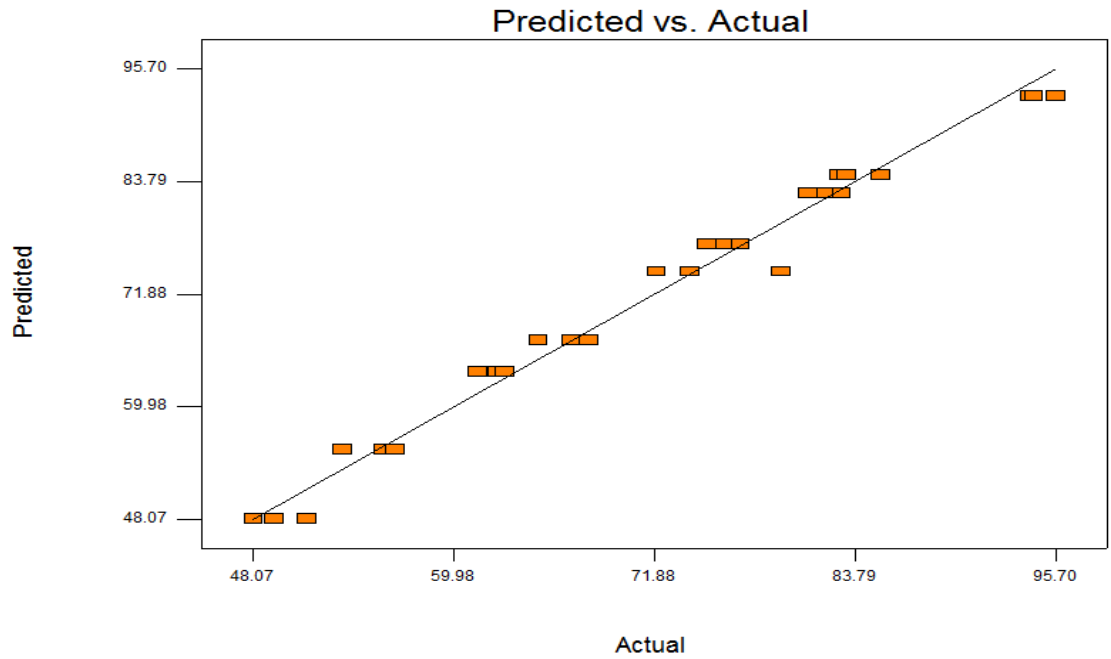
Figure: 4.7. Actual value versus predicated value of percentage oil yield of Kali jira
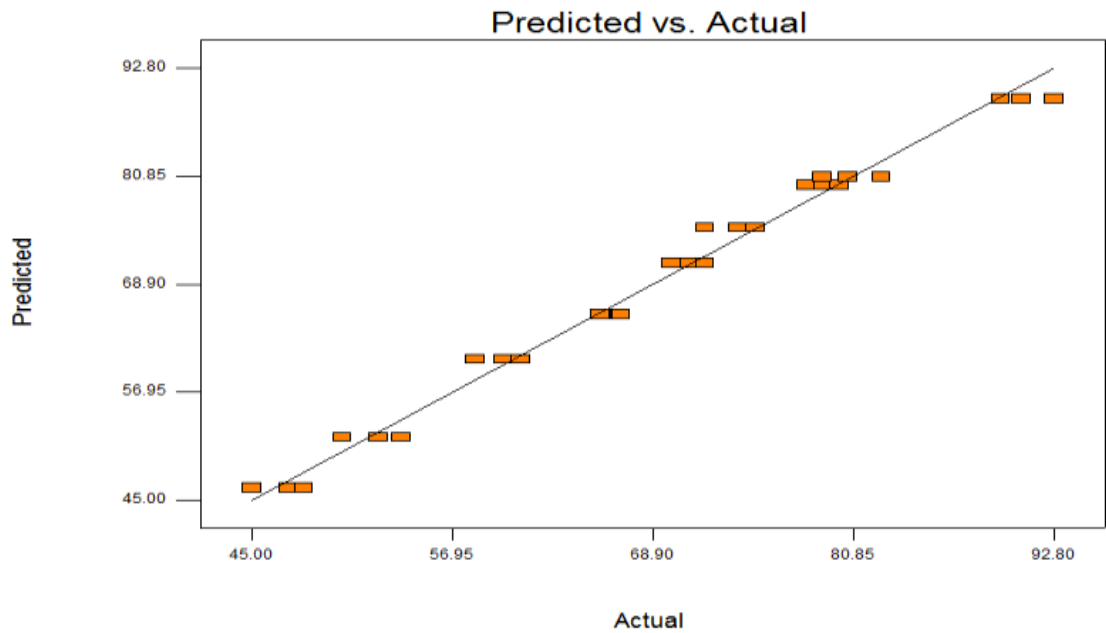
Figure: 4.8. Actual value versus predicated value of percentage oil yield of Kalonji

Figures 4.5, 4.6, 4.7 and 4.8 show that there is no interaction among each factor. This shows us an increment in time will increase the quantity of black cumin oil extracted. Extraction at six hours did give a significant optimum value with the particle size of 0.25-0.5 mm on oil yield.

The residual, difference between the actual value of the experiment and the predicated value (which was calculated using equation:

Yield Kali jira (%) = +71.72+7.98* A[1]-0.33 * A[2]-16.01 * B[1]+2.88* B[2]-1.36* A[1]B[1]-0.1* A[2]B[1]-0.58* A[1]B[2]+0.86* A[2]B[2] and

Yield   Kalonji   (%)   =   +68.98+7.64*A[1]-0.99*A[2]-16.04*B[1]+3.21*B[2]0.81* A[1]B[1]+0.33*A[2]B[1]-0.83*A[1]B[2]-0.19*A[2]B[2]

for some of the runs is shown in the Table 4.12 and Table 4.13).

Table: 4.12. Difference between the experimental (actual) value and predicated value for Kalonji variety

Standard	Actual value	Predicated value	Residual
1	58.3	60.58	-2.28
2	60.00	60.58	0.58
3	61.00	60.58	0.42
4	50.38	51.95	-1.57
5	52.56	51.95	0.61
6	53.90	51.95	1.95
7	47.22	46.30	0.92
8	45.00	46.30	-1.30
9	48.10	46.30	1.80
10	79.00	79.83	-0.83
11	78.00	79.83	-1.83
12	80.00	79.83	0.17
13	71.00	71.19	-0.19
14	70.00	71.19	-1.19
15	72.00	71.19	0.81
16	66.90	65.54	1.36
17	65.78	65.54	0.24
18	67.00	65.54	1.46
19	90.84	89.44	1.40
20	92.90	89.44	3..36
21	89.60	89.44	0.16
22	79.00	80.81	-1.81
23	80.50	80.81	-0.31
24	82.50	80.81	1.69
25	74.00	75.16	-1.16
26	75.00	75.16	-0.16
27	72.00	75.16	-3.16

Table: 4.13 Difference between the experimental (actual) value and predicated value for Kali jira variety

Standard order	Actual value	Predicated value	Residual
1	61.40	63.69	-2.29
2	62.60	63.69	-1.09
3	63.00	63.69	-0.69
4	53.41	55.38	-1.97
5	55.80	55.38	-0.42
6	56.50	55.38	1.12
7	49.32	48.07	1.25
8	48.10	48.07	0.03
9	51.30	48.07	3.23
10	82.00	82.58	-0.58
11	81.00	82.58	-1.58
12	83.00	82.58	0.42
13	74.00	74.27	-0.27
14	72.00	74.27	-2.27
15	79.40	74.27	5.13
16	65.00	66.96	-1.96
17	67.00	66.96	-0.045
18	68.00	66.96	1.04
19	94.20	92.84	1.36
20	94.40	92.84	1.56
21	95.70	92.84	2.86
22	82.24	84.53	-1.69
23	83.30	84.53	-1.23
24	85.30	84.53	0.77
25	76.00	77.22	-1.22
26	77.00	77.22	-0.22
27	75.00	77.22	-2.22

4.4. Characterization of the extracted oil

Using process parameters that gave a maximum oil yield (particle size range 0.25-0.5mm, extraction time of 6 hour and the two varieties) oil was extracted and physical and chemical properties were studied.

4.4.1. Moisture and volatile matter of oil

The moisture and volatile matter of oil was determined by oven method, 5 gm of oil was taken and put in oven and the weight was recorded at 1 hour and 2 hours (Singh et al., 1981).

The result obtained is summarized in the table below:

Table: 4.14. Moisture and volatile matter of oil

Time (hr)	W at t= 0 hr	W at t=1 hr	W at t= 2 hr	Weight(gm) loss by (2 hr -0 hr)
Weight (gm) Kali jira	5.0	4.92	4.92	0.08
Weight (gm) Kalonji	5.0	4.91	4.91	0.09

From equation (Moisture and volatile matter of oil $= \frac{W_1}{W} \times 100\%$)

W_1 is loss in gm in material on drying = 5.0gm- 4.92gm= 0.08gm

W is weigh in gram of oil taken for the test = 5.0gm

4.4.2. Specific gravity

Density bottle (Pycnometer) method used to determine the specific gravity of oil as the detail experimental procedures were stated in section 3.6.2. From equation (Specific gravity at 30⁰C $= \frac{A-B}{C-B}$); $A_{KALI\ JIRA}$ is weight in gram of dry bottle (pycnometer) with oil at 30⁰C= 72.2676 $A_{KALONJI}$ is weight in gram of dry bottle (pycnometer) with oil at 30⁰C= 71.7580

B is weight in gram of dry bottle (pycnometer) at 30⁰C= 24.416

C is weight in gram of weight bottle (pycnometer) with water at 30⁰C= 76.56

Substituting the above equation:

Specific gravity=   (A-B)／(C-B)

Specific gravity KALI JIRA = (72.2676-24.416)／(76.56-24.416) = 47.8516 / 52.144

$$= 0.91768$$

Specific gravity KALONJI = (71.7580-24.416)／(76.56-24.416) = 47.342 / 52.144

$$= 0.90790$$

Hence the density of oil can be determined using:

$SG = \rho_{oil} / \rho_{water}$

Where: ρ_{oil} = density of black cumin seed oil

ρ_{water} = density of water =1000 kg/m^3

ρ_{oil} of Kali jira = SG* ρ_{water} = 0.91768 *1000 kg/m3

Therefore, density of Kali jira oil was 917.68 kg/m3

ρ_{oil} of Kalonji = SG* ρ_{water} = 0.90790 *1000 kg/m3

Therefore, density of Kalonji oil was 907.90 kg/m3

4.4.3. Kinematic viscosity

Dynamic viscosity of Kali jira oil, which was read from vibro viscometer, was 2.8mpa.sec at temperature of 29.9ºC.

