Proximate Composition and Antioxidant Properties of Five Seeds: A Comparative Study

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CONTENTS

1. INTRODUCTION

Seeds play important roles in human nutrition and health since ancient time. The seeds having numerous fat-soluble bioactive and polyphenols, together with their corresponding antioxidant activities, have increasingly been consumed. Hence, these seeds can be considered as a valuable source of dietary supplements and functional foods due to their health- promoting bioactive components, polyphenols, and corresponding antioxidant activities. The phytochemicals from these super seeds demonstrate bioavailability in humans with promising health benefits. Additional long- term and well-design human intervention trials are required to ascertain the health-promoting properties of these seeds.

Seeds are extremely nutritious and contain many essential components your body needs to stay strong and healthy. They are an excellent source of fiber, protein, vitamins, minerals, antioxidants, and contain significant amounts of healthy fats such as mono unsaturated and poly unsaturated facts. Seeds, when consumed as part of a balanced diet, can benefit our body in a number of ways. In fact, many seeds like chia seeds, flax seeds, sesame seeds and pumpkin seeds are gaining popularity across the world due to their health benefits.

Plant seeds have been consumed since ancient times due to their nutritional value, especially for their macro- and micro-nutrients as well as their polyphenols with antioxidant activity in recent times. Polyphenols are compounds with a di- or trihydroxyphenyl group that are directly involved in the stabilization of reactive species by donating a hydrogen atom or transferring an electron. Moreover, these phenolic compounds can be mainly found in the coats (hull, husk, or skin, for instance) covering the cotyledon(s) of seeds such as reported for lentil, pea, beans, peanuts, and pistachio (Arjeh *et al*, 2020) and can be separated from the seed matrix by extraction with appropriate solvent.

Historically, plants (fruits, seeds, vegetables, medicinal herbs etc.) have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities like antioxidants, anti-inflammatory, anti tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial or anti-viral activities. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals have more than one function. There is however, much scope for further systematic research in screening Indian medicinal plant for these phytochemicals and assessing their potential in protecting against different types of diseases.

All plants produce chemical compounds as part of their normal metabolic activities. Plants produce primary and secondary metabolites with divergent functions. The primary metabolites; amino acids, simple sugars, proteins and lipids are involved in cellular processes. Secondary metabolites are chemically active compounds; flavonoids, alkaloids, terpenoids, steroids, saponins etc. which are produced in response in stress with complexity in structure and more restriction in distribution than the primary metabolites.

Pumpkin is a genus of plants belonging to the Cucurbitaceae family comprising several species, the most common of which are *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita moschata*. *Cucurbita maxima*, one of at least four species of cultivated squash, is one of the most diverse domesticated species. This species originated in South America from the wild *Cucurbita andreana* over 4000 years ago. The two species hybridize quite readily but have noticeably different calcium levels. Only long-vining plants are known in this species. Pumpkin are characterised by a content of carotenoids, polyphenolic compounds, mineral components and vitamin C. The level of bio active compounds is affected by the pumpkin cultivar, which determines its antioxidant properties. The antioxidant potential of 19 pumpkin cultivars of Cucurbita pepo and *Cucurbita moschata* species was determined and compared using ABTS, DTPH, FRAP, chelating activity and ORAC assay. Results of the study confirmed the high diversity of pumpkin cultivars in terms of their antioxidant activity. It was also shown that the antioxidant activity is more affected by the type of cultivar than by the pumpkin species.

Pumpkin (Cucurbita sp.) seeds are a key food source for humans because they are a very good source of proteins (24−36.5%) and oil (31.5−51%). Pumpkin seeds have long been valued as an important natural food for men's health. Pumpkin seed extract is useful for immunomodulation, reproductive health and therapeutic advantage over a wide range of disease conditions. The intake of whole extract of pumpkin seeds has been correlated with reduced benign prostate hyperplasia-associated symptoms. The antioxidative property of pumpkin seed extract could also improve fertility, and it helps to prevent arteriosclerosis, high blood pressure and heart diseases; it also stimulates metabolism of accumulated fats.

Chia (*Salvia hispanica* L.) is a small seed that comes from an annual herbaceous plant, *S.hispanica.* In recent years, usage of Chia seeds has tremendously grown due to their high nutritional and medicinal values. Chia was cultivated in Mesopotamia cultures, but then disappeared for centuries until the middle of the 20th century, when it was rediscovered. Chia seeds contain healthy omega – 3 fatty acids, polyunsaturated fatty acids, dietary fiber, proteins, vitamins, and some minerals. Besides this, the seeds are an excellent source of polyphenols and antioxidants, such as caffeic acid, rosmarinic acid, myricetin, quercetin, and others. Today, chia has been analysed in different areas of research. Researches around the world have been investigating the benefits of chia seeds in the medicinal, pharmaceutical, and food industry. Chia oil is today one of the most valuable oils on the market. Different extraction methods have been used to produce the oil.

Chia, *Salvia hispanica*, is a medicinal and dietary plant species used since ancient times. Its product is a dry indehiscent fruit which is commonly called seed. In recent times, there was an increasing attention and diffusion of the seeds of the plant for their health benefits and uses in cooking. In fact, seeds are a rich source of nutrients first of all the polyunsaturated omega-3 fatty acids that protect from inflammation, enhance cognitive performance and reduce the level of cholesterol. Seeds are also rich in polyphenols derived from caffeic acid that are antioxidant compounds protecting the body from free radicals, ageing and cancer. In addition, carbohydrate-based fibers, present at high concentration levels, are associated with reducing inflammation, lowering cholesterol and regulating bowel function.

Chia seeds are oval and grey with black and white spots, having a diameter around 2 millimetres (0.08 in). The seeds are hygroscopic, absorbing up to 12 times their weight in liquid when soaked and developing a mucilaginous coating that gives chia-based foods and beverages a distinctive gel texture. There is evidence that the crop was widely cultivated by the Aztecs in pre-Columbian times and was a staple food for Mesoamerican cultures. Chia seeds are cultivated on a small scale in their ancestral homeland of central Mexico and Guatemala and commercially throughout Central and South America.

Sunflower seed and sprout contain high concentration of niacin and vitamins A, B and C. The sunflower (*Helianthus annuus* L.) Seed and sprout is a ubiquitous crop with abundant nutrients and biological activities. There are three types of commonly used sunflower seeds: linoleic (most common), high oleic, and sunflower oil seeds. Each variety has its own unique levels of monounsaturated, saturated, and polyunsaturated fats. For commercial purposes, sunflower seeds are usually classified by the pattern on their husks. If the husk is solid black, the seeds are called black oil sunflower seeds. The crops may be referred to as oilseed sunflower crops. These seeds are usually pressed to extract their oil. Striped sunflower seeds are primarily eaten as a snack food; as a result, they may be called confectionery sunflower seeds. The term “sunflower seed” is actually a misnomer when applied to the seed in its pericarp (hull). Botanically speaking, it is a cypsela. When dehulled, the edible remainder is called the sunflower kernel or heart.

Flax seed (*Linum usitatissimum* L.) is an oil seed used in industrial and natural health products. Flax seed accumulate many biologically active compounds and elements including linolenic acid, lignin, polysaccharides, alkaloids, cyanogenic glycosides and cadmium. Most biological and clinical studies of flax seed have focused on extract containing alpha –linolenic acid. Other flax seed compounds have received less attention and their activity is not well described. The benefits of conception of whole flax seed fractions such as oil, mucilage and protein indicate that consideration of the entire portfolio of bioactive present is required to associate biological activities with specific compounds. Flax seed is one of the oldest cultivated crops, continuous to be widely grown for oil, fiber, and food. Flax seed contain soluble and insoluble fiber. Whole flax seed is widely accepted as a healthy food that has anticancer activity. Controlled experimental diets have demonstrated numerous beneficial effects of flax seed conception.

Sesame (*Sesamum indicum*) is a flowering plant in the genus Sesamum, also called benne. Numerous wild relatives occur in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods. World production in 2018 was 6 million metric tons (5,900,000 long tons; 6,600,000 short tons), with Sudan, Myanmar, and India as the largest producers. Sesame seeds have been grown in tropical regions throughout the world since prehistoric times. Sesame seeds, a rich source of proteins, is one of the first crops processed for oil production. Its non-culinary application includes its use as an ingredient in soap, cosmetics, lubricates and medicines.

Sesame seeds also contain two unique substances: sesamin and sesamolin known to have a cholesterol lowering effect in humans and to prevent high blood pressure. Both of these were also reported to increase the hepatic mitochondrial and the peroxisomal fatty acid oxidation rate in experimental animals. Cephalin, a phospholipid from sesame seed has been reported to possess haemostatic activity. The oil has wide medical and pharmaceutical applications. It is mildly laxative, emollient and demulcent. The seeds and fresh leaves may be used as a poultice. The antibacterial activity of seeds against staphylococcus and streptococcus as well as common skin fungi, such as athlete's foot fungus has also been well recognized. The oil is also known to maintain high density lipoprotein cholesterol (HDL) and lower low density lipoprotein cholesterol (LDL) refined sesame oil is rich with antioxidant components like lignans allowing for greater shelf-life of foods plus improving their flavour and taste. In addition to its use as an antioxidant sesame oil contains a large amount of linoleate in triglyceride form that selectively inhabit malignant melanoma growth. Off-late, the work has also been oriented towards the production of biodiesel from sesame seed oil as a viable alternative to the diesel fuel.

The ethno-botanical and medicinal uses of this commercially important, nutritionally rich oilseed need to be explored for better utilization. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3,000 years ago. Sesamum has many other species, most being wild and native to sub-Saharan Africa. *S.indicum*, the cultivated type, originated in India. It tolerates drought conditions well, growing where other crops fail. Sesame has one of the highest oil contents of any seed. With a rich, nutty flavour, it is a common ingredient in cuisines around the world. Like other foods, it can trigger allergic reactions in some people.

Seeds are good source of compounds with phenolic functionality including phenols, lignin, lignans, coumarins, tannins, phenolic acids, and flavonoids which are important tn the human diet. These phenolic secondary metabolites comprise of an aromatic benzene ring with one or more hydroxyl groups (e.g., polyphenolics) that can exist as a simple monomeric phenolics (simple phenolics) or a complex polymerized polyphenolic molecule. These molecules generally play a critical role in plant defence mechanisms including pathogen or insect attack, ultraviolet light, and mechanical damage in plants. They also play an important role in human health, protecting against damage induced by reactive oxygen or nitrogen species. Phenolics are classified based upon their carbon skeleton and accordance with the number of phenol units present.

Flavonoids are complex phenolic that can be classified into six subclasses that include flavonoids, flavanones, isoflavanons, anthocyanins, and present principally as glycosylated, esterified and polymerised derivative forms in fruits. Flavanols play an important role as antioxidants; for example, they protect ascorbic acid from autoxidation in juices and which can lead to juices and which can lead to juice discoloration.

Although flavonoids are abundant in fruit or beverages can be a significant source of dietary flavonoids, levels will vary depending on the varieties, environmental conditions, soil, and climatic factors. Berries are a good source of quercetin and its derivatives whereas the most abundant dietary flavanone glycoside is present in citrus fruits.

Polyphenols are compounds with a di- or trihydroxy phenyl group that are directly involved in the stabilization of reactive species by donating a hydrogen atom or transferring an electron [Quideau *et al*, 2011]. Moreover, these phenolic compounds can be mainly found in the coats (hull, husk, or skin, for instance) covering the cotyledon(s) of seeds such as reported for lentil, pea, beans, peanuts, and pistachio [Singh *et a*l, 2017; Arjeh *et al*, 2020; Lorenzo *et al*, 2018] and can be separated from the seed matrix by extraction with appropriate solvent [Moizer *et al*, 2016]. It is also relevant to mention that some natural extracts rich in polyphenols (such as those obtained from Rosmarinus officinalis L., grape seed and skin, and olive pulp) are generally recognized as safe (GRAS) can be applied in food as antioxidants [Oswell *et al*, 2018; Zhang *et al*, 2015].

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms. Antioxidants are man – made or natural substances that may prevent or delay some types of cell damage. In recent years much attention has been devoted to natural antioxidant and their association with health benefits. Plants are potential sources of natural antioxidants and produce various anti oxidative compounds that have therapeutic potentials. Antioxidant – based drug formulations are used for the prevention and treatment of many complex diseases.

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Oxidative stress occurs due to oxidative biochemical reactions taken place in the cells or organ. During metabolic reactions, aerobic cells produce reactive oxygen species (ROS) such as superoxide anion (O2-), hydrogen peroxide (H2O2), hydroxyl radical (OH), and organic peroxides as normal products of the biological reduction of molecular oxygen (Sharma *et al*, 2010). The disparity between the amount of ROS and antioxidant substances leads to damage of important bio-molecules and membrane system of the cells which creates severe impact on the whole organism. The oxidation of lipid, DNA, protein, carbohydrate and other biological molecules by toxic ROS may cause DNA mutation or/and serve to damage target cells or tissues; and this often results in cell senescence and death. These ultimately leads to several chronic and degenerative diseases including gastritis, reperfusion injury of many tissues, atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases etc.

About the Plants

Pumpkin seed (*Cucurbita maxima* Duch.)

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Cucurbitales
Family	:	Cucurbitaceae
Genus	:	*Cucurbita*
Species	:	*maxima*

Pumpkin (*Cucurbita maxima* Duch; Family-Cucurbitaceae) is one of the most popular vegetables worldwide. The nutrition value of pumpkin seed is familiar. The major nutritionally related components of pumpkin seeds are proteins (30–51 %) and oils (up to 40 %). They are also rich in carbohydrates and microelements. The seed extract contains triglycerides with palmitic, stearic, oleic, and linoleic acid as the dominant fatty acids. Other important components present in the pumpkin oils are tocopherols, sterols, phospholipids, and hydrocarbons. Animal studies have shown that pumpkin, pumpkin seeds, pumpkin seed powder, and pumpkin juice can reduce blood sugar.

Cucurbita maxima, commonly referred to as winter squash, is a warm-season annual vegetable vine that trails along the ground or climbs up structures using tendrils. It has yellow fruit-bearing flowers that are produced in the fall. Pumpkin seeds are a good source of healthful oils, magnesium, and other nutrients that enhance the health of the heart, bones, and other functions. Seeds, in general, are considered excellent source of potassium, magnesium, and calcium. Plant seeds are also a good source of polyunsaturated fatty acids (PUFAs) and antioxidants. The fatty acids in pumpkin seeds contain a range of beneficial nutrients, such as sterols, squalene, and tocopherols. Researchers have described the fatty acid profile of seeds, grains, and legumes as "favourable".

Sesame seed (*Sesamum indicum* L.)

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Pedaliaceae
Genus	:	*Sesamum*
Species	:	*indicum*

Sesame (*Sesamum indicum* L.) is a flowering plant in the genus Sesamum, also called benne. Numerous wild relatives occur in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3,000 years ago. Sesamum has many other species, most being wild and native to sub-Saharan Africa. *S. indicum*, the cultivated type, originated in India. It tolerates drought conditions well, growing where other crops fail. Sesame has one of the highest oil contents of any seed. With a rich, nutty flavour, it is a common ingredient in cuisines around the world. Like other foods, it can trigger allergic reactions in some people.

It tolerates drought conditions well, growing where other crops fail. Sesame has one of the highest oil contents of any seed, with a rich, nutty flavour, it is a common ingredient in cuisines around the world. Sesame seeds contain the lignans sesamolin, sesamin, pinoresinol, and lariciresinol. The by-product that remains after oil extraction from sesame seeds, also called sesame oil meal, is rich in protein and is used as feed for poultry and livestock. Sesame seeds contain significant amount of phytic acid, which is considered as antinutrient in that it binds to certain nutritional elements consumed at the same time, especially minerals, and prevent their absorption by carrying them along as they pass through the small intestine. Sesame seeds are also an important source of dietary fiber and micronutrients such as minerals, lignans, tocopherols, and phytosterols.

Sunflower seed (*Helianthus annuus* L.)

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Asterales
Family	:	Asteraceae
Genus	:	*Helianthus*
Species	:	*annuus*

Sunflower seeds are technically the fruits of the sunflower plant (*Helianthus annuus* L.). Helianthus is a genus comprising of about 70 species of annual and perennial flowering plants in the daisy family Asteraceae commonly known as sunflowers. The seeds are harvested from the plant's large flower heads, which can measure more than 12 inches (30.5 cm) in diameter. There are two main types of sunflower crops. One type is grown for the seeds you eat, while the other which is the majority farmed is grown for the oil. The sunflower seeds you eat are encased in inedible black-and-white striped shells, also called hulls. Those used for extracting sunflower oil have solid black shells. sunflower seeds have a mild, nutty flavour and a firm but tender texture. They're often roasted to enhance the flavour, though you can also buy them raw.

Sunflower seeds are more commonly eaten as snack than as part of a meal. They can also be used as garnishes or ingredients in various recipes. The seed can also be sprouted and eaten in salads. The sunflower seeds are rich in oleic and linoleic acid and low in saturated fats and sodium. They also contain magnesium, potassium and fibre. They help to lower blood pressure and serum cholesterol levels. Sunflower seeds are a good or excellent source of nearly a dozen essential vitamins and minerals, two of them being zinc and selenium. Zinc is an integral part of the immune system, as it helps both to develop and maintain proper function of immune cells. Additionally, zinc functions as an antioxidant to fight off free radicals. Selenium also plays a role in fighting inflammation and infection, along with boosting immunity, to ensure our bodies are producing a proper response to any intruders in the body. This mineral is an important part of achieving mental health and preventing neurodegenerative disorders.

Chia seed (*Salvia hispanica* L.)

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Lamiaceae
Genus	:	*Salvia*
Specie	:	*hispanica*

Salvia hispanica, commonly known as chia is a species of flowering plant in the mint family, Lamiaceae, native to central and southern Mexico and Guatemala. It is considered as pseudo cereal, cultivated for its edible, hydrophilic chia seed, grown and commonly used as food in several countries of western South America, western Mexico, and the south-western United States. Chia is an annual herb growing up to 1.75 metres (5 feet 9 inches) tall, with opposite leaves that are 4–8 cm long and 3–5 cm wide. Its flowers are purple or white and are produced in numerous clusters in a spike at the end of each stem. The seeds are small ovals with a diameter around 1 mm. They are mottle-coloured, with brown, grey, black, and white. The seeds are hydrophilic, absorbing up to 12 times their weight in liquid when soaked. While soaking, the seeds develop a mucilaginous coating that gives chia-based beverages a distinctive gelatinous texture.

The seeds are hygroscopic, absorbing up to 12 times their weight in liquid when soaked and developing a mucilaginous coating that gives chia-based foods and beverages a distinctive gel texture. Chia seeds may be added to other foods as a topping or put into smoothies, breakfast cereals, energy bars, granola bars, yogurt, tortillas, and bread. They also may be made into a gelatin-like substance or consumed raw. The gel from ground seeds may be used in place of eggs in cakes while providing other nutrients, and is a common substitute in vegan and allergen-free baking. Unlike flax seeds, whole chia seeds do not need to be ground because the seed coat is delicate and readily digested, possibly improving nutrient bioavailability. The fatty acids of chia seed oil are mainly unsaturated, with linoleic acid (17–26% of total fat) and linolenic acid (50–57%) as the major fats.

Flax seed (*Linum usitatissimum* L.)

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Malpighiales
Family	:	Linaceae
Genus	:	*Linum*
Species	:	*usitatissimum*

Linum usitatissimum L. in the family Linaceae. It is also known as common flax or linseed, is a flowering plant. It is cultivated as a food and fibre crop in the region of the world with temperate climates. Textiles made from flax are known in Western countries as linen. The flax seeds have a high amount of soluble fiber. The fiber flax is harvested before the seed is mature, when the quality of fiber is best. The seed at this stage does produce an oil of good quality. Flax seeds are very high in fiber and provide good amounts of protein. They're also rich in fat and one of the best plant-based sources of heart-healthy omega-3 fatty acids. Flax seeds contain 42% fat, with 1 tablespoon (10 grams) providing 4.3 grams. This fat content is composed of 73% polyunsaturated fatty acids, such as omega-6 fatty acids and the omega-3 fatty acid alpha-linolenic acid (ALA) 27% monounsaturated and saturated fatty acids. Flax seeds are one of the richest dietary sources of ALA. In fact, they're only exceeded by chia seeds. ALA is an essential fatty acid, which means that our body cannot produce it. Thus, we need to obtain it from the food you eat.

Significance of the study

There is, nowadays, an ever-growing interest for a natural alternative in exchange for all that is obtained by chemical synthesis. Natural substances with antioxidant properties acquire an increasingly wider application in the food industry, cosmetics, and pharmaceutical industry as effective remedies in counteracting the damaging action of free radicals and in stopping pro-oxidant processes that can cause various pathological states. The use of synthetic antioxidants is increasingly restricted because of their suspected activity of carcinogenesis promoters, as well as because of the consumers' rejection of the synthetic food additives. Recently consumers prefer ingredients of natural origin, which can be extracted from plants, food by-products, and other natural sources. Thus, the administration of natural antioxidants is of paramount importance with strong positive perspectives in maintaining the human body's normal redox status.

Wild flora is an inexhaustible source of bioactive compound, with a main contribution in nutrition and health. As a result, many plant species have been investigated in searching for novel antioxidants and other bioactive compounds. Antioxidants have attracted more and more attention as potential agents for preventing and treating oxidative stress-related diseases. Natural antioxidants are expected to be an alternative to the synthetic ones because of their potential health benefits. Seeds may have the potential to confer beneficial health effects due to their antioxidant activity and the total phenolic compounds and flavonoids.

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Seeds are important sources of dietary fibre, nutrients and antioxidants. Among dietary plant products, sunflower seeds are characterized by high antioxidant potential. We have many seeds which are edible, and can be used as medicines. The aim of this study is to determine and compare the nutrients, total phenolic compounds, total flavonoids content, and the antioxidant capacity of five seeds which are regarded as super foods because of their high nutritional quality.

2. AIMS AND OBJECTIVES

AIMS

The aim of the present study is to compare and evaluate the proximate composition and antioxidant potential of five seeds of plants such as *Salvia hispanica L., Linum usitatissimum L., Cucurbita maxima Duch., Sesamum indicum L., and Helianthus annuus L.*

OBJECTIVES

- Collection of selected experimental seeds
- Preparation of seed extracts
- Analysis of Proximate composition (moisture, ash, crudefibre, starch, carbohydrate, protein and lipid)
- Determination of Nutritive value
- Qualitative analysis of phytochemical constituents in seeds
- Quantitative analysis of Total Phenol Content (TPC) in seeds
- Estimation of Flavonoid content in Seeds
- To determine the antioxidant properties of seed extracts by invitro antioxidant assay

3. REVIEW OF LITERATURE

All biological processes in an organism must remain in homeostasis. When the pro-oxidant load and antioxidant defence are unbalanced, reactive oxygen species (ROS) are produced, and free radicals are generated [Zamora *et al*, 2018]. Oxidative stress is characterised by the amount of ROS produced and is closely related to development of some diseases such as cancer caused by oxidative lesions in DNA. However, there are other mechanisms that protect organisms against oxidation, including good nutrition (Zamora *et al*, 2018). Thus, the interest in finding compounds with antioxidant activity such as flavonoids has increased. Among them, apigenin (a plant-derived food polyphenol, with sources such as chamomile tea and celery) seems to have strong antioxidant activity in neurological disorders (Saha *et al*, 2017).

Phenolic compounds occur in all seeds as a diverse group of secondary metabolites. Hence, they are a component of the human diet although data for dietary intakes and metabolic fate are limited. Their role in oxidation processes, as either antioxidants or substrates in browning reactions, is examined. They are characterised by high chemical reactivity and this complicates their analysis.

Phenolic compounds are one of the most important classes of phytochemicals with both functional and health promoting properties. Fruits are the excellent sources of these compounds, and improved methodology for the extraction, isolation, separation, identification, and quantification of the full range of the phenolic content of fruits is critical for understanding the health potential of various fruits, as well as good sources for these compounds. (Ali *et al*, 2010)

Phenolic compounds are a group of secondary plant metabolites, many with health-promoting properties that are present in all parts of plants. They have an aromatic structure, including either one or more hydroxyl groups giving them the ability to stabilize free radicals and protect biological tissues against damage related to reactive oxygen species. (Samman *et al,* 1996). Phenolic compounds are concentrated in the fruit of plants, and therefore, the fruit can be an important dietary source of these phytochemicals, which exist as monomers, or bound to one another. (Das, 2012)

Polyphenolic compounds are classified into different subclasses based upon the number of phenol ring systems that they contain, saturation, and length of the carbon chain that bind the rings to one another. The phenolic acids present in fruit tissues protect the plant against disease, infections, UV radiation, and insect damage. For this reason, the beneficiary effects of phenolic compounds are continually being investigated for their health-promoting properties and for meeting increased consumer demand for healthy nutritious food (Kahkonen, 1999). Due to the functional properties of polyphenolic compounds, there is increased interest on improving extraction, separation, and quantification techniques of these valuable bioactive compounds, so they can be used as value-added ingredients in foods, pharmaceuticals, and cosmetics.

Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects. These molecules are found in a variety of fruits, seeds and vegetables. Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water. (Reis, 2013). They consist of two benzene rings connected by a short three carbon chain. One of the carbons in this chain is connected to a carbon in one of the benzene rings, either through an oxygen bridge or directly, which gives a third middle ring (Rahman, 2015). The flavonoids can be divided into six major subtypes, which include chalcones, flavones, isoflavonoids, flavanones, anthoxanthins and anthocyanins. Many of these molecules, particularly the anthoxanthins give rise to the yellow color of some petals, while anthocyanins are often responsible for the red color of buds and the purple-red color of autumn leaves.

The pumpkin seeds (*C.maxima* Duch.) from the Cucurbitaceae family are usually considered as industrial waste products and thrown out. In some cases, the seeds are utilized as uncooked, Cooked, or roasted, although simply for domestic purpose.

The nutritional and therapeutic importance of pumpkin seed was studied by Syed et al in 2019.The study describes the seeds are considered a by-product of the pumpkin fruit, they are cheaper and their utilization in different food products may lead to enhancement in their nutritional value at a lower cost. Health benefits of pumpkin seed was studied by Dan Brennan, (2020).

Pumpkin seeds protect against formaldehyde induced major organ damages (Mollika *et al,* 2002). In addition to nutrient composition, it is composed of various biologically active components such as polysaccharides, protein, peptide, sterols, and para-amino benzoic acids by (Adams *et al.,* 2011).

Yadav *et al* (2010) studied the medicinal and biological potential of pumpkin. In this study they found that the pumpkin is one of the famous edible plants that is utilized for many disorders due to the occurrence of many edible components and phytochemicals.

The potential of pumpkin seeds as a functional food ingredient (Joachim and James, 2020). Arunima & Vivek (2021) studies the nutritional, phytochemical, and antimicrobial attributes of seeds and kernels of different pumpkin cultivars. They found that the seeds of pumpkin are a nutritional treasure as they are a robust source of good quality fat, protein, fibers, antioxidants, and other phytochemical compounds.

A study was done by Maria *et al,* (2021), and it explains the nutritional value, phytochemical potential, and therapeutic benefits of pumpkin. In this study they explained the pumpkin seed is used as a medicine for anti-inflammatory, antioxidant, antiviral, and antidiabetic properties. Glycaemic response and antioxidant activity of Pumpkin seed powder Blended biscuits was studied by (Malkanthi *et al*, 2018). The present study aimed to evaluate glycemic response and antioxidant activity of pumpkin seed powder (Cucurbita maxima) blended biscuits.

Chia seeds contain healthy ω-3 fatty acids, polyunsaturated fatty acids, dietary fiber, proteins, vitamins, and some minerals. Besides this, the seeds are an excellent source of polyphenols and antioxidants, such as caffeic acid, rosmarinic acid, quercetin, and others. (Masa *et al*, 2019)

Yingbin *et al* (2018) studied the phytochemical and biological characteristics of mexican chia seed oil. The purpose of this research was to investigate the chemical profile, nutritional quality, antioxidant and hypolipidemic effects of the oil.

A study conducted by Bruna *et al (*2017) reveals that the chia seeds are also rich in polyphenols derived from caffeic acid that are antioxidant compounds, protecting the body from free radicals, aging and cancer.

Katunzi-Kilewela *et al*, (2021) studied on the nutritional, health benefits and usage of chia seeds. This article reviews the nutritional content, bioactive compounds, and nutraceutical functionality of chia seeds and their use in the development of functional foods.

Chemical characterisation of chia for use in food products was studied by Silveira *et al*, (2014). Many of its newly developed functional foods contain bioactive compounds including dietary fiber, antioxidants and other substances. Chia seeds contain high levels of lipids (34.4%) and are rich in Omega-3, Omega-6 and Omega-9, which constituted 62, 17.4 and 10.5% of the total lipids, respectively. Chia seed also contain fibers (23.7%) and proteins (19.6%). Their extracted phenolic compounds (32.35 µgGAE. mLextract-1) showed antioxidant activity.

Nutritional, phytochemical and therapeutic potential of chia seed was studied by Zia-ud Din *et al* (2021). This study provides an overview for the potential applications of chia seed in various sectors of health and nutrition. Dietary fibers and high amount of phytochemicals could be an effective health promoting factors. Besides the nutritional advantages of chia seed, good amount of phytochemicals can play anti-oxidant role resulting in improving the health of an individual.

Avilene *et al.* (2021) studied the Assessment of the Phytochemical and Nutrimental Composition of Dark Chia Seed. In this study, comprehensive characterization of extractable and non-extractable phenolic compounds of dark chia seed *Salvia hispanica* L. were studied using high-performance liquid chromatography, electrospray ionization, quadrupole time-of-flight (HPLC-ESI-QTOF) and discuss potential health benefits associated with the presence of a number of nutritional and bioactive compounds.

Improvement of chia seeds with antioxidant activity, essential amino acids, and dietary fiber by controlled germination bioprocess was conducted by Mario *et al* (2017). The purpose of this study was to obtain functional flour from germinated chia seeds under optimized conditions with increased antioxidant activity, phenolic compounds, essential amino acids, and dietary fiber with respect to un-germinated chia seeds.

A study was conducted by Kvetoslava *et al (2016)*, on Characterization of protein fractions and antioxidant activity of chia seeds. In this study they explained the potential alternative source of high-quality protein, fats, carbohydrates, high dietary fibre, vitamins and mineral elements.

Effect of addition of flaxseed flour on phytochemical, physicochemical, nutritional, and textural properties of cookies was studies by Parvinder *et al* in 2019. The impact of the study was to explain the nutritional, physiochemical, phytochemical (total phenolic content and total flavonoids content) properties of flax seed.

Tawheed and Thakur made a comparative study on proximate composition, phytochemical screening, antioxidant and antimicrobial activities of *Linum usitatisimum* L.(flaxseeds) and was published in 2014. In this study they explained that the seeds are excellent nutritional package with their gold mine of healthy minerals. niacin and folic acid contents.

A study on phytochemicals and uses of flaxseed, was conducted by Madiha *et al* (2018). The study provides extraction methods and latest fractionation techniques for isolation of bioactive compounds of essential oils obtained from flaxseeds.

Variations in oil and some phytochemical contents in flaxseed cultivars was reported by Hossom *et al* (2011). In this study they observed that flaxseeds, containing about 36 to 40% of oil, are the richest (among crop plants) source of polyunsaturated fatty acids (PUFA) essential in the human diet. PUFA are highly susceptible to oxidation.

Flaxseed is a functional food source (Dave, 2001). The implications of diets containing flaxseed or its components for human nutrition and disease prevention are analysed in this paper. Results of the first meta-analysis examining the relationship between intake of flaxseed or its components and risk reduction of disease in humans is presented.

Parvinder *et al* (2018) reported the recent advances in utilization of flaxseed as potential source for value addition. The studies found that flaxseed carries functional ingredients and provide health benefits.

Effect of incorporation of flaxseed to wheat rusks: Antioxidant, nutritional, sensory characteristics, and in vitro DNA damage protection activity was studied by Rajwinder et al (2018). The objective of this study was to evaluate the nutritional value and acceptance of rusks prepared from three flaxseed cultivars (LC-2023, Jawahar-27, Jeevan) containing four different concentrations of flaxseed powder (FP) as partial replacement for wheat flour.

The study conducted by Dilipkumar (2011) explained the role of sunflower seeds in health and nutrition. The study explains the potential usages of sunflower seeds in health and nutrition. It also reveals that sunflower seed preparations have found widespread use in traditional medicine. Sunflower seed possesses high food and nutritive value, providing a good source of healthy unsaturated fats, proteins, nutrients, and phytochemicals.

Nutritional and therapeutic potential of sunflower seeds was studied by Faquir *et al.,* (2012). The purpose of this paper is to provide a comprehensive overview of multiple functions of sunflower seeds including their nutritional and nutraceutical benefits.

Hip Seng *et al* (2013) conducted the study on the Antioxidant potential of *Pleurotus porrigens* extract and application in sunflower oil during accelerated storage. The aim of the study is to evaluate the total phenolic content (TPC), 2, 2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging and ferric reducing/antioxidant power (FRAP) were also evaluated. P. porrigens' SFIII exhibited highest DPPH scavenging, while n-BUT showed highest FRAP; TPC was found highest in crude extract.

Adeleke and Babalola (2013) explained oilseed crop sunflower as a source of food. From this work they reported sunflower as an important oilseed crop native to South America and currently cultivated throughout the world. Also, they found that sunflower contains mineral elements and phytochemicals such as dietary fiber, manganese, vitamins, tocopherols, phytosterols, triterpene glycosides, α-tocopherol, glutathione reductase, flavonoids, phenolic acids, carotenoids, peptides, chlorogenic acid, caffeic acid, alkaloids, tannins, and saponins; and these compounds contribute to their functional and nutraceutical development.

In vitro phytochemical investigation of *H. annus* seeds was conducted by Rubab *et al.*, (2016). They explained that the seeds of *H. annus* contain various chemical components and biological activities with special emphasis to the anti-oxidant, antimicrobial, cytotoxic and thrombolytic assay. The results of standard phytochemical analysis revealed the presence of tannins, saponins, flavonoids, carbohydrates, steroids, fixed oils and vitamins.

Sunflower petals were found to have triterpene glycosides, which have anti-inflammatory activity (Ukiya *et al.*, 2007). Rafael and Manuel (1991) studied *in vitro* oleate desaturase in developing sunflower seeds. The *in vitro* oleoyl phosphatidylcholine desaturase (ODS) activity in developing seeds of normal and high oleic sunflower lines has been studied.

Isolation of phenolic constituents and characterization of antioxidant markers from sunflower seed extract (Yoshiaki *et al*.,2013). They explained the antioxidative effect of the phenolic constituents from the sunflower seeds. The evaluation was based on the oxygen-radical absorbance capacity (ORAC), and the fraction containing caffeic acid derivatives showed a high antioxidant potency.

Antioxidant, phytochemical and physicochemical properties of sesame seed studied by Emmanuel *et al., (*2020). The aim of this study was to examine the antioxidant, phytochemical and physicochemical properties of sesame seeds grown in Ghana. They evaluated that the sesame seeds possess significant antioxidant activity. They also contain significant amounts of phenolics, flavonoids, nutrients and minerals. This suggests that dietary uptake of sesame seeds could be potentially protective against diverse diseases.

The study conducted by Lin *et al* (2016) explains the phytochemical contents, antioxidant and antiproliferative activities of selected black and white sesame seeds. The study reported the phytochemical profiles and the antioxidant and antiproliferative activities of six varieties of sesame seeds.

Phytochemical Profile and Antioxidant Activity of Sesame Seed by-products for Stability and Shelf-Life Improvement of Refined Olive Oil was studied by Mohamed *et al*., (2022). This study was designed to evaluate the effect of a natural antioxidant on the quality of refined olive oil (ROO) stored at 60 ± 1 °C for up to 48 days.

Senouwa *et al., (2018)* studied metabolome profiling of different coloured sesame seeds which provides new insight into their antioxidant activities. From this study they found that the pharmacological proprieties of black seeds might be related to their high content of flavonoids and essential amino acids. These findings expand information on phytochemical composition of different colored sesame seeds and provide resources for their comprehensive use and quality improvement.

Selin and Elhussein (2018) conducted the study on Assessment of sesame cake as a source of high-added value substances. This study aims to present pharmacological and therapeutic effects of sesame cake extract by pointing out its application in pharmaceutical, cosmetic and food industries.

Alege *et al*. (2014) developed the Phytochemical studies and genetic diversity in sesame. The results revealed high genetic diversity among the 23 sesame genotypes showed that the phenolic contents had negative correlation with tannin while flavonoid had positive correlation with saponin and alkaloid.

4. MATERIALS AND METHODS

4.1: Extract preparation of seeds

a. Solvents used

Organic solvents such as Acetone, Chloroform, distilled water and Ethanol were employed for the extraction of different bioactive compounds.

b. Extraction

In this study, the plant metabolites were extracted using cold extraction (Percolation). About 8g of the cleaned, fresh sample was transferred into clean screw cap bottles of 50ml capacity. 40ml of various solvents were added and soaked it for a week separately

4.2: Preliminary Phytochemical analysis

The extract using different solvents were screened for the qualitative analysis of different classes of natural compounds, using the methodology of *Sofowora* (1982) and Kepem (1986). The major pharmaceutically valuable compounds, investigated by the present studies were:

4.2.1: Detection of Alkaloids

Extracts were dissolved individually in dil.HCI and filtered and then subjected to the following tests.

a) Hager's Test- Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

b) Dragendoff's Test- Filtrates were treated with I ml of Dragendoff's reagent. Formation of reddish orange precipitation indicated the presence of alkaloids.

4.2.2: Detection of Carboxylic acids

1 ml of each of various extracts was separately treated with a few ml of saturated solutions of sodium bicarbonate. Observation of effervescence (due to liberation of CO_2) indicated the presence of carboxylic acids.

4.2.3: Detection of Coumarins

1 ml each of alcoholic extracts was treated with alcoholic 10% NAOH solution. Production of dark yellow colour indicated the presence of Coumarins.

4.2.4: Detection of flavonoids

a) Alkaline reagent Test - Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on addition of dilute acid indicates the presence of flavonoids.

b) Lead acetate Test - Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitates indicate the presence of flavonoids.

2.5 ml of each of the various extracts were separately dissolved in 1 ml each of alcohol (stock solution) and subjected to the following test.

c) Ferric chloride Test- I ml each of stock solution was added with a few drops of neutral $FeCl_3$, solution, Formation of blackish red indicated the presence of flavonoids.

4.2.5: Detection of Phenols

a) The extract (0.5 ml) was dissolved in 5 ml of distilled water. To this, a few drops of neutral ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

b) Ferric chloride Test - A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

4.2.6: Detection of protein and amino acids

5 ml each of the various extracts were dissolved in 5 ml of water separately and were subjected to the following tests.

a) Biuret test - 1 ml of each of the various extracts was warmed gently with 10% NaOH solution and a drop of diluted $CuSO_4$, solution. Formation of reddish violet colour indicated the presence of proteins and amino acids

b) Ninhydrin test- 1 ml each of the various extracts was separately treated with a few drops of ninhydrin solution, Change in colour showed the presence of proteins and amino acids.

4.2.7: <u>Detection of Quinones</u>

a) 1 ml of the various extracts was separately treated with alcoholic KOH solution. Quinones give colouration ranging from red to blue

b) A small amount of extracts was treated with con. HCl and observed for the formation of yellow precipitation (or colouration)

4.2.8: <u>Detection of Resins</u>

One ml of various extracts was diluted with water. Formation of bulk black precipitate indicates the presence of resins.

4.2.9: <u>Detection of Saponins</u>

a) Froth Test - Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of Saponins.

b) Foam Test - 0.5 gm of extracts was shaken with 2 ml of water. If foam produced persists for few minutes indicates the presence of Saponins.

4.2.10: <u>Detection of Steroids and Phytosterols</u>

5 ml of each of various extracts were dissolved in 5 ml of chloroform separately (stock solution) and was subjected to the following test.

a) Salkowski Test - 1 ml each of conc. H_2SO_4 was added to the stock solution and allowed to stand for 5minutes after shaking. Turning of golden yellow colour in the lower layer indicated the presence of steroids and Phytosterols.

4.2.11: <u>Detection of Tannins</u>

a) Gelatin Test - Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

5 ml each of the various extracts was dissolved in minimum amount of water separately filtered add filtrate were then subjected to the following test.

i) Ferric chloride Test- To the above filtrate a few drops of $FeCl_2$ solution were added. The colour change indicates the presence of tannins

ii) Basic lead acetate test- To the filtrate a few drops of aqueous basic lead acetate solution are added. Formation of reddish brown precipitate indicated the presence of tannins.

4.2.12: Detection of Xanthoprotein

1ml of various extracts was treated separately with a few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitation indicated the presence of xanthoprotein.

4.2.13: Detection of Terpenoids

An amount of 0.8 g of selected plant samples was taken in a test tube. Then poured 10 ml of ethanol in it, shaken well and filtered to take 5 ml extract. Then add 2 ml of chloroform were mixed in exact and 3 ml of sulphuric acid were added in the extract. Formation of reddish

4.2.14: Detection of Glycosides

a) Keller Killani Test - Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops 26 solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of conc. H_2SO_4 A brown ring at the interphase indicates the presence of cardiac glycosides.

b) Bromine water Test - Test solution was dissolved in bromine water and observed for the formation of yellow precipitates to show a positive result of the presence of glycosides.

4.2.15: Detection of Lignins

Phosphoglucinol with con.HCI was added with the test solution. Formation of pink colour indicates the presence of lignins.

4.2.16: Detection of Carotenoids

1g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% H_2SO_4 was added. A blue colour at the interphase showed the presence of carotenoids.

4.2.17: Detection of Phlobatannins

Powdered plant sample was mixed with distilled water in a test tube. Then shake it well, and filtered to take plant extract. Then to each plant extract 1% aqueous hydrochloric acid was added and then boiled with the help of hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

4.2.18: <u>Detection of Diterpenes</u>

a) Copper acetate test - Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of Diterpenes.

4.2.19: <u>Detection of Sugars</u>

5 ml each of the various extracts was dissolved separately in distilled water filtered and then subjected to the following tests.

a) Molisch's Test- To the filtrate a few drops of alcoholic alpha- naphthol and 2 ml of conc. H_2SO_4 were added slowly through the sides of the test tube. Formation of reddish brown precipitate indicated the presence of sugars.

b) Fehling's Test - A small portion of the various filtrate were treated with 1 ml of Fehling's solution 1 and 2 and then heated gently, Change in colour indicated the presence of sugars.

4.3: <u>Proximate and Nutritive Analysis of Seeds</u>

4.3.1: <u>Determination of Moisture Content</u>

The moisture content was determined according to AOAC method. The sample was taken in a flat bottom dish (pre-weighed); kept overnight in an oven at 100°C and weighed. The loss in weight was regarded as a measure of moisture content.

4.3.2: <u>Determination of Crude fiber</u> (Fibre – Muslin cloth method)

For determination of Crude fiber, 2 g material were treated with 200ml of 1 25% H2SO4 with 30 min boiling. After filtration and washing, the residue was treated with 1,25% NaOH with 30 min boiling, then filtered, washed with hot distilled water. The residue was dried overnight at 80-100 °C and weighed (m_1). It was then ignited and the ash weighed (m_2). Loss in the weight gives the weight of crude fiber calculated using the formula.

Crude fibre (%) = $(100 \times m_1 - m_2) / m_0$, where m_1 is the weight of ashing dish with content before ashing, m_2 is the weight of ashing dish with content after ashing, m_0 is the weight of sample.

4.3.3: <u>Estimation of Starch</u> (Sedimentation method)

Starch content in seeds was estimated by sedimentation method. A 5 gm sample was ground with 100 ml distilled water using a mortar and pestle. The mixture was separated through a cheese cloth and again added 50 ml distilled water. The filtrate was allowed to stand at overnight. After that starch was settled out. The filtrate was decanted off. So, the starch was left in the beaker. After that, 100 ml water added to rinse the starch. The process was repeated where the water was decanted off again. Lastly the wet starch was sundried to get a white powder. The percentage yield of isolated starches was determined using equation.

Starch%= (Sample weight- final sample weight) / (Sample weight)) x 100.

4.3.4: <u>Estimation of Protein by Lowry's Method</u> (Lowry *et al*, 1951)

1g of the sample was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer. Centrifuge it and used the supernatant for protein estimation. Pipetted out 0.2-1.0 ml of the working standard into a series of test tubes. Pipetted out 0.5 ml of the samples extracts in another test tube. Made up the volume to I ml in all the test tubes. A tube with 1 ml of water serves as the blank. Added 5 ml of reagent C (Alkaline copper solution) to each tube including the blank. Mixed well and allowed to stand for 10 min. Then added 0.5 ml of reagent D (Folin- Ciocalteau Reagent) mixed well and incubated at room temperature for 30 min. Blue colour was developed and read the colour at 660nm.

4.3.5: <u>Estimation of Total Carbohydrate content</u> (Hedge & Hofreiter, 1962)

The carbohydrate content was detected by Anthrone method. Take 1g of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCI and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceases. Made up the volume to 100 ml and centrifuged, collected the supernatant and take 0.5 ml for analysis. Prepared the standard by taking 0.2 -1.0 ml of the working standards, 1.0 ml of water serves as blank and made up the volume to 1.0 ml in all the tubes with distilled water, then added 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630nm.

4.3.6: <u>Estimation of Lipids</u> (Folch *et al*, 1957)

Estimation of total lipid content in the sample was carried out using the method of Folch et al. (1957). Weighed 2g of the sample into a wide mouthed boiling tube and added 20ml of ethanol: diethyl ether (3:1) mixture to this and stirred well. Then it was kept in a thermostatic water bath for 2 hours at 550 C and cooled. The contents were then centrifuged at 3000rpm for 10 minutes and decanted the clear supernatant to a pre-weighted petri dish. The pellet was collected into the boiling tube, added 20ml of ethanol: diethyl ether mixture and again extracted for 2 hours. Centrifuged the contents and supernatant was decanted to the same Petri dish. Added 20ml of chloroform: methanol (1:1) mixture to the residue and extracted for 1 hour at 500 C. Centrifuged and decanted the supernatant to the same petri dish was recorded. The quantification of total lipid content was carried out by reducing the weight of Petri dish before extraction (W 1) from weight of petri dish after extraction (W 2).

Total lipid (%) = (W 2 – W 1) x 100

4.3.7: Determination of Nutritive value

The nutritional value of seed was calculated as per the formula used by Nile and Khobragade (2009).

Nutritive value= (4 x percentage of protein) + (9 x percentage of fat) + (4 x percentage of carbohydrate)

4.4: Antioxidant potential of Seeds

4.4.1: Determination of Total Phenolic content (Slinkard & Singleton, 1977)

The total phenolic content of extracts was determined using the Folin-Ciocalteau's Phenol reagent. Pipetted out 0.5ml of sample into test tubes. Made up volume in each test tube to 3ml with distilled water. Add 0.5ml of Folin-Ciocaltueau reagent. After 3 minutes, added 2ml of 20% sodium carbonate solution to each test tube. Mixed thoroughly. Place the tubes in boiling water for exactly I minute. Cool and measure the absorbance at 650 nm against a reagent blank. The total phenol content was determined using the standard graph of pyrocatechol.

4.4.2: Determination of Flavonoid content (Boham and Kocipai, 1974)

2.5gm of powdered sample was mixed with 80% of aqueous methanol and let it kept for 24 hrs. Discarded the supernatant, the residue re-extracted three times with same volume of methanol with Whitman filter paper. Sample filtrate was transferred to a pre-weighted Petri dish (**W_1**) and evaporated to dryness over a water bath. The content in the petri dish is cooled and weight of the dry petri dish (**W_2**) was recorded.

Total Flavonoid (%)= ((W_2 - W_1) / Sample weight) *100

4.4.3: DPPH Free Radical Scavenging Assay (Shimada *et.al* 1992).

The DPPH free radical scavenging assay was determined by the method of Shimada et.al (1992). 0.1mM DPPH (2,2-diphenyl -1-picrylhydarzil) was prepared in methanol solution. 0.5g of sample was homogenized using 5ml of methanol and centrifuged the contents. The supernatant was collected, different aliquots (0.5 and 1 ml) were prepared and final volume was made up to 1 ml using methanol. To this mixture added 2ml of 0.1mM DPPH solution (control) and reaction mixtures were measured at 517 nm against methanol as blank. The assay was carried out in triplicates. Lesser values of absorbance of the reaction mixture indicate higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the formula.

DPPH Scavenged (%) = (Absorbance of control – absorbance of test) ÷ (Absorbance of control)) × 100

4.4.5: Reducing Power Assay (Oyaizu,1986).

Antioxidant capacity as per reducing assay was measured according to a method reported by Oyaizu (1986). 1g of sample was extracted in 10ml phosphate buffer, contents were centrifuged and supernatant was collected for the assay. Volumes of 2.5ml of different concentrations of the extract were mixed with 2.5ml phosphate buffer solution (0.2M, pH = 6.6) and 2.5ml of 1% potassium ferric cyanide in test tubes. The mixtures were placed in water bath for 20 minutes at 50^0 C. After that 2.5 ml of 10% trichloro acetic acid (TCA) was added and mixed thoroughly. Then 2.5ml was taken from the mixture and added 2.5 ml of distilled water and 0.5ml of 0.1% $FeCl_2$ solution. The reaction mixture was allowed to stand for 10 minutes. Then the absorbance of the mixture was read at 700 nm against phosphate buffer as blank. The assay was carried out in triplicates. Higher values of absorbance of the reaction mixture indicate greater reducing power. Ascorbic acid standard was used as a positive control.

Figures

Fig 4.1 Extract of Flax

Fig 4.2 Extract of Sesame

Fig 4.3 Extract of Sunflower

Fig 4.4 Extract of Chia

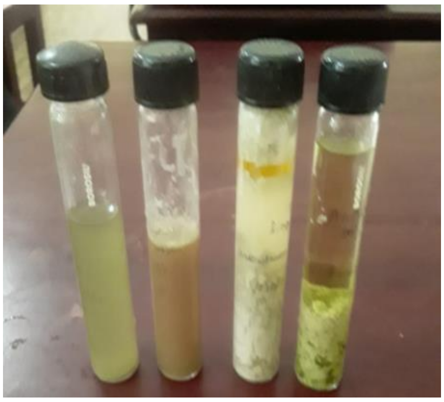

Fig 4.5 Extract of Pumpkin

Fig 4.6: Coumarin

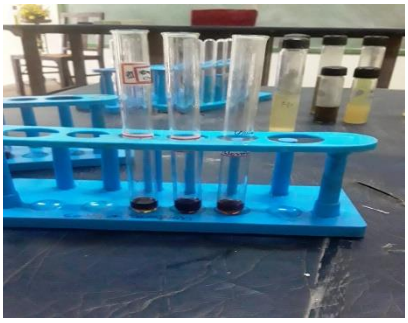

Fig 4.7: Flavonoid

Fig 4.8: Xanthoprotein

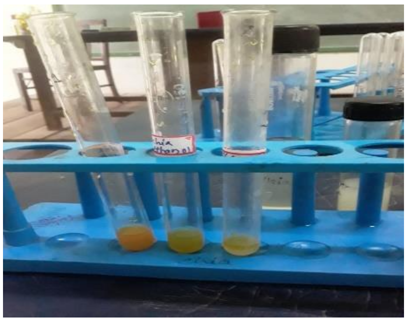

Fig 4.9: Quinone

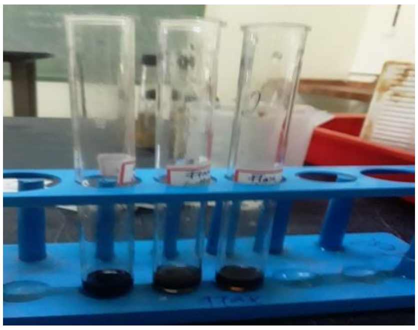

Fig 4.10: Resin

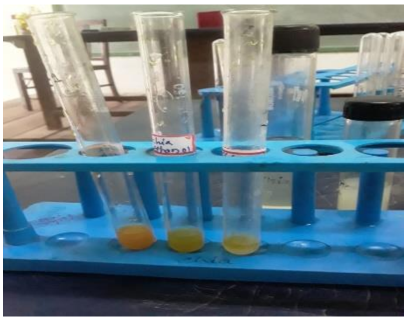

Fig 4.11: Glycosides

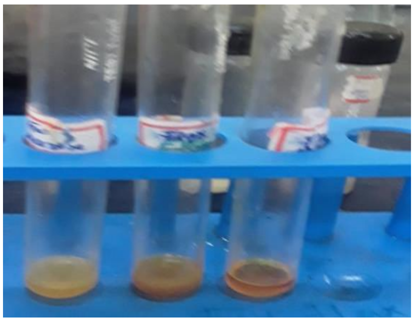

Fig 4.12: Tannin

5. RESULTS AND DISCUSSION

Dried seeds were powdered then the preliminary phytochemical analysis, determination of proximate composition and antioxidant properties were analysed using the above procedures. and the observations were tabulated.

5.1. Phytochemical Analysis of Seeds

The results of phytochemical screening of seeds reveals the presence of some secondary metabolites that may be responsible for the bioactivity. Table 5.1 shows the phytochemical constituents present in seed extracts.

Table 5.1. Preliminary Phytochemical Analysis

SI. No.	**Phytochemicals**	**Chia**	**Flax**	**Pumpkin**	**Sesame**	**Sunflower**
1.	Flavanoid	+	+	+	+	+
2.	Alkaloid	-	-	-	-	-
3.	Terpinoid	+	+	+	+	+
4.	Carotenoid	+	+	+	+	+
5.	Quinones	-	+	-	-	-
6.	Carboxylic acid	+	-	+	-	+
7.	Resin	-	-	-	-	-
8.	Sugar	+	+	-	+	+
9.	Coumarin	+	+	+	+	+
10.	Glycoside	-	+	-	+	-
11.	Saponin	-	+	+	-	+
12.	Diterpene	-	+	-	+	-
13.	Xanthoprotein	+	+	+	-	+

14.	Phenol	-	+	+	+	-
15.	Tannin	-	+	-	+	+
16.	Phytosterol	-	-	-	+	-
17.	Protein	+	+	+	+	+

Phytochemicals are naturally present in the plants and shows biologically significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanism. The preliminary phytochemical analysis of the five seeds showed the presence of Alkaloids, Glycosides, Coumarins, Quinones, Diterpenes, Saponins, Phytosterols, Phenols, Tannins, Carboxylic acid, Xanthoprotein, Proteins, and Sugars

5.2. Proximate analysis of seeds

The proximate analyses of Moisture, ash, crude fibre, starch, carbohydrate, protein and lipid were conducted according to standard analytical procedures.

5.2.1. Determination of Moisture, ash, crude fibre and starch

Moisture content in seeds falls in the range of 3.56 to 5.38% (Table 5.2). Ash content of seeds, which is an indicator for mineral elements was found to be highest in Chia seeds which is closely comparable with ash values of other seeds (Table 5.2). Higher ash content value indicates that it is rich in different mineral sources. Ash is the inorganic residue from the incineration of organic molecule. According to Pomeranz and Clifto (1981) ash contents of seeds and tubers should be in the range of 1.5 to 3.5% for consumption. In this study the ash content of all seeds falls within this range.

Table 5.2: Composition of moisture, ash content, crude fibre and starch

Sl. No.	Seeds	Moisture (%)	Ash (%)	Crude fibre (%)	Starch (%)
1.	Chia seeds	4.11	4.9	24	5.8
2.	Flax seeds	3.56	4.2	22	6.8
3.	Pumpkin seeds	5.18	3.9	15.5	11.2
4.	Sesame seeds	5.24	3.8	12.5	6.7
5.	Sunflower seeds	5.38	4.6	11	7.2

Highest value of crude fibre was found in Chia seeds which had 24.5% crude fibre followed by Flax seed (22.8%). According to Bartosz *et al* (2019) Chia seeds are a good source of fibre and can be recommended for diabetes and people with hypercholesterolaemia. Moreover, they can be as supplement in the daily diet because of the high content of omega-3. Starch content was observed to be greater in pumpkin seed (11.2%) (Table 5.2). Starch content in other seeds ranges from 5.8 to 7.2%.

5.2.2. Nutritional Analysis of seeds: Estimation of total Carbohydrates, Proteins, Lipids and Nutritive Value

The overall composition of major nutrients is presented in Table 5.3.

Table 5.3: Nutritional composition of seeds

Sl. No.	Seeds	Carbohydrates (%)	Protein (%)	Lipid (%)
1.	Chia seeds	38.61	16.98	28.5
2.	Flax seeds	29.78	19.03	32.5
3.	Pumpkin seeds	42.56	23.73	39.1
4.	Sesame seeds	23.18	17.07	40.3
5.	Sunflower seeds	20.64	21.86	43.5

Results of the quantitative analysis revealed that pumpkin contained the highest amount of carbohydrate followed by Chia seed and flax seed. The highest quantity of protein was also found in Pumpkin seed. The quantity of proteins in other seeds ranges from 16.98% to 21.86%. From the results it was found that all the five seeds are good sources of protein. Lipid content was found to be higher in sunflower seed (43.5%) which was followed by sesame (40.3%).

5.1.2.1. Estimation of Nutritive value

The nutritive value of seeds is presented in Table 5.4. Nutritive value was calculated on the basis of quantity of carbohydrate, proteins and lipids present in a material. Greater nutritional values ensure higher acceptability and greater calorific value. It can be considered as the energy obtained from the material when completely utilized. Among the five seeds analyzed, nutritive value was found to be higher in pumpkin seed (611.4 Cal/100g). Nutritive value of sunflower and sesame seeds was found to be 555.9 and 522.09 Cal/100g respectively.

Table 5.4: Nutritive value of seeds

Sl. No.	Seeds	Nutritive Value (Cal/100g)
1.	Chia	469.2
2.	Flax	483.2
3.	Pumpkin	611.4
4.	Sesame	522.09
5.	Sunflower	555.9

5.3. Antioxidant Properties of Seeds

The oxidative stress generated by free radicals is eliminated by antioxidants. Total Phenolic content, total flavonoids, DPPH free radical scavenging activity and reducing power assay were investigated.

5.3.1. Total Phenolic Content (TPC)

Among the seeds analyzed in our study Sunflower had the highest content of total phenols. Total phenolic content in the seed samples is depicted in the figure 5.1. Highest value of total phenolics was found in Sunflower which had 29 mg/g total phenols on dry weight basis. The phenol content in the five seeds ranged from 3.3 to 29 mg/g (Figure 5.1). Lowest value of phenol was observed in pumpkin seed (3.3mg/g). From the results it was observed that phenolic content shows great variability among the seeds analysed.

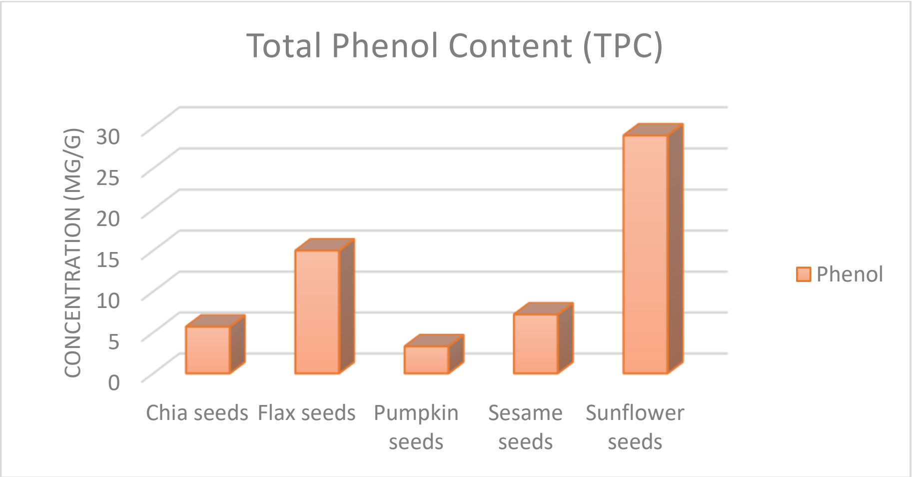

Figure 5.1: Total Phenol content in Seed extracts

Seeds are a good source of compounds with phenolic functionality including phenols, lignins, lignans, coumarins, tannins, phenolic acids, and flavonoids which are important in the human diet. Higher plants synthesize several thousand known different phenolic compounds. The ability to synthesize phenolic compounds has been selected throughout the use of evolution in different plant lineages, thus permitting plants to cope with the constantly changing environmental challenges over evolutionary time. Plant phenolics are considered to be a key role as defense compounds when environmental stress, such as high light, low temperatures, pathogen infection, herbivores, and nutrient deficiency can lead to an increased production of free radicals and other oxidative species in plants. Fruits and seeds are good source of natural antioxidants and that polyphenolics are its major antioxidants (Shui and Leong, 2006).

5.3.2. <u>Estimation of Flavonoids</u>

Flavonoid content determined in seeds is depicted in the figure 5.2. Sunflower seeds had the highest total flavonoid content (5.6 %) followed by pumpkin seeds (3.2%). Flax and Sesame seeds showed similar flavonoid content of 1.6%. Flavonoids play a variety of biological activities in plants, animals and bacteria. In plants flavonoids have long been known to be synthesized in particular sites and responsible for color, aroma of flowers, fruits to attract pollinators consequently fruit dispersion, help in seed, spore germination, growth and development of seedling. Flavonoids protects plant from different biotic and abiotic stresses and act as unique UV filter, function as signal molecules, allelopathic compound, phytoalexins, detoxifying agents, anti-microbial defensive compound. Flavonoids have roles against frost hardiness, drought resistance and may play a vital role in plant heat acclimation and freezing tolerance (Samanta *et al*., 2011).

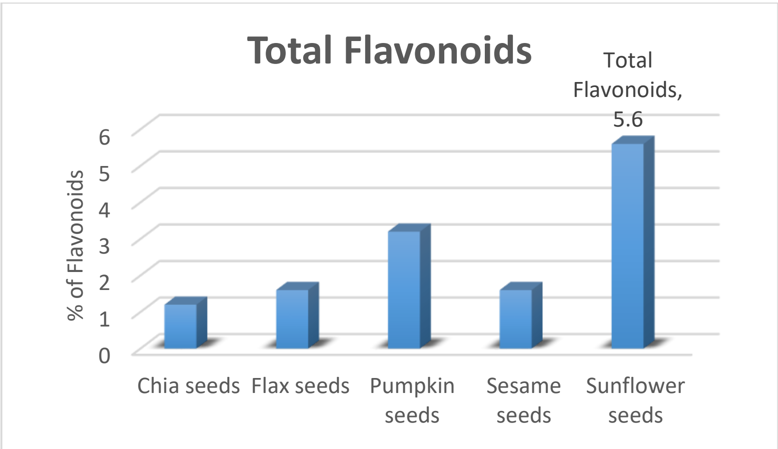

Figure 5.2: Flavonoid content in Seed extracts

Flavonoids are widely spread in different foods and beverages (such wine and tea), but the sources with the highest levels are fruits and vegetables (Liu *et al*, 2007). Among the legume seeds, soybean seeds generally contained significantly higher amount of total measured flavonoids (892–917 µg/g), while cowpea and peanut seeds contained a significantly higher amount of quercetin (214–280 µg/g and 133–289 µg/g, respectively) (Wang *et al*, 2008).

Among the temperate fruits, the highest levels of flavonoids are found in berries, such as black elderberry (1358.66 mg/100 g) and black chokeberry (1012.98 mg/100 g) (Maatta *et al*, 2004; Wu *et al*, 2004). In the drupes group, some fruits such as plum and sweet cherry have higher levels of flavonoids than the rest of the group, 101.67 mg/100 g and 185.05 mg/100 g, respectively (Pascual *et al*, 2000; Arts *et al*, 2000). In the pomes group, apple has the highest level of flavonoids (56.35 mg/100 g) (Arts et al, 2000; Vrhovsek *et al*, 2004).

5.3.3. INVITRO ANTIOXIDANT ACTIVITY

5.3.3.1. Reducing power assay

The invitro antioxidant activity of seed extracts were determined using reducing power assay. This assay was based on the principle that substances, which have reduction potential, react with potassium ferricyanide to form potassium ferrocyanide which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm (Bhalodia *et al*, 2013). The higher the absorption of the reaction mixture, the greater the reducing capacity of the extracts. The absorbance of the intensity of the color noted at 700 nm of seed samples is presented in figure 5.3. Sunflower seed extract showed higher antioxidant activity (1.702) followed by sesame seeds (0.857). Absorbancy value of other seed extracts ranged from 0.288 to 0.334 (Figure 5.3).

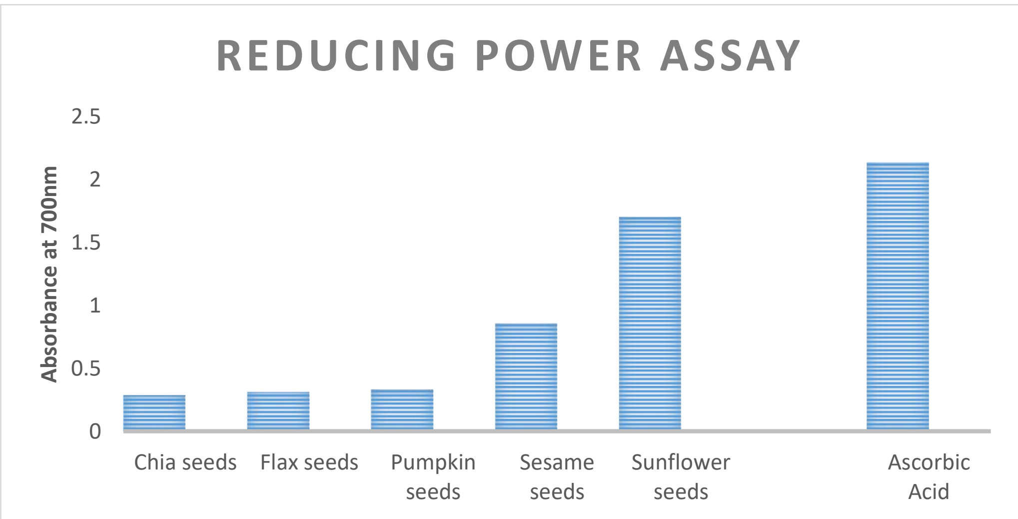

Figure 5.3: Reducing Power Assay

Among the Seeds, Sunflower has significantly higher content of total phenols, flavonoids and reducing power activity than those of other seeds studied. Additionally, we found a high correlation between the total phenolics content and the antioxidant activity in all the seeds analyzed (Figure 5.1 & 5.3). The Antioxidant activity and phenolic compound profiles of six fractions obtained from sunflower seed extract were studied by Karamac *et al* (2012). The results of all antioxidant activity tests showed good correlations among each other and with the phenolic contents for the individual fractions (Karamac *et al*., 2012). Maatta *et al* (2009) found similar correlation between the total phenolics and the antioxidant activity in apple cultivars. According to them the antioxidant content and activity, vary considerably depending on the apple cultivars analyzed. Arts *et al* (2009) evaluated the kinetics of DPPH radical scavenging by some tropical fruits. According to them antioxidant potential of these fruits could be attributed to different compounds evaluated such as vitamins and phenolics.

5.3.3.2. <u>DPPH Free Radical Scavenging Assay</u>

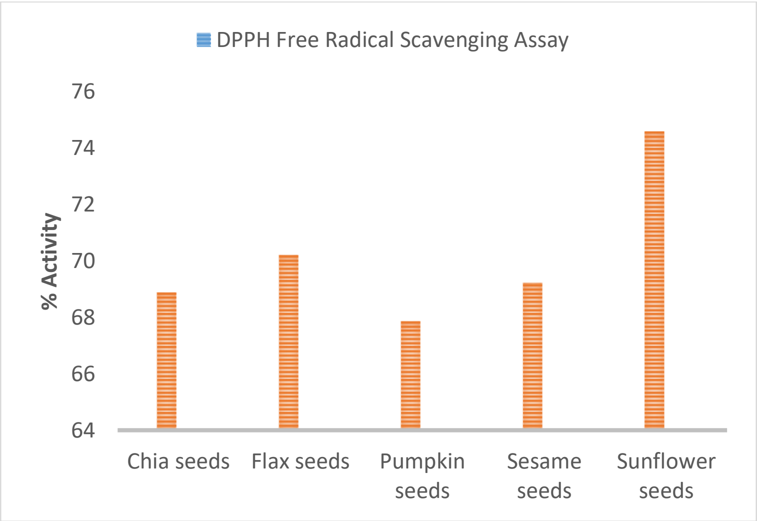

Figure 5.4: DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging activity of seed extracts (Figure 5.4) revealed that all the seed extracts possessed activity greater than 65% and higher activity was observed in sunflower seed extract (74.59%). Hydrogen donating ability of the antioxidants present in the seed extracts can have effect of DPPH. According to Huang *et al* (2005), polyphenols and tocopherols scavenge DPPH free radicals.

6. SUMMARY & CONCLUSION

Seeds are nutritional power houses and they contain many essential components our body needs to stay strong and healthy. They are an excellent source of fiber, protein, vitamins, minerals, antioxidants, and contain significant amounts of healthy fatty acids such as mono unsaturated and poly unsaturated fatty acids. Seeds, when consumed as part of a balanced diet, can benefit our body in a number of ways. In fact, many seeds like chia seeds, flax seeds, sesame seeds and pumpkin seeds are gaining popularity across the world due to their particular health benefits. The good thing is that seeds are everywhere and are extremely versatile, meaning they can be easily incorporated in to a variety of different recipes. They are also packed with antioxidants and fat-burning compounds, which are beneficial for losing weight and help us to maintain good health.

The aim of the study was to evaluate the proximate composition and antioxidant potential of five seeds such as *Salvia hispanica L. Linum usitatissimum L., Cucurbita maxima Duch., Sesamum indicum L., and Helianthus annuus L.* Phenols and other phytochemicals known to possess natural antioxidant property which curbs the effect of free radicals and the oxidative damage caused by them. Natural antioxidants are expected to be an alternative to the synthetic ones because of their potential health benefits. Therefore, the objectives of this study were to compare and evaluate the proximate composition and antioxidant properties of the above five seeds. The Preliminary Phytochemical analysis of the seed extracts showed the presence of alkaloids, glycosides, coumarins, quinones, diterpenes, saponins, phytosterols, phenols, tannins, carboxylic acid, xanthoprotein, proteins, sugars. The proximate analyses of moisture, ash, crude fibre, starch, carbohydrate, protein and lipid were conducted according to standard analytical procedures. The antioxidant property was determined with the quantification of total phenols and flavonoids and the invitro antioxidant activity was evaluated with the reducing power assay DPPH free radical scavenging assay.

Moisture content in seeds falls in the range of 3.56 to 5.38%. Ash content was found to be highest in Chia seeds which is closely comparable with ash values of other seeds. Highest value of crude fibre was also found in Chia seeds which had 24.5% crude fibre followed by Flax seed (22.8%). Starch content was observed to be greater in pumpkin seed (11.2%).

Quantitative analysis of major nutrients revealed that pumpkin contained the highest amount of carbohydrate followed by Chia seed and flax seed. The highest quantity of protein was also found in Pumpkin seed. Quantity of proteins in other seeds ranges from 16.98% to 21.86%. From the current study it was found that all the five seeds are good sources of protein. Lipid content was found to be higher in sunflower seed (43.5%) which was followed by sesame (40.3%). Among the five seeds analyzed, nutritive value was found to be higher in pumpkin seed (611.4cal/100g). Nutritive value of sunflower and sesame seeds was found to be 555.9 and 522.09 cal/100g respectively. From the results it was found that all the seeds under study are of high nutritional value.

Among the seeds analyzed in our study Sunflower had the highest content of total phenols. The phenol content in the five seeds ranged from 3.3 to 29 mg/g. Lowest value of phenol was observed in pumpkin seed (3.3mg/g). Phenolic content shows great variability among the seeds analyzed. In the case of flavonoids also, Sunflower seed had the highest total flavonoid content (5.6%) followed by Pumpkin (3.2%). The invitro antioxidant activity of seed extracts were determined using reducing power assay and DPPH free radical scavenging assay. The results of reducing power assay of seed extracts showed that sunflower seed had higher antioxidant activity (1.702) followed by sesame seeds (0.857). The DPPH free radical scavenging activity of seed extracts revealed that all the seed extracts possessed activity greater than 65% and higher activity was observed in sunflower seed extract (74.59%).

From the above study it was found that among the five seeds, Sunflower has significantly higher content of total phenols, total flavonoids and invitro antioxidant activity than those of other seeds studied. Additionally, we found a high correlation between the total phenolics content and the antioxidant activity in all the seeds analyzed. The presented data for total phenolic and flavonoid content are a basis for assessment of the preventive role of seeds against free radical effect. The results of this study provide information on the characteristics of these seeds, information that could be useful for product development, medicinal applications, food production as sources of bioactive compounds, and also for food supplements or functional foods. Based on the above findings, it can be concluded that the various phytochemical compounds are present in all the five seeds and are nutritionally promising.

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