Chapter 1
Natural Products

1.1 Ayurveda

Ayurvedic medicine is a system of Vedic traditional medicine native to the Indian subcontinent and is a form of alternative medicine. The oldest known Ayurvedic texts are the *Susrutha Samhita* and the *Charaka Samhita*. These classical Sanskrit texts are among the foundational and formally compiled works of Ayurveda. By the medieval period, Ayurvedic practitioners developed a number of medicinal preparations and surgical procedures for the treatment of various ailments. Practices that are derived from Ayurvedic medicine are regarded as part of complementary and alternative medicine, and along with Siddha Medicine and Traditional Chinese medicine, forms the basis for systems medicine. Concerns have been raised about Ayurvedic products; U.S. studies showed that up to 20% of Ayurvedic U.S. and India manufactured patent medicines sold through internet contained toxic levels of heavy metals such as lead, mercury and arsenic [1].

Ayurveda is an intricate system of healing that originated in India thousands of years ago. We can find historical evidence of Ayurveda in the ancient books of wisdom known as the Vedas. In the Rig Veda, over 60 preparations were mentioned that could be used to assist an individual in overcoming various ailments. The Rig Veda was written over 6,000 years ago, but really Ayurveda has been around even longer than that. It is a science of life. We all are part of nature. Just as the animals and plants live in harmony with nature and utilize the laws of nature to create health and balance within their beings, we, too, adhere to these very same principles. Therefore, it is fair to say that Ayurveda is a system that helps to maintain health in a person by using the inherent principles of nature to bring the individual back into equilibrium with its true self. [2, 3].
1.1.1 Principles

There are two ways to approach Ayurvedic principles and terminology: either one may focus on the historical foundation or, alternatively, a description may take an ethnographic approach and focus on the forms of traditional medicine prevalent across India today. Much like the medicine of classical antiquity, Ayurveda has historically taken the approach of enumerating bodily substances in the framework of the five classical elements earth, water, fire, air and ether. Moreover, Ayurveda names seven basic tissues (dhatu). They are plasma (rasa), blood (rakta), muscles (mamsa), fat (meda), bone (asthi), marrow (majja), and semen (shukra) [4, 5].

Mahābhūta Ayurveda states that a balance of the three elemental substances, the Doshas, equals health, while imbalance equals disease. There are three doshas: Vata, Pitta and Kapha [6]. One Ayurvedic theory states that each human possesses a unique combination of these doshas, which define this person’s temperament and characteristics. Each person has a natural state, or natural combination of these three elements, and should seek balance by modulating their behavior or environment [7]. In this way they can increase or decrease the doshas they lack or have an abundance of respectively. Another view present in the ancient literature states that dosha equality is identical to health, and that persons with imbalance of dosha are proportionately unhealthy, because they are not in their natural state of balance. Prakriti is one of the most important concepts in Ayurveda. Ayurveda gives us a model to look at each individual as a unique makeup of the three doshas and to thereby design treatment protocols that specifically address a person’s health challenges. When any of the doshas (Vata, Pitta or Kapha) become accumulated, Ayurveda will suggest specific lifestyle and nutritional guidelines to assist the individual in reducing the dosha that has become excessive. We may also suggest certain herbal supplements to hasten the healing process. If toxins in the body are abundant, then a
cleansing process known as Pancha Karma is recommended to eliminate these unwanted toxins [8, 9].

According to Ayurveda, every individual has unique needs for balance. Since, diet is one of the most important ayurvedic tools for achieving balance, ayurvedic healers generally design individualized diets for people they see, based on various factors such as age and gender, the doshic tendencies that need to be balanced at a given time, the strength of the body tissues and the digestive fires, and the level of ama (toxins) in the body. The place where a person lives and the season are also factors that affect dietary dos and don'ts [10-12].

1.2 Natural product: A study

1.2.1 Introduction to natural product

By definition, the word *natural* is an adjective referring to something that is present in or produced by nature and not artificial or man-made. When the word *natural* is used in verbiage or written, many times it is assumed that the definition is something good or pure. However, many effective poisons are natural products. The term *natural products* today is quite commonly understood to refer to herbs, herbal concoctions, dietary supplements, traditional Chinese medicine, or alternative medicine. The information presented here will be restricted to the discovery and development of modern drugs that have been isolated or derived from natural sources, while in some cases, such discovery and development have been based on herbs, folklore, or traditional or alternative medicine, the research and discovery of along with the development of herbal remedies or dietary supplements typically present different challenges with different goals [13].