Dynamic viscosity of Kalonji oil, which was read from vibro viscometer, was 3.2mpa.sec at temperature of 30.7ºC.

Substituting the dynamic viscosity of Kali jira oil =2.8 mpa.s = 2.8.*106 kg/m.s, dynamic viscosity of Kalonji oil = 3.2mpa.sec = 3.2 *106 kg/m.s and

Density of Kali jira oil = 917.68 kg/m^3 in equation and Density of Kalonji oil = 907.90 kg/m^3

Kinematic viscosity (V) $= \frac{\mu}{\rho}$

Kinematic viscosity Kalonji = dynamic viscosity / density of oil

$$= (3.2 * 106 \text{ kg/m.s}) / 907 \text{ kg/m}^3$$

$$= 35.25 \text{ m}^2/\text{s}$$

Kinematic viscosity Kali jira $= (2.8 * 106 \text{ kg/m.s}) / (917.68 \text{ kg/m}^3)$

$$= 30.51 \text{ m}^2/\text{s}$$

Table: 4.15. Dynamic and Kinematic viscosity of the two varieties

Varieties	Dynamic Viscosity	Kinematic viscosity
Kali jira	2.8*106 kg/m.s	30.51m^2/s
Kalonji	3.2 *106 kg/m.s	35.25 m^2/s

4.4.4. pH value of oil

The pH value of black cumin seed oil was triplicated, and the results obtained are summarized in table 4.16 below

Table: 4.16. pH value of black cumin seed oil

Run	pH Kali jira	pH Kalonji	Mean ± SD Kali jira	Mean ± SD Kalonji
1	5.11	5.10		
2	5.12	5.11	5.12 ±0.01	5.11±0.01
3	5.13	5.12		

Therefore, the pH value of black cumin seed oil was slightly acidic. In preparation of skin and hair care materials, the preferable pH value is in the range of 3.5-6.5 (Mueller *et al*., 2000). The obtained pH value of black cumin seed oil is in the range to be used in producing cosmetic materials.

4.4.5. Saponification value of the oil

Table: 4.17. Saponification value of black cumin seed oil

Run	Volume of HCL for the blank (ml)	Volume of HCL for the sample (ml) Kali jira	Volume of HCL for the sample (ml) Kalonji	Mass of sample (gm)	SV Kali jira	SV Kalonji
1	37.6	18.3	18.5	2	194.9	192.1
2	37.6	18.4	18.6	2	193.5	190.7
3	37.6	18.7	18.9	2	189.3	186.5
Mean ± SD					192.6±1.81	189.8±1.80

Hence, the saponification value of black cumin seed oil was 192.6 and 189.8 mg KOH/gm of Kali jira and Kalonji respectively oil. High saponification value implies greater proportion of fatty acids of low molecular weight. The values obtained for saponification value of black cumin seed oil was favorably comparable with the saponification value of olive oil (185-196) which is a well-known vegetable oil in cosmetics industry. High saponification value of the black cumin seed oil suggests the use of the oil in production of liquid soap, cosmetics, shampoos and creams.

4.4.6. Acid value

Table: 4.18. Acid values for black cumin seed oil (triplicate result obtained is summarized)

Run	Titration volume of Kali jira	Titration volume of Kalonji	AV Kali jira	AV Kalonji	%FFA Kali jira	%FFA Kalonji
1	0.11	0.09	0.61	0.49	0.305	0.245
2	0.14	0.13	0.78	0.72	0.39	0.36
3	0.08	0.07	0.44	0.39	0.22	0.19
Mean ±SD			0.61±0.4	0.53±0.3	0.3±0.21	0.26±0.21

From Table 4.18 properties of black cumin seed oil the average acid value of black

cumin seed oil of Kali jira and Kalonji is 0.61±0.4 and 0.53±0.3 respectively which is relatively smaller. The low acidity of oil is an indication of oil which is free from hydrolytic rancidity and enables the direct use of such oil without further neutralization (Arogba, 1999). Therefore, the result obtained indicated that black cumin seed oil can used directly without further neutralization. The low free fatty acids content (0.3 and 0.26) was indicative of low enzymatic hydrolysis. This can be an advantageous that black cumin seed oil cannot develop off (rancidity) flavor during storage.

4.4.7. Iodine value

The iodine value (IV) is the amount of iodine (in gram) necessary to saturate 100 g of oil sample. The iodine value is used to determine the unsaturation of oils and in assessing the stability of oil in industrial application (Xu et al., 2007). The lower the iodine value of oil, which reflects its characteristics such as higher resistance to oxidation, the longer shelf life and higher quality. Whereas the higher the iodine value of oil, the lower the quality.

Testing of iodine value of black cumin seed oil fat has been conducted at ACCT Department lab, FFST, CVASU and it was found to be 118 ±0.922gm/100gm of Kali jira and Kalonji 116±0.132 gm/100gm of oil. The result indicated that black cumin seed oil has high iodine value, which indicates high resistance to oxidation and longer shelf life. The oil can be classified as a non- drying oil since its iodine value is lower than 100. Certainly, the oil can also be used extensively as lubricants and hydraulic brake fluids.

Table: 4.19. Physical and chemicals parameters of black cumin essential oil

Physical parameter	Values of Kali	Values of Kalonji
Specific Gravity (g/cm3)	0.91768	0.90790
pH	5.12 ±0.01	5.11±0.01
Kinematic viscosity	$3.051*10\text{-}7 \text{ m}^2/\text{s}$	$3.525*10\text{-}7 \text{ m}^2/\text{s}$
Density	917.68kg/m^3	907.90kg/m^3
Moisture and volatile matter of oil (%)	1.6	1.8

Table: 4.20. Chemicals parameters of black cumin essential oil

Chemicals parameters	Values of Kali jira Mean ± SD	Values of Kalonji Mean ± SD
Free Fatty Acid (%)	0.3±0.21	0.26±0.21
Iodine Value (g/100g)	118 ±0.922	116±0.132
Acid Value (mg KOH/g)	0.61±0.4	0.53±0.3
Saponification Value	192.6±1.81	189.8±1.80

Black cumin oil extracted through solvent extraction was tested for various physical & chemical characteristics and fatty acid profile (Table 19 and Table 20).

Means values for physical parameters of essential oil including specific gravity, pH and density values were 0.91768±0.001 and 0.90790±0.002, 5.12±0.01 and 5.11±0.01, 917.68 and 907.90, 917.68kg/m^3 and 907.90kg/m^3 Kali jira and Kalonji respectively. Likewise, means for chemical parameters like free fatty acid, iodine, acid value and saponification value were 0.3±0.21 and 0.26±0.21, 118±0.922g/100g and 116±0.132g/100g, 0.62±0.007mg KOH/g and 0.52±0.004mg KOH/g, 192.65±0.97 and 189.6±1.594, respectively.

Physical parameters like specific gravity, refractive index, are important in quantitative estimation of fat and oils and values observed in the present research remained in the ranges described in literature (Ramadan and Morsel, 2002; Atta, 2003; Cheikh-Rouhou et al., 2007). Chemical attribute like iodine value indicates the presence of unsaturated fatty acids and higher value is an indication of the presence of lower amount of saturated fats and vice versa. The iodine value usually ranged from 15.0 to 150mg/100g in vegetable oils. The present findings are in corroboration with the values reported earlier by Atta (2003) and Cheikh-Rouhou et al. (2007).

Results of physical and chemicals characteristics were in line with studies of Ramadan and Morsel (2002), Atta (2003), Takruri and damesh, (1998) and Al-Jassir et al. (1992). Moreover, results of fatty acid profile provide evidence of its rich nutritional profile in terms of polyunsaturated fatty acids.

4.5. Determination of the functional groups present using FT-IR

Infra-red (IR) spectrum was recorded on a Perkin-Elmer FT-IR spectrometer. About 1 mg of the isolated compound was prepared as KBr pellets and employed for recording the IR spectrum (frequencies between 4000 and 400 cm-1).
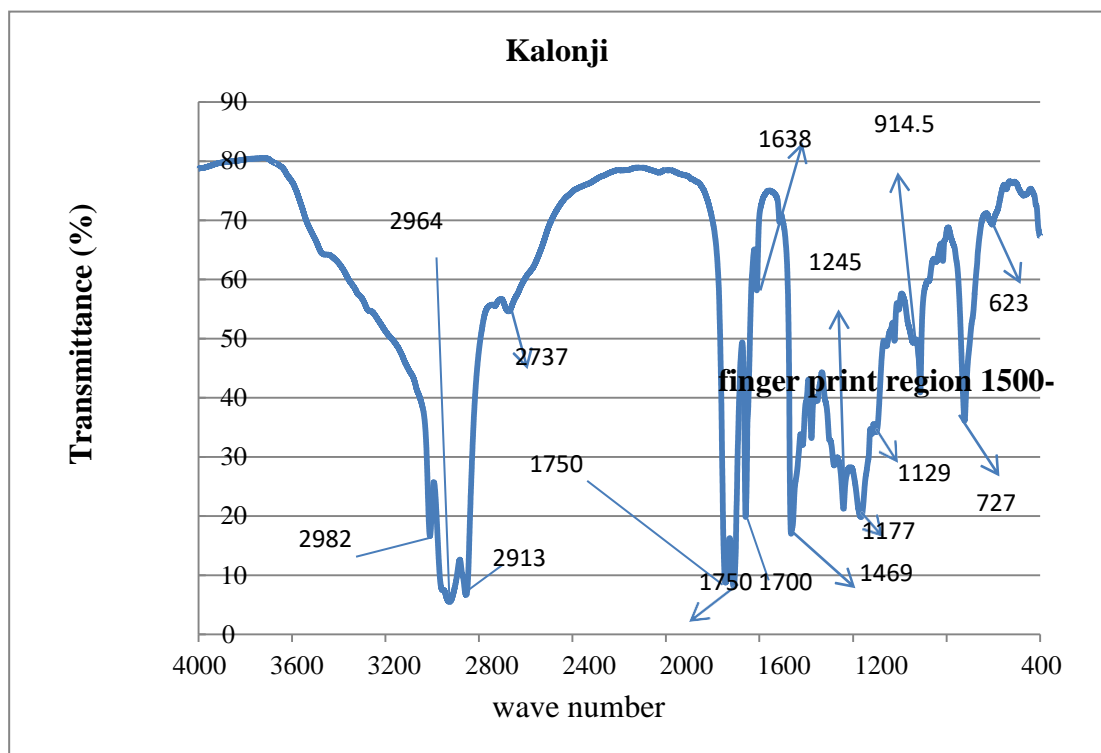
Figure: 4.9. FI-IR Kalonji varieties
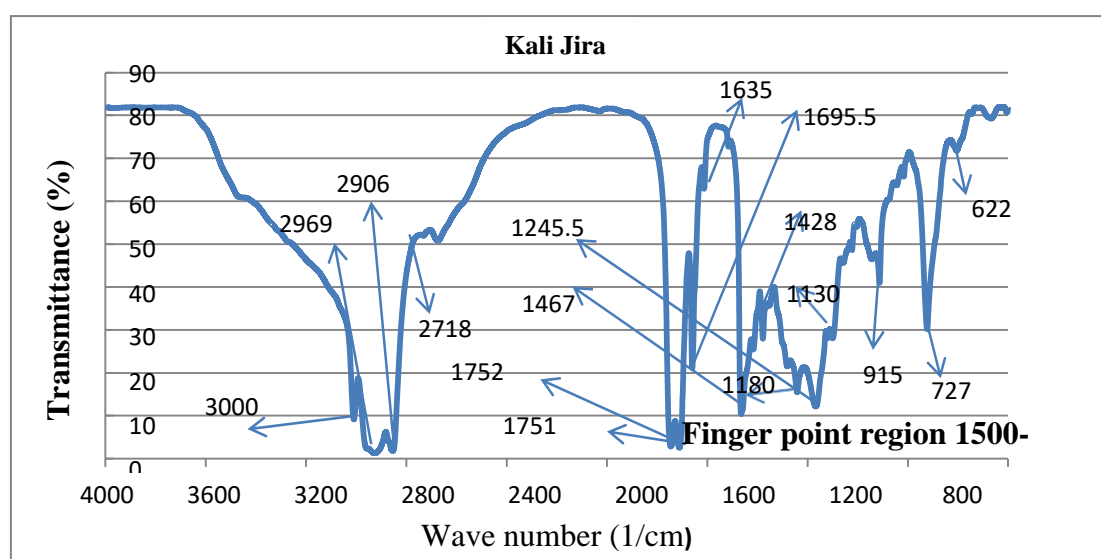
Figure: 4.10. FT-IR of Kali jira varieties

The functional groups present in the essential oil were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph obtained from an FT-IR spectrophotometer with those of an IR correlation chart. FTIR spectra of both oils appear fairly similar. In the FT-IR spectrum of *Nigella sativa* essential oil the absorption band or frequency from 3100 cm^{-1}-2800 cm-1 in the graph 2982cm^{-1}, 2964cm^{-1}and 2913cm^{-1} showed the presence of medium indicate is the region of functional groups such as (1) Hydrogen's stretching, (2) double bond's stretching and (3) deformation and bending of other bonds. C-H stretch for alkane and stretching of methyl ester. The stretch band at 2738 cm-1 C=O revealed the presence of aldehyde. The range of wave number 1740-1760 cm-1 esterified carboxyl group C=O, from 1600-1650cm^{-1} asymmetric carboxyl stretching, and the peak is 1630-1650 cm^{-1} free carboxyl group compound. A medium-weak band between 1680-1600 cm^{-1} showed the presence of alkenes C=C stretch. A wave number (1230–700 cm^{-1}) corresponds to carbohydrate radical from the triglyceride structure of oils. From the spectra of selected oils, the bands at 1130, 1180 and 915 cm^{-1} are evident, which are correlated to stretching vibration of C-O ester group. A strong absorption band between 900 cm^{-1} – 675 cm^{-1} indicated the presence of aromatic C=C but from 1500-400 finger print region.

FTIR spectra of both oils appear similar. However, careful examination of FTIR spectra of both oils reveals some significant differences either in number of peaks at region 3100–2800 cm^{-1} (assigned with) or in the peak intensities at frequencies of especially at 2982 and 3000cm^{-1}, marked with in Fig.4.9 and Fig.4.10 except the above peak Kali jira and Kalonji black cumin essentials were almost the same functional group peaks.

4.6 GC-MS

4.6.1. Library list of total components of Kali jira, and Kalonji & area (%)

Table: 4.21. Library lists of total components of Kali jira & area (%)

No	Retention time	Compound name	matching	% of total
1	38.245	Methyl tetradecanoate	99	2.24
2	41.750	Penta decanoic acid, methyl ester	99	0.36
3	44.387	9- hexadecenoic acid, methyl ester (z)	99	1.41
4	44.387	7 -hexadecanoic acid, methyl ester (z)	99	1.41
5	45.161	Penta decanoic acid,14-methyl ester	94	14.98
6	45.161	Hexadecanoic acid, methyl ester	94	14.98
7	50.512	10,13-Octa decadienoic acid, methyl ester	93	44.35
8	50.512	9,12- Octadecadienoic acid, methyl	93	44.35
9	50.512	11,14 Octa decadienoic acid, methyl ester	93	44.35
10	50.691	9- Octa decenoic acid, methyl ester(E)	99	26.17
11	50.691	6- Octadecadecenoic acid, methyl ester(Z)	99	26.17
12	50.691	9-Octadecenoic acid(-Z) methyl ester	99	26.17
13	50.799	11-Octadecenoic acid, methyl ester	99	2.65
14	50.799	Cis-13-Octadecenoic acid, methyl ester	99	2.65
15	50.799	9-Octadecenoic acid, methyl ester(E)	99	2.56
16	51.421	Methyl stearate	99	4.56
17	53.216	Tetra cosanoic acid, methyl ester	99	-0.15
18	56.316	Cis-11,14-Eicosadienic acid, methyl ester	99	2.89
19	56.316	11,13- Eicosadienic acid, methyl ester	99	2.89
20	56.316	10,13- Eicosadienic acid, methyl ester	93	2.89
21	56.464	Cis-11- Eicosedienic acid, methyl ester	99	0.55
22	56.464	Cis-13- Eicosedienic acid, methyl ester	99	0.55
23	56.464	Cis-methyl 11-eicosenate acid	99	0.55

Table: 4.22. Library lists of total components of Kalonji & area (%)

No	Retention time	Compound name	matching	% of total
1	25.788	Longifolene	99	0.51
2	38.242	Methyl tetradecanoate	99	0.52
3	41.371	2,6,10-Dodecatrien-1-ol,3-7,11-trimethyl	41	0.26
4	41.371	Cyclopentane, 1-buthyl-2-ethyl	38	0.26
5	41.371	Cis-2,6-Dimethyl -2,6-octadiene	38	0.26
6	44.385	9-hexadecenoic acid, methyl ester(Z)	99	0.31
7	44.385	(Z)-methyl hexadec-11-enoate	96	0.31
8	45.183	Pentadecanoic acid, 14- methyl ester	95	12.76
9	45.183	Hexadecanoic acid, methyl ester	91	12.76
10	50.585	8,11-Octadecadienoic acid, methyl ester	93	50.05
11	50.585	E,Z-13,12-Nanodecatrienene	90	50.05
12	50.585	9,12-Octadecadienoic acid(Z,Z)-methyl ester	89	50.05
13	50.747	9-Octadecenoic acid, methyl ester(Z)	99	24.87
14	50.747	8-Octadecenoic acid, methyl ester	99	24.87
15	50.835	11-octadecenoic acid, methyl ester	99	1.81
16	50.835	9- Octadecenoic acid, methyl ester	99	1.81
17	50.835	Cis-13- Octadecenoic acid, methyl ester	99	1.81
18	51.436	Methyl stearate	99	4.05
19	56.328	Cis-11,14-Eicosadienoic acid, methyl ester	99	3.83
20	56.328	11-14- Eicosadienoic acid, methyl ester	99	3.83
21	56.328	9,12-Octadecadienoic	99	3.83
22	56.466	Cis -13-Eicosenoic acid, methyl ester	99	0.63
23	56.466	Methyl 9- eicosenoate	99	0.63
24	56.466	11-Eicosenoic acid, methyl ester	98	0.63
25	57.230	Methyl 18- methylnonadecanoate	99	0.40
26	57.230	Eicosanoic acid, methyl acid	99	0.40

4.6.2. Fatty acid composition of Kali jira and Kalonji

Table: 4.23. Fatty acid composition of Kali jira

No.	R.T (min)	Fatty acid	Library search Compound name	Matching qual. (%)	% of total
1	38.245	Myristic acid(14:0)	Methyl tetradecanoate	99	2.24
2	44.387	palmitic acid (16:1)	9-hexadecenoic acid, methyl ester (z)	99	1.41
3	44.387	palmitic acid (16:1)	7-hexadecanoic acid ester (z), methyl	99	1.41
4	45.161	palmitic acid(16:0)	Hexadecanoic acid, methyl ester	94	14.9
5	50.512	Linoleic acid(18:2)	10,13-Octa decadienoic acid, methyl ester	93	44.3
6	50.691	Oleic acid(18:1)	9-Octadecenoicacid, methyl ester(E)	99	26.1
7	50.799	Oleic acid(18:1)	9-Octadecenoicacid, methyl ester(E)	99	2.65
8	53.216	Myristic acid(14:0)	Tetracosanoic acid, methyl ester	99	-0.15
9	56.316	Eicosadienoic acid (20:2)	Cis-11,14-Eicosadienic acid, methyl ester	99	2.89
10	56.464	Eicosenoic acid(20:1)	Cis-11-Eicosedienic acid, methyl ester	99	0.55
11	56.464	Arachic acid(20:0)	Cis-methyl 11-eicosenate acid	99	0.55
Saturated fatty acid					**17.62**
Monounsaturated					**32.19**
Polyunsaturated					**47.24**

Table: 4.24. Fatty acid composition of Kalonji

No.	R.T (min)	Fatty acid	Library search Compound name	Matching qual. (%)	% of total
1	38.242	Myristic acid(14:0)	Methyl tetradecanoate	99	0.52
2	41.371	Linoleic acid (18:2)	Cis-2,6-Dimethyl-2,6-octadien0ic acid, methyl ester	41	0.26
3	44.385	Linoleic acid (18:1)	9-hexadecenoic acid, methyl ester(Z)	99	0.31
4	45.183	palmitic acid (16:0)	Hexadecanoic acid, methyl ester	91	12.8
5	50.585	Linoleic acid (18:2)	8,11-Octadecadienoic acid, methyl ester	93	50.1
6	50.747	Oleic acid (18:1)	9-Octadecenoic acid, methyl ester(Z)	99	24.9
7	50.835	Oleic acid (18:1)	11-octadecenoic acid, methyl ester	99	1.81
8	56.328	Eicosadienoc acid (20:2)	11-14-Eicosadienoic acid, methyl ester	99	3.83
9	56.466	Eicosadienoc acid (20:2)	Cis-13-Eicosenoic acid, methyl ester	99	0.63
10	57.230	Eicosenoic acid (20:1)	Eicosanoid acid, methyl acid	99	0.40
Saturated fatty acid					**13.68**
Monounsaturated					**27.62**
Polyunsaturated					**54.14**

In the present investigation, linoleic acid was the dominating fatty acid followed by oleic and palmitic acids, while eicosadienoic acid and eicosenoic acid were also present both varieties but Arachic acid available only in Kali jira. Investigations led by Ramadan and Morsel (2002), Atta (2003), Ashraf *et al. (*2006), Cheikh-Rouhou *et al. (*2007) supported the findings of the present research. Results of Nickavar *et al. (*2003) observed some variations in the concentrations of myristic (2% and 0.3% greater) and eicosadienoic acid (1.35% and 1.59% less) , Eicosadienoic acid is present

in the two varieties but this acid not present the above researcher. However, they reported proportion of polyunsaturated fatty acids similar to that of tested black cumin seeds.

Fatty acid profile of black cumin essential oil is presented in Table 4.23 and 4.24. It is obvious from the results of Kali jira and Kalonji that linoleic, oleic and palmitic acids were the dominant fractions amounting 44.35± 0.51 and 50.05±0.78, 26.17 ±0.05 and 24.87±0.06 and 14.98±0.68% and 12.76±0.73, respectively while Myristic, eicosenoic and arachic acids were present in quantities of Kali jira and Kalonji 2.24±0.03 and 0.52±0.03%, 2.89±0.05 and 3.83±0.05 %, 0.55±0.01% and no arachic acids of the essential oil, respectively. Similarly, linoleic, oleic acid and palmitic were also present with percentage of 44.35±2.3% and 0.31±0.05%, 2.65±0.05% and1.81±0.05%, 1.41±0.08 and 0.26±0.05% Kali jira and Kalonji respectively. It is evident from the results that saturated and monounsaturated fatty acids Kali jira and Kalonji were found to be 17.62 and 13.68 % and 32.19 and 27.62 %, respectively, while polyunsaturated fatty acids 47.24 % and 54.14 % respectively.

4.6.3. Functional components of Kali jira and Kalonji varieties

Table: 4.25. Functional components of Kali jira variety

No.	Functional components	Compound name	Matching qual. (%)	% of total
1	α-Thujene	alpha-Thujene	91	0.21
2	α-Pinene	alpha-pinene	92	0.05
3	β-Pinene	2-beta-pinene	91	0.07
4	ρ-Cymene	Benzene,1-methyl-2-(-1-methylethyl)-	94	31.46
5	Dihydrothymoquinone	2,2,5-Cyclohexadiene-1,4-dion,2-methyl-5-(1-methylethyl)-	93	2.05
6	Thymoquinone	2,5-Cyclohexadiene-1,4-dion,2-methyl-5-(1-methylethyl)-	97	20.04
7	Thymol	Silane,trimethyl[5-methyl-2-(1-methylethyl) phenoxy)-	42	2.37

Table: 4.26. Functional components of Kalonji variety

No.	Functional components	Compound name	Matching qual. (%)	% of total
1	α-Thujene	alpha-Thujene	96	0.56
2	α-Pinene	alpha-pinene	92	0.05
3	β-Pinene	2-beta-pinene	88	0.04
4	ρ-Cymene	Benzene,1-methyl-2-(-1-methylethyl)-	94	31.29
5	Dihydrothymoquinone	2,2,5-Cyclohexadiene-1,4-dion,2- methyl-5-(1-methylethyl)-	93	2.25
6	Thymoquinone	2,5-Cyclohexadiene-1,4-dion,2-methyl-5-(1-methylethyl)-	92	20.34
7	Thymol	Silane,trimethyl[5-methyl-2-(1-methylethyl) phenoxy)-	47	2.49
8	longifoline	longifoline	99	0.51

Thymoquinone is more concentrated in black cumin essential oil ranging from 18-24% (Burits and Bucar, 2000) whereas extraction of essential oil by means of n-hexane retains meager quantity as in case of present investigation. Some organic extracts of black cumin seed also reported to possess appreciable amounts of thymoquinone. In one such study, (Singh et al., 2005) estimated thymoquinone contents of 11.8% in acetone seed extract. Moreover, thymoquinone belongs to class of compounds known as terpenoids; most members are volatile in nature and their heating losses ranged from 20-50% (Ceccarini et al., 2004) that could also be a possible reason for lower quantity of thymoquinone in essential oil.

Some other studies conducted elsewhere showed varying pictures about the composition of black cumin essential oil. Burits and Bucar (2000) analyzed essential oil using GC-MS and characterized many components including thymoquinone; 27.8%–57.0%, ρ-cymene; 7.1%– 15.5%, carvacrol; 5.8%–11.6%, t-anethole; 0.25%–2.3%, 4-terpineol; 2.0%–6.6% and longifoline; 1.0%–8.0% Mozzafari *et al*. (1998), Nickavar et al. (2003), Ashraf et al. (2006) and Wajs et al. (2008) determined ρ-

cymene as major component of black cumin seed essential oil. Among these research investigations, results coined by Burits and Bucar (2000), Mozzafari et al. (2000) and Nickavar et al. (2003) are in agreement with the present findings. However, Wajs et al. (2008) reported composition of active ingredient that differed slightly from the results of the present study. Overall, black cumin is naturally bestowed with rich volatile oil (0.40-1.50%) that contains 18.4-24% thymoquinone and 46% monoterpenes such as p-cymene and α-pinene (Al- Jassir, 1992; El-Tahir et al., 1993; Ashraf et al., 2006).

Black cumin essential oil was evaluated for its active ingredients through GC-MS generally this study was the two varieties of Kali jira and Kalonji respectively; indicated that it contains thymoquinone, dihydrothymoquinone, p-cymene, α-thujene, β-pinene, thymol and α-pinene as major constituents Kali jira and Kalonji respectively i.e. 20.04 and 20.34, 2.05 and 2.25, 31.46 and 31.29, 0.21 and 0.56, 0.07 and 0.04, 2.34 and 3.49, 0.05 and 0.05, respectively (Table 4.25 and 4.26).

In this study Kali jira varieties have higher values than Kalonji than functional components of thymol, dihydrothymoquinone, α-Thujene and β-Pinene, and longifoline available only in Kalonji variety by 0.51 %.

4.6.4. GC-MS Kali jira and Kalonji chromatogram
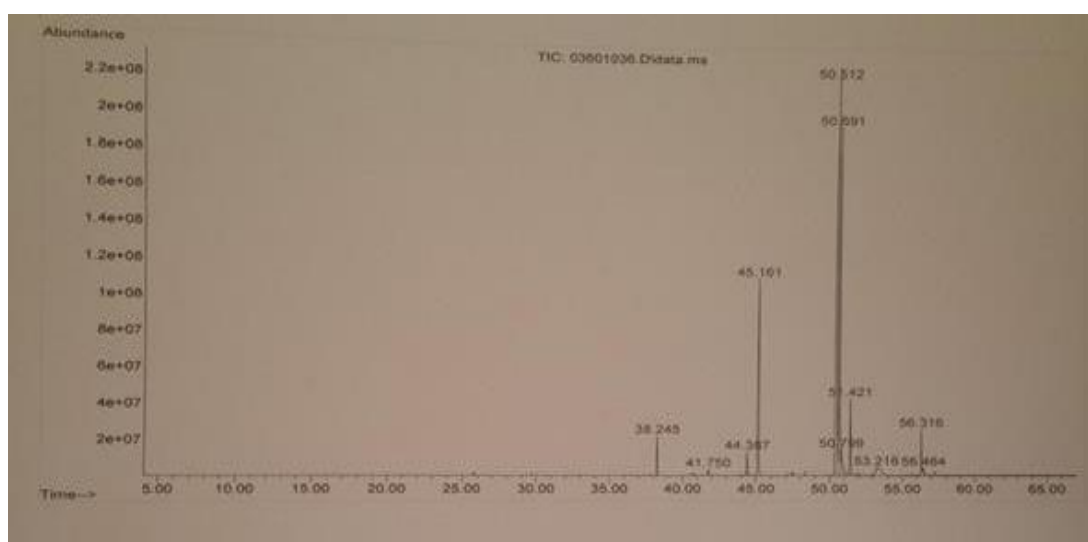
Figure: 4.11. Chromatogram of Kali jira
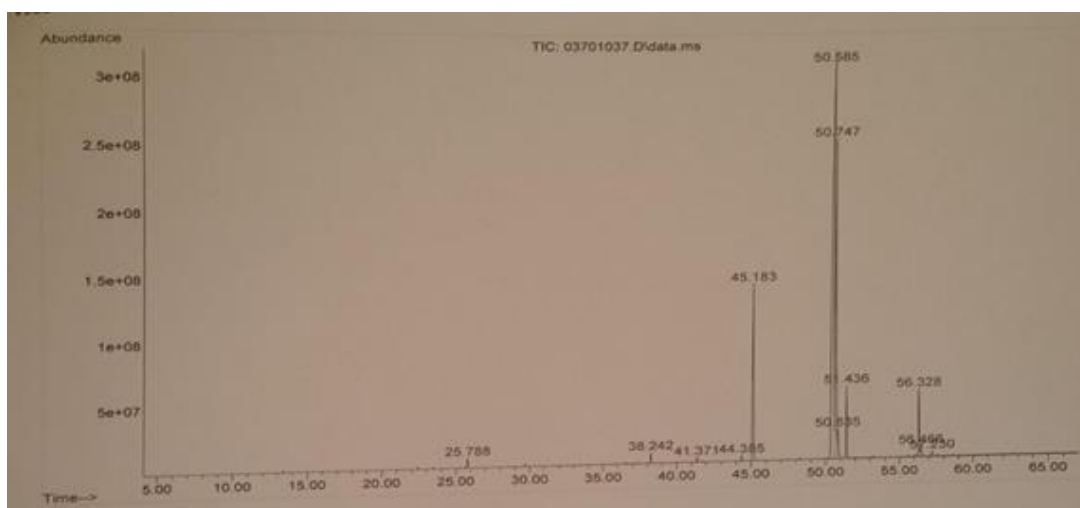
Figure: 4.12. Chromatogram of Kalonji

A total of 26 and 23 different components of Kalonji and Kali jira respectively, with different retention times, were eluted from the GC column as indicated by the chromatogram (Fig.4.11 and 4.12) and were further analyzed with an electron impact MS voyager detector. Identification of constituents was done based on their retention time and mass spectra library search. The mass spectrographs of the identified constituents are given in the above figures. The relative number of individual components was calculated based on GC peak areas.

Comparison of the GC-MS spectrograph obtained with the instruments data bank together with computer matching with Wiley 275 and BCSIR laboratories provided with computer controlling the GC-MS system revealed that the essential oil of black cumin seed contained different organic compounds that eluted at different retention times depending on the boiling point of the eluted component. The instruments data bank was also able to identify Kali jira black cumin essential oil the presence of Methyl tetradecanoate (2.24%), Pentadecanoic acid, methyl ester (0.36%),9-hexadecenoic acid, methyl ester(z)(1.41%),7-hexadecanoic acid, methyl ester (z)(1.41%), Pentadecanoic acid,14-methyl ester (14.98%),Hexadecanoic acid, methyl ester(14.98%),10,13-Octa decadienoic acid, methyl ester (44.35%), 9,12-Octadecadienoic acid, methyl ester(E,E)(44.35%),11,14 Octadecadienoic acid, methyl ester (44.35%), 9-Octadecenoic acid, methyl ester(E) (26.17%), 6- Octadecadecenoic acid, methyl ester (Z) (26.17%), 9-Octadecenoicacid(-Z) methyl ester (26.17%), 11-

Octadecenoic acid, methyl ester (2.65%), Cis-13-Octadecenoicacid, methyl ester (2.65%), 9-Octadecenoic acid, methyl ester(E) (2.56%), Methyl stearate (4.56%), Tetracosanoic acid, methyl ester (-0.15%), Cis-11,14-Eicosadienicacid, methyl ester (2.89%), 11,13-Eicosadienicacid, methyl ester (2.89%), 10,13-Eicosadienic acid, methyl ester (2.89%), Cis-11-Eicosadienoic acid, methyl ester (2.89%), Cis-13-Eicosedienic acid (0.55%), methyl ester (0.55%) and Cis-methyl 11-eicosenateacid (0.55%) and RT, 38.245, 41.750, 44.387, 44.387, 45.161, 45.161, 50.512, 50.512, 50.512, 50.691, 50.691, 50.691, 50. 799, 50.799, 50.799, 51.421, 53.216, 56.316, 56.316, 56.316, 56.464, 56.464 and 56.464 minutes respectively and the instruments data bank was also able to identify Kalonji black cumin essential oil the presence Longifolene (25.788%), Methyltetradecanoate (38.242%), 2,6,10- Dodecatrien-1-ol,3-7,11-trimethyl (41.371%), Cyclopentane, 1-buthyl-2-ethyl (41.371%), Cis-2,6-Dimethyl-2,6octadiene (41.371%), 9-hexadecenoic acid, methyl ester(Z) (44.385%), (Z)-methyl hexadec-11-enoate (44.385%), Pentadecanoic acid, 14- methyl ester (45.183%), Hexadecanoic acid, methylester (45.183%), 8,11-Octadecadienoicacid, methylester (50.585%), E,Z-13,12 Nano decatrienene (50.585%), 9,12-Octadecadienoic acid (Z,Z)-methyl ester (50.585%), 9-Octadecenoic acid, methyl ester(Z) (50.747%), 8-Octadecenoic acid, methyl ester (50.747%), 11-octadecenoic acid, methyl ester (50.835%), 9- Octadecenoic acid, methyl ester(50.835%), Cis-13 Octadecenoic acid, methyl ester (50.835%), Methyl stearate(51.436%), Cis-11,14 Eicosadienoic acid,methyl ester (56.328%), 11-14-Eicosadienoic acid,methyl ester (56.328%), 9,12 Octa decadienoic (56.328%), Cis-13 Eicosenoic acid, methylester (56.466%), Methyl 19eicosenoate (56.466%), 11-Eicosenoic acid, methylester (56.466%), Methyl18-methyl nonadecanoate (57.230%) and Eicosanoic acid, methyl acid (57.230%) and with retention times of 25.788, 38.242, 41.371, 41.371,41.371, 44.385, 44.385, 45.183, 45.183, 50.585, 50.585, 50.585, 50.747, 50.747, 50.835, 50.835, 50.835, 51.436, 56.328, 56.328, 56.328, 56.466, 56.466, 56.466, 57.230 and 57.230 respectively.

4.6.5. MS Spectrum

Table: 4.27. Kali jira variety Mass Spectrums

Data Filename	03601036.D	Sample Name	Kali jira
Sample Type	liquid	Position	36
Instrument Name	AAU	User Name	Nazrul
Acq. Method	Essential oil. M	Acquired Time	3/10/2018 12:45:45 PM
IRM Calibration Status Comment	Not Applicable	DA Method	default.m
Expected Barcode		Sample Amount	
Dual Injection Vol.	1	Tune Name	ATUNE.U
Tune Path	D:\MassHunter\GCMS \1\5977\	MS Firmware Version	6.00.21
Acquisition Time #2	2018-03-10 09:45:45Z	Operator Name	Nazrul
Run Completed Flag	True	Acquisition SW Version	Mass Hunter GC/MS Acquisition B.07.02.1938 08-Sep-2014 Copyright © 1989-2014 Agilent Technologies, Inc.

User chromatography
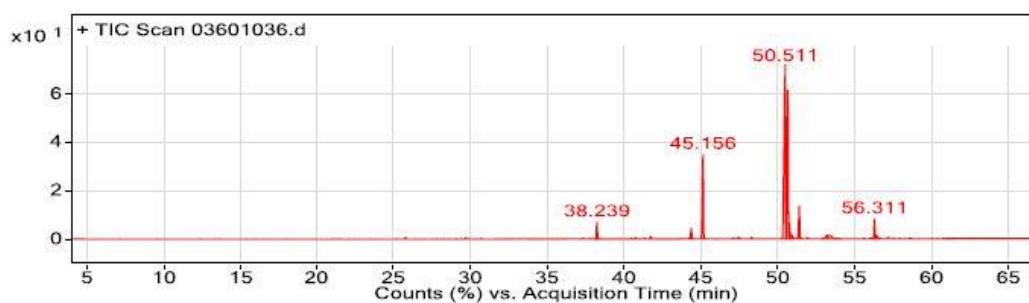
Figure: 4.13. Mass Spectrums of TIC of Kali jira variety

Table: 4.28. Kalonji variety mass spectrums

Data Filename	03701037.D	Sample Name	Kalonji
Sample Type	liquid	Position	37
Instrument Name	AAU	User Name	Nazrul
Acq. Method	Essential oil. M	Acquired Time	3/10/2018 12:59:47 PM
IRM Calibration	Not Applicable	DA Method	default.m
Expected Barcode		Sample Amount	
Dual Injection Vol.	1	Tune Name	ATUNE.U
Tune Path	D:\MassHunter\GCMS \1\5977\	MS Firmware Version	6.00.21
Acquisition Time #2	2018-03-10 09:59:47Z	Operator Name	Nazrul
Run Completed Flag	True	Acquisition SW Version	Mass Hunter GC/MS Acquisition B.07.02.1938 08-Sep-2014 Copyright © 1989-2014 Agilent Technologies, Inc.

User chromatography
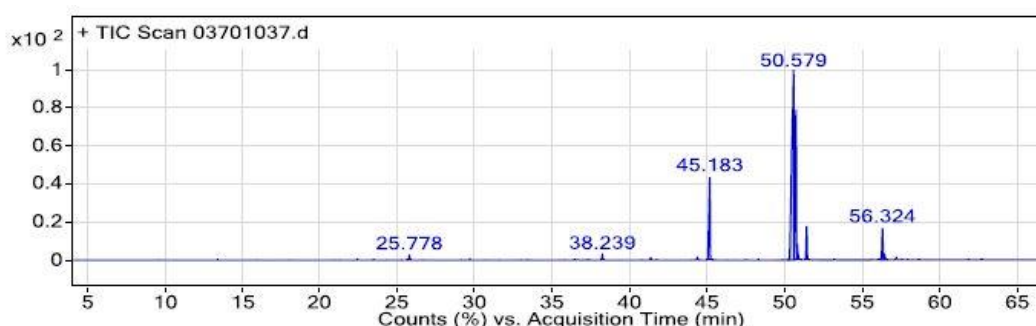
Figure: 4.14. Mass spectrum TIC of Kalonji variety

Table: 4.29. Integration list peak

Peak	Start	RT	End	Height	Area	Area %
1	25.684	25.778	25.886	7723668.01	29083744.35	1.01
2	38.131	38.239	38.387	8490508.94	30179068.38	1.05
3	44.999	45.183	45.357	139468764.8	731565620.1	25.53
4	50.229	50.579	50.619	319881204.4	2865729163	100
5	50.619	50.753	50.794	251198476.7	1430632293	49.92
6	50.794	50.834	51.09	23219630.67	114163426.8	3.98
7	51.305	51.426	51.642	56980960.67	236616845.8	8.26
8	56.203	56.324	56.405	52999317.13	222447853.7	7.76
9	56.405	56.459	56.607	7869885.12	37659485.21	1.31

Table: 4.30. Comparison of physicochemical and functional properties of oils

Oil properties	Measured value of Kali jira variety	Measured value of Kalonji variety	Commercial Specification
Moisture (%)	5.29±0.12	5.43 ±0.13	3.8-7.0
Specific gravity	0.91768	0.90790	0.911-0.935
pH	5.12 ±0.01	5.11±0.01	Less than 6
Kinematic Viscosity	30.51m2/s	35.25m2/s	(2.5-4.0)m2/s
Saponification (mg KOH/g)	192.6±1.81	189.8±1.80	180-210
Acid value	0.61±0.4	0.53±0.3	Less than 2
Iodine value(g/100g)	118±0.922	116±0.132	95-120
Moisture and volatile matter of oil (%)	1.6	1.8	>2
Thymoquinone (%)	20.04	20.34	18-24
ρ-Cymene (%)	31.46	31.29	20-40
Dihydrothymoquinone (%)	2.05	2.25	1.5-5
Linoleic acid (omega-6) (%)	44.35	50.1	< 50
Oleic acid (%)	26.1	24.9	>22.5

Commercial essential oils were analyzed specific gravity, kinematic viscosity, saponification value, iodine value, moisture content and volatile matter of oil and pH and other specification of Commercial essential oil values obtained from (www.Natural sourcing *N.sativia* oil).

The physicochemical properties of the oils were within the specification of commercial black cumin seed oil. The total content of oil was not compared due to the lack of data under this specific property. However, it can be said that the higher thymoquinone value in Bangladeshi black cumin (local varieties of Kali jira and Kalonji) seed essential oil will give higher opportunity to be used in the cosmetics industry since high thymoquinone in oil guarantees the use of oil in cosmetics industry (Burits and Bucar, 2000; Nizikou et al., 2010**;** Burt, 2004; Atta, 2003).

4.7 Antioxidant activity of Black cumin seed fixed oil

Black cumin fixed oil is rich in antioxidants like tocopherols, carotenoids, etc. which can replace normal shortening (Ramadan, 2007). In order to justify the nutraceutical and functional value of black cumin seed fixed oil, carotenoids and total tocopherol content were determined and data is presented in Table-4.31.

Table 4.31. Thymoquinone, Total Tocopherol and Carotenoids contents of Black cumin fixed oil

Functional components	Mean value
Thymoquinone (mg/kg oil)	201.31 ± 13.17
Total Tocopherol (mg/kg oil)	361.71 ± 10.23
Carotenoids (mg/kg oil)	88.95 ± 3.91
Total Tocopherol and Carotenoids (mg/kg oil)	450.66 ± 16.21

The data depicted in table-4.31 showed that total tocopherol content of black cumin fixed oil was 361.71 ± 10.23 mg/kg while carotenoid content was observed to be 88.95 ± 3.91 mg/kg. It could be concluded based on obtained results that higher total tocopherol and carotenoids content may be responsible for imparting functional

properties to black cumin fixed oil by preventing the oxidation. Al-Saleh, Billedo. A.G and El-Doush. (2006) reported that tocopherol content together with thymoquinone and thymol is principally responsible for enhancing the shelf life of black cumin oil. Tocopherol is also responsible for providing hypercholesterolemia effect to black cumin fixed oil (Gylling et al., 1999)

Figure: 4.15. Antioxidant activity of black cumin (Kali jira) fixed and essential oils.
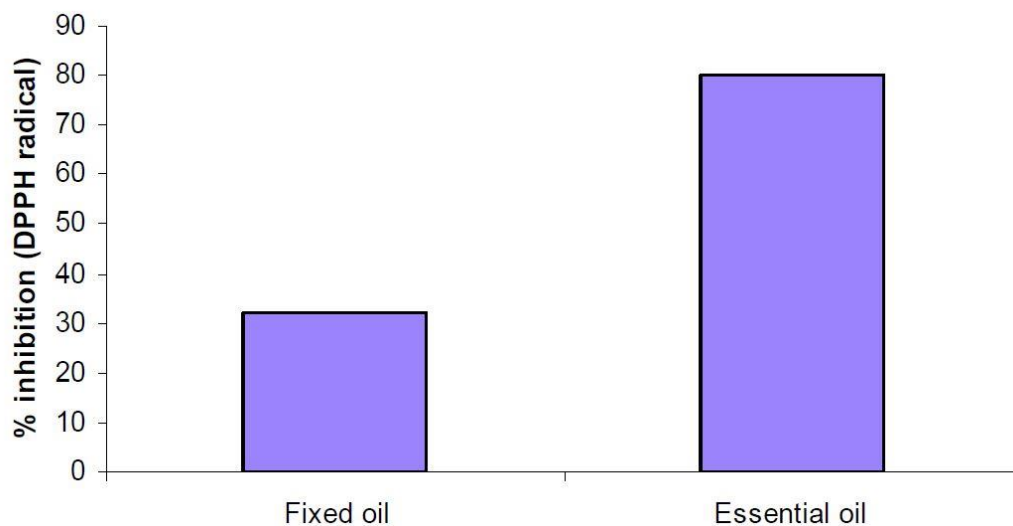
Figure: 4.16. DPPH radical scavenging activities of black cumin (Kali jira) fixed and essential oils

Antioxidant potential of fixed and essential oils of Kali jira variety was also assayed. Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was determined; black cumin fixed, and essential oils inhibited lipid peroxidation by 25.62 and 92.56%, respectively (Fig. 4.15). Later, DPPH assay was also conducted that is another module to study the antioxidant potential of test materials; black cumin fixed, and essential oils inhibited DPPH radical formation by 32.32 and 80.25%, respectively (Fig. 4.16).

Chapter V: Discussion

This work was intended to study the influence of different factors (Particle sizes and extraction time) on the quality and quantity of oil extracted from black cumin seeds varieties, essential oil used for cosmetics. Variability of these operating conditions is the pre-dominant factors for the quality and quantity of local varieties of Kali jira and Kalonji essential oil.

There are different methods of essential oil extraction from black cumin seed varieties. In this thesis, Soxhlet extraction was used. From the experimentation it was found that maximum oil yield of 91.11% and 94.77% for Kalonji and Kali jira variety respectively was obtained at particle size range of 0.5-0.25mm and extraction time of 6 hour. A minimum oil yield of 46.8 % and 49.77% was obtained at particle size range of 2.5-1.4mm and 2-hour extraction time for Kalonji and Kali jira variety respectively, the observed quantitative difference in the quantity of the oil was due to particle size and extraction time as well as seed variety variability. Thus, determination of appropriate size of the particle and optimal time for the recommended particle size needs to have a consideration to get the maximum amount of the required product.

From design expert software the analysis of ANOVA P value < 0.0001 for particle size and extraction time with black cumin local varieties of Kali jira and Kalonji indicate that operating parameters have significant effect on oil yield.

The result obtained was increased as we compare it from the literature using hexane as a solvent. The quality of the oil could be affected due to several reasons like purities with the seed, genotype of the seed, operating conditions, maturity stage, drying condition, and type of soil and extraction equipment. From the investigation, particle size and time was the dominant factor for the change in quality of the oil.

Analysis using Gas Chromatography-Mass Spectrometer was found to be the best method to identify even the minor components, functional component and fatty acid composition of particular oil along with major components, and determination of functional group by FTIR. Characterization of essential oil enumerated that

polyunsaturated fatty acids were the dominating fraction i.e. 47.24 % for Kali jira whereas Kalonji 54.14% as compared to saturated and monounsaturated fatty acids i.e. 17.62 and 32.19 for Kali jira whereas Kalonji 13.68 and 27.62 % respectively. Thus, investigation of optimal operating condition has to be taken in to consideration.

Thymoquinone is more concentrated in black cumin essential oil ranging from 18-24% (Burits and Bucar, 2000) whereas extraction of fixed oil by means of n-hexane retains meager quantity as in case of present investigation. Some organic extracts of black cumin seed also reported to possess appreciable amounts of thymoquinone. In one such study, Singh et al. (2005) estimated thymoquinone contents of 11.8% in acetone extract seed. Moreover, thymoquinone belongs to class of compounds known as terpenoids; most members are volatile in nature and their heating losses ranged from 20-50% (Ceccarini et al., 2004) that could also be a possible reason for lower quantity of thymoquinone in fixed oil. Consumption of different isomeric forms like α-, β-, γ- and δ-Tocopherols contribute substantially towards the health improvement. Presence of these important phytochemicals has also been highlighted in two earlier studies; initially conducted by Ramadan and Mörsel (2002) and later by Al-Saleh et al. (2006). According to Ramadan and Mörsel (2002), one gram of black cumin fixed oil contains 284, 40, 225, 48μg of α-, β-, γ- and δ-Tocopherols, respectively. They also reported the presence of β-carotene amounting 593μg/g oil. Similarly, Al-Saleh et al. (2006) reported the concentrations of 10.41 and 6.95mg/kg-seed of α- and γ- Tocopherols, respectively. They observed the concentration of thymoquinone (3098.5±1519.66 mg/kg) in black cumin seeds.

Black cumin essential oil is also important owing to its rich phytochemistry. Some other studies conducted in different part of the world showed varying picture about the composition of black cumin essential oil. Burits & Bucar (2000) analyzed essential oil using GC-MS and characterized many components including thymoquinone; 27.8%– 57.0%, ρ-cymene; 7.1%–15.5%, carvacrol; 5.8%–11.6%, t-anethole; 0.25%–2.3%, 4- terpineol; 2.0%–6.6% and longifoline; 1.0%–8.0% (Mozzafari et al., 2000; Nickavar et al., 2003). Afterwards, Ashraf et al. (2006) and Wajs et al. (2008) determined p-cymene as major component of black cumin seed essential oil. Among these research investigations, results reported by Nickavar et al.

(2003) and Burits & Bucar (2000) are in agreement with the present findings. Overall, black cumin is naturally bestowed with antioxidant rich volatile oil (0.40-1.50%) that contains 18.4-24% thymoquinone and 46% monoterpenes such as p-cymene and α-pinene (Al-Jassir, 1992; El-Tahir et al., 1993; Singh et al., 2005; Ashraf et al., 2006). Several other scientists explored the antioxidant potential of black cumin oil and its various fractions containing active ingredients. Because of the ease and convenience, DPPH assay has widespread use in free radical scavenging assessment (Thaipong et al., 2006; Erkan et al., 2008; Scherer & Godoy, 2009). According to Burits & Bucar (2000), IC_{50} value (DPPH assay) for different test compounds like essential oil, thymoquinone and carvacrol were found to be 460.0, 211.0 and 28.8 mg/mL, respectively. In another investigation, black cumin seeds inhibited DPPH radical formation and mean IC_{50} (μM) was found to be 515 ± 20.1 while ABTS. + (TEAC, mM Trolox) assay gave readings of 2.0 ± 0.7, 2.4 ± 0.3 and 2.5 ± 0.6 after 1, 4 and 6 minutes of reaction (Erkan et al., 2008)

Chapter VI: Conclusions

N. sativa seeds and oils are abundant in nutrient and antioxidants. The results were compared with commercially available black cumin seed essential oil specification and standard except the Moisture and volatile matter of oil which is slightly below the level, other properties both varieties in desired range but Kali jira varieties have higher value than Kalonji except pH, Moisture and volatile matter of oil, thymol, thymoquinone, linoleic. Generally due to the presence of linoleic acid, thymoquinone and high saponification value of oil suggests and guarantees the use of oil for cosmetics industry and in production of liquid soap, shampoos and creams. Due to the above properties and characteristic Kali jira varieties are preferable for making cosmetics to Kalonji variety. Black cumin holds nutraceutical potential against various physiological threats owing to its rich phytochemistry especially due to the presence of thymoquinone, tocopherols, etc. Finally, fixed and essential oil supplementation in food products especially bakery items is feasible and can be employed to achieve the allied health claims.

Chapter VII: Recommendations and Future perspectives

I recommend the project to be implemented from the point of application of two varieties of Black cumin oil extraction:

1. Further research on supercritical fluid extraction and cold press on improvement of the production black cumin essential oil with feasibility study.
2. Further research work on comparison of different extraction technology such as steam distillation, supercritical fluid extraction and cold press with solvent extraction.
3. The country should invite and encourage investors who are interested to work in value added products like essential oil production from black cumin instead of importing the oil from abroad. This may create job opportunities to the employees and save valuable foreign exchange.
4. Further research work on the remained varieties of Bangladeshi black cumin varieties.
5. Further research work on the investigation of temperature, solvent type and other factors.
6. Further research works on of Black cumin seed essential oil for shampoo, cream and soap application.
7. Further research work on Black cumin seeds essential oil thymoquinone application for anti-oxidation.
8. Further research work on Black cumin seeds essential oil MS analysis of the two varieties mass to charge ration further fragment.
9. Adding up of black cumin fixed/essential oils formulate enrich food product.

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Appendix A: Formulas and Equations used for characterization of the oil

1. Moisture content (%) of the kernel $= \frac{W_1 - W_2}{W_1} \times 100\%$ AG.1

   Where: W_1 = original weight of the sample before drying

   W_2 = weight of the sample after drying

2. Moisture and volatile matter of oil $= \frac{W_1}{W} \times 100\%$ AG.2

   Where: W_1 = loss in gram of the material on drying

   W = weight in gram of oil taken for the test

3. Specific gravity at $30^0C = \frac{A - B}{C - B}$ ………………...…….AG.3

   Where: A = weight in gm of density of bottle with oil at 30^0c

   B = weight in gm of density of bottle at 30^0c

   C = weight in gm of density of bottle with water at 30^0c

4. Kinematic viscosity $V = \frac{\mu}{\rho}$. ………..……………….AG.4

   Where: μ = dynamic viscosity

   ρ = density of oil

5. Saponification value $= \frac{56.1\,(B - S)N}{W}$ …………….................…AG.5

   Where:

   B= volume in ml of standard hydrochloric acid required for the blank

   S= volume in ml of standard hydrochloric acid required for the sample

   N= normality of hydrochloric acid

   W= weight in gm of the oil / fat taken for the test

6. Unsaponification matter $= \frac{100\,(A - B)}{W}$ ………………….…...AG.6

   B= 0.282VN

Where: A = Weight in gm of the residue

B = Weight in gm of free fatty acids in the extract as oleic acid

N =Normality of standard sodium hydroxide solution

V = Volume in ml of standard sodium hydroxide solution

W = weight in gm of the sample

7. Acid value $= \dfrac{56.1 \times V \times N}{W}$ ...…………………… AG.7

Where: V= volume in ml of standard sodium hydroxide solution

N= Normality of sodium hydroxide solution

W= weight in gm of sample

8. Percent free fatty acid (as oleic acid) $= \dfrac{AV}{1.99}$. …………………….AG.8

Where: AV= acid value

Appendix B: Fatty acid composition of black cumin seed oil

Table B1: Fatty acid composition of black cumin seed oil of Kali jira

```
Search Libraries:    D:\MassHunter\Library\NIST14.L    Minimum Quality:    0

Unknown Spectrum:    Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#    RT   Area%          Library/ID              Ref#    CAS#    Qual
────────────────────────────────────────────────────────────────────────
 1  38.245  2.24 D:\MassHunter\Library\NIST14.L
                 Methyl tetradecanoate           104286 000124-10-7 99
                 Methyl tetradecanoate           104288 000124-10-7 96
                 Methyl tetradecanoate           104289 000124-10-7 96

 2  41.750  0.36 D:\MassHunter\Library\NIST14.L
                 Pentadecanoic acid, methyl ester 117468 007132-64-1 99
                 Pentadecanoic acid, methyl ester 117471 007132-64-1 98
                 Pentadecanoic acid, methyl ester 117470 007132-64-1 98

 3  44.387  1.41 D:\MassHunter\Library\NIST14.L
                 9-Hexadecenoic acid, methyl ester, 128700 001120-25-8 99
                  (Z)-
                 7-Hexadecenoic acid, methyl ester, 128697 056875-67-3 99
                  (Z)-
                 9-Hexadecenoic acid, methyl ester, 128693 001120-25-8 99
                  (Z)-

 4  45.161 14.98 D:\MassHunter\Library\NIST14.L
                 Pentadecanoic acid, 14-methyl-, me 130843 005129-60-2 94
                 thyl ester
                 Hexadecanoic acid, methyl ester  130822 000112-39-0 94
                 Hexadecanoic acid, methyl ester  130813 000112-39-0 90

 5  50.512 44.35 D:\MassHunter\Library\NIST14.L
                 10,13-Octadecadienoic acid, methyl 153881 056554-62-2 94
                  ester
                 9,12-Octadecadienoic acid, methyl  153898 002566-97-4 93
                 ester, (E,E)-
                 11,14-Octadecadienoic acid, methyl 153880 056554-61-1 93
                  ester

 6  50.691 26.17 D:\MassHunter\Library\NIST14.L
                 9-Octadecenoic acid, methyl ester, 155754 001937-62-8 99
                  (E)-
                 6-Octadecenoic acid, methyl ester, 155752 002777-58-4 99
                  (Z)-
                 9-Octadecenoic acid (Z)-, methyl e 155751 000112-62-9 99
                 ster

 7  50.799  2.65 D:\MassHunter\Library\NIST14.L
                 11-Octadecenoic acid, methyl ester 155737 052380-33-3 99
                 cis-13-Octadecenoic acid, methyl e 155747 1000333-58-3 99
                 ster
                 9-Octadecenoic acid, methyl ester, 155758 001937-62-8 99
                  (E)-

 8  51.421  4.56 D:\MassHunter\Library\NIST14.L
                 Methyl stearate                  157879 000112-61-8 99
                 Methyl stearate                  157884 000112-61-8 99
                 Methyl stearate                  157883 000112-61-8 98
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
9	53.216	-0.15	D:\MassHunter\Library\NIST14.L			
			Tetracosanoic acid, methyl ester	228681	002442-49-1	99
			Tetracosanoic acid, methyl ester	228678	002442-49-1	98
			Tetracosanoic acid, methyl ester	228680	002442-49-1	96
10	56.316	2.89	D:\MassHunter\Library\NIST14.L			
			cis-11,14-Eicosadienoic acid, methyl ester	180766	1000333-61-8	99
			11,13-Eicosadienoic acid, methyl ester	180763	056599-57-6	98
			10,13-Eicosadienoic acid, methyl ester	180764	030223-50-8	93
11	56.464	0.55	D:\MassHunter\Library\NIST14.L			
			cis-11-Eicosenoic acid, methyl ester	182571	1000333-63-8	99
			cis-Methyl 11-eicosenoate	182558	002390-09-2	99
			cis-13-Eicosenoic acid, methyl ester	182570	1000333-52-1	99

Table B2: Fatty acid composition of black cumin seed oil of Kalonji

```
Search Libraries:    D:\MassHunter\Library\NIST14.L    Minimum Quality:    0

Unknown Spectrum:    Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#    RT   Area%           Library/ID              Ref#    CAS#     Qual

 1   25.788  0.51 D:\MassHunter\Library\NIST14.L
                  Longifolene                      68502 000475-20-7 99
                  Longifolene                      68498 000475-20-7 99
                  Longifolene                      68503 000475-20-7 99

 2   38.242  0.52 D:\MassHunter\Library\NIST14.L
                  Methyl tetradecanoate           104286 000124-10-7 99
                  Methyl tetradecanoate           104288 000124-10-7 97
                  Methyl tetradecanoate           104289 000124-10-7 96

 3   41.371  0.26 D:\MassHunter\Library\NIST14.L
                  2,6,10-Dodecatrien-1-ol, 3,7,11-tr 85750 004602-84-0 41
                  imethyl-
                  Cyclopentane, 1-butyl-2-ethyl-   27961 072993-32-9 38
                  cis-2,6-Dimethyl-2,6-octadiene   17326 002492-22-0 38

 4   44.385  0.31 D:\MassHunter\Library\NIST14.L
                  9-Hexadecenoic acid, methyl ester, 128700 001120-25-8 99
                   (Z)-
                  9-Hexadecenoic acid, methyl ester, 128693 001120-25-8 98
                   (Z)-
                  (Z)-Methyl hexadec-11-enoate     128656 000822-05-9 96

 5   45.183 12.76 D:\MassHunter\Library\NIST14.L
                  Pentadecanoic acid, 14-methyl-, me 130843 005129-60-2 95
                  thyl ester
                  Hexadecanoic acid, methyl ester  130822 000112-39-0 91
                  Hexadecanoic acid, methyl ester  130813 000112-39-0 91

 6   50.585 50.05 D:\MassHunter\Library\NIST14.L
                  8,11-Octadecadienoic acid, methyl 153872 056599-58-7 93
                  ester
                  E,Z-1,3,12-Nonadecatriene        123183 1000131-11-3 90
                  9,12-Octadecadienoic acid (Z,Z)-, 153889 000112-63-0 89
                  methyl ester

 7   50.747 24.87 D:\MassHunter\Library\NIST14.L
                  9-Octadecenoic acid, methyl ester, 155754 001937-62-8 99
                   (E)-
                  9-Octadecenoic acid (Z)-, methyl e 155748 000112-62-9 99
                  ster
                  8-Octadecenoic acid, methyl ester 155719 002345-29-1 99

 8   50.835  1.81 D:\MassHunter\Library\NIST14.L
                  11-Octadecenoic acid, methyl ester 155737 052380-33-3 99
                  9-Octadecenoic acid, methyl ester, 155758 001937-62-8 99
                   (E)-
                  cis-13-Octadecenoic acid, methyl e 155747 1000333-58-3 99
                  ster

 9   51.436  4.05 D:\MassHunter\Library\NIST14.L
```

1

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Methyl stearate	157879	000112-61-8	99
			Methyl stearate	157885	000112-61-8	99
			Methyl stearate	157883	000112-61-8	99
10	56.328	3.83	D:\MassHunter\Library\NIST14.L			
			cis-11,14-Eicosadienoic acid, meth yl ester	180766	1000333-61-8	99
			11,14-Eicosadienoic acid, methyl e ster	180762	002463-02-7	99
			9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3	96
11	56.466	0.63	D:\MassHunter\Library\NIST14.L			
			cis-13-Eicosenoic acid, methyl est er	182570	1000333-52-1	99
			Methyl 9-eicosenoate	182550	1000336-50-5	99
			11-Eicosenoic acid, methyl ester	182565	003946-08-5	98
12	57.230	0.40	D:\MassHunter\Library\NIST14.L			
			Methyl 18-methylnonadecanoate	184595	1000352-20-6	99
			Eicosanoic acid, methyl ester	184598	001120-28-1	99
			Eicosanoic acid, methyl ester	184599	001120-28-1	98

Appendix B: Fatty acid composition and functional component
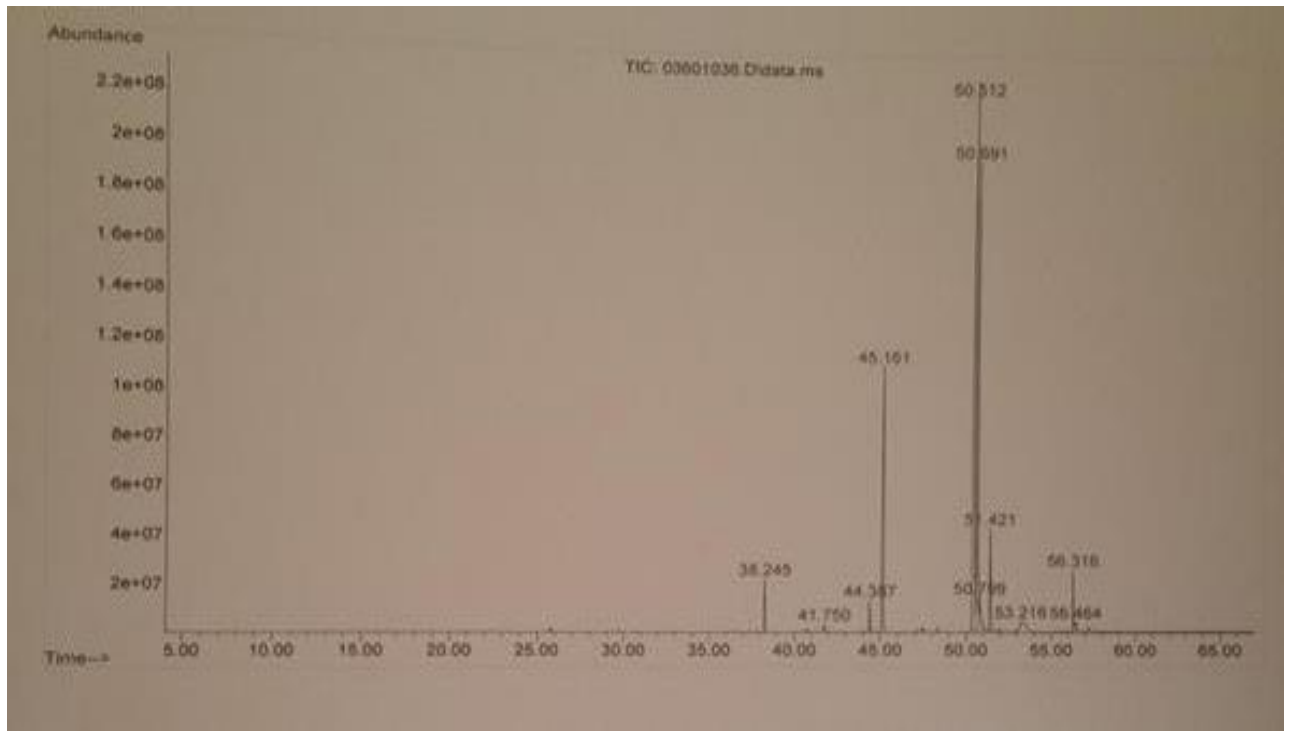

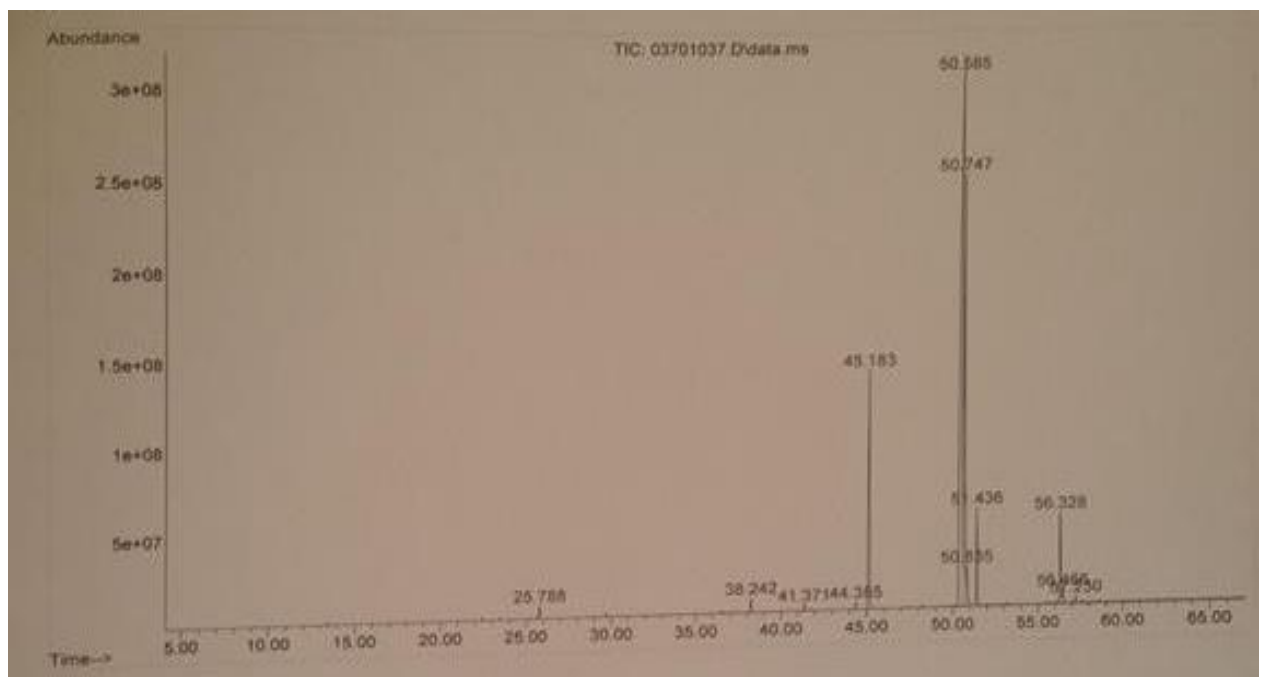**Figure C1: Chromatogram of Kali jira black cumin essential oil**

Figure C2: Chromatogram of Kalonji black cumin essential oil

Appendix D: Laboratory equipment's and samples photo

Figure D1: Water Distiller.   **Figure D2: Mixer.**   **Figure D3: Deionizer**.

**Figure D4: Flame photo meter used   Figure D5: Furnace used to ash
to detect Na & K   the sample.**

**Figure D6: UV-visible Spectroscopy used to measure absorbance of Ur
analyst of interest,**

**Figure D7: Atomic Absorption spectrophotometer used for mineral
analysis,**

Figure D8: Soxhlet fat extractor used for fat analysis.

Figure D9: Centrifuge used to separate components of a complex mixture
Figure D10: Kjeldahl analyzer unit which is used for protein analysis that is distillation and titration.

Figure: D11 Black cumin seed on cross miller, black cumin seed after drying on tray of alumni foil, cross miller respectively.

Figure D12: Sieve shaker, Sieve in top view, Samples of black cumin extracted essential oil respectively.
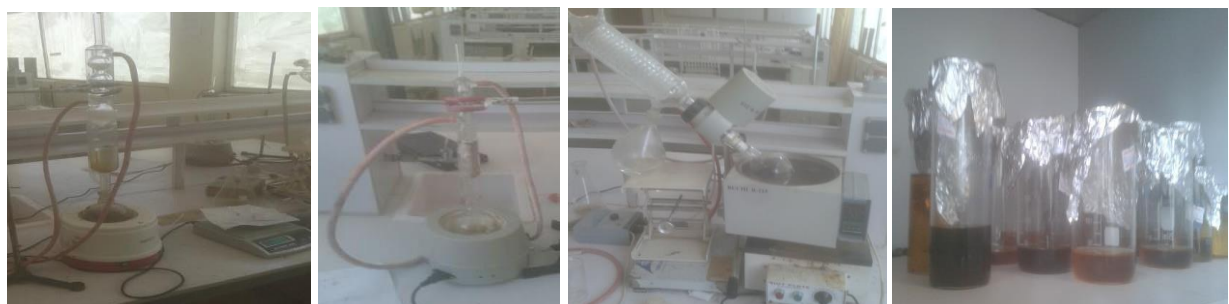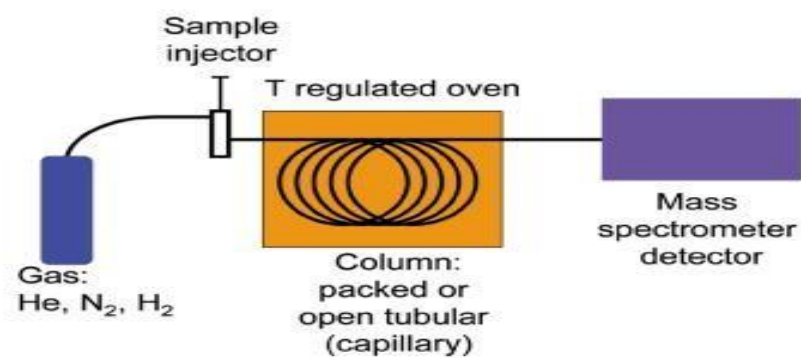

Figure D13: Soxhlet extractor, Rotary evaporated and sample of essential oil extracted in beaker

Figure D14: Essential oil extracted 6-hour, 2 hour and 4 hour and Rotary evaporator respectively.

Figure D15: Total essential oil sample in sample container.

Figure D16: Gas Chromatography-Mass spectrometer schematic diagram

Figure D17: Fourier Transform Infrared Sample holder of liquid and equipment

Figure D18: Black cumin essential of Kali jira and Kalonji respectively

Brief Biography

Md. Imran Bin Kayes passed the Secondary School Certificate Examination in 2009 and then Higher Secondary Certificate Examination in 2011. Mr. Imran obtained his B.Sc. (Hon's) in Food Science and Technology in 2016 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Technology, Faculty of Food Science and Technology, CVASU. He has been working as a Programme Assistant (Nutrition) in the United Nations World Food Programme since January 2019. He published one scientific article as co-author in an international peer-reviewed journal. He has immense interest to work in nutritive value and antioxidant activity analysis of food substances, heavy metals detection in food products, microbiological analysis of foods, community nutrition, emergency nutrition, public health etc.