1.2.2 History and background of the use of natural products as therapeutic agents

Natural products are generally either of prebiotic origin or originate from microbes, plants, or animal sources. As chemicals, natural products include such classes of compounds as terpenoids, polyketides, aminoacids, peptides, proteins, carbohydrates, lipids,
nucleic acid bases, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and so forth. Natural products are not just accidents or products of convenience of nature but they are a natural expression of the increase in complexity of organisms. Interest in natural sources to provide treatments for pain, palliatives, or curatives for a variety of maladies or recreational use reaches back to the earliest points of history. Nature has provided many things for humankind over the years, including the tools for the first attempts at therapeutic intervention. Neanderthal remains have been found to contain the remnants of medicinal herbs. Earlier describes the uses of over 1000 different herbs. This work served as the basis for Tibetan medicine translated from Sanskrit during the eighth century Chinese herb guides document the use of herbaceous plants as far back in time as 2000 BC. In fact, the Chinese material medical has been repeatedly documented over centuries starting at about 1100 BC. For a variety of different reasons, the interest in natural products continues to this very day. The first commercial pure natural product introduced for therapeutic use is generally considered to be the narcotic morphine [14, 15].

1.2.3 Natural products research and development - An update
The World Health Organization estimates that approximately 80% of the world’s population relies primarily on traditional medicines as sources for their primary health care. Over 100 chemical substances that are considered to be important drugs that are either currently in use or have been widely used in one or more countries in the world have been derived from a little under 100 different plants. Approximately 75% of these substances were discovered as a direct result of chemical studies focused on the isolation of active substances from plants used in traditional medicine. Natural-products-based anticancer drug discovery continues to be an active area of research throughout the world [16, 17].
1.2.4 Future of natural products

Structure–activity studies on leads generated from natural products sources combined with computerized graphic model building will become increasingly more prevalent. Such activity in turn will result in the discovery of molecules with optimal activity, improved bioavailability, fewer side effects, and very desirable therapeutic indices. However, abuse of our natural resources will certainly limit our ability to learn from nature. Alternatively, the highly focused and single-minded research into natural products can in and of itself lead to the depletion of sources of natural products and even the eventual extinction of species. In the hunt for natural products another obstacle that will have to be more commonly addressed is the issue of intellectual property rights. The search for and the development of natural products will result in the creation of a variety of different types of alliances between industry, government, individuals, universities, and hopefully even foster a spirit of international cooperation [18-21].

1.3 Phytonutrients/Phytochemicals

"Phyto" comes from the Greek word "phuton" meaning "plants" and hence the chemicals/nutrients found in plants are called phytochemicals or phytonutrients. The terms are used interchangeably but "phytonutrients" is increasingly becoming more popular for the positive association with "nutrient" rather than "chemical".

In fact, sometimes preparation may even make the phytonutrients more readily available to us. For example, the sulfur compounds from garlic or onions are released, when chopped and exposed to air, or lycopene in tomatoes become more concentrated when processed and made into tomato sauce. There are several sources for lycopene is available in different natural products but tomato is richest source among all. It is estimated that there are tens of thousands of phytochemicals. However, only about 1,000 of these were identified and only about a hundred were actually analyzed and tested.
Researchers have found that all plants contain compounds that protect them from diseases [22]. When we eat these plants, the very same protective compounds, called phytochemicals, are made available to our bodies. In the same way, it protects our blood stream, cells, tissues, membranes, organs and immune functions from diseases. An outstanding fact that proves an amazing creator is that in each plant, it is believed there are hundreds of different phytochemicals. A simple tomato not only has lycopene, but has several other phytochemicals, which cannot even yet be identified [23]. Studies have shown that individuals with high intake of the four plant-based food groups' fruits, vegetables, whole grains and legumes have a much lower risk of degenerative diseases such as cancer, diabetes, heart disease, hypertension, etc.

1.3.1 How does phytonutrient help to prevent these diseases?
To understand the role of phytonutrients, we need to backtrack a little and understand how diseases are formed.

An example: When free radicals run rampant in our body, through the air we breathe, the food we eat, or merely from stress, they cause deterioration and destruction of our healthy cells. This process ultimately results in degenerative diseases in the weakest parts of our body that succumb to the attack. When we eat food that has phytonutrients, it will quickly activate a group of enzymes that go around cleaning up the free radicals before, they cause any harm to the body, and it works like the antioxidant. In fact, many phytonutrients are antioxidants [24, 25].

1.3.2 Survey of naturally occurring Antioxidants
It is recommended as better to ingest phenolic antioxidants in the common diet than to add antioxidant preparations to foods. The task is not that difficult as most plant foods contain phenolic compounds, usually 0.5– 5.0% of dry weight. The content of antioxidant substances in the US foods has recently been published, but various nonconventional foods rich in antioxidants have been produced as well [26, 27].
The importance of cereals as sources of antioxidants is often undervalued because of the relatively low content of antioxidants, but the consumption of cereals is high. The milling procedure may be different and non-extractable phenolics are not accounted for standard analytical methods, even when they can be released from the non-extractable form by action of digestive enzymes. Extrusion cooking; a progressing food processing technology destroys polyphenolics, but some free phenolic acids are released by their decomposition. Whole-wheat flour and the bran have the highest phenolic content. Rice is also a good source of phenolic antioxidants but the antithrombotic and antioxidative activity correlates with the content of neither group of phenolics so that, other factors also contribute to the resulting antioxidant activity. Several common vegetables are rich sources of antioxidants; wastes from their processing could be used for the production of antioxidants. Potatoes should be mentioned in the second place because of the high potato consumption. Among other vegetables, for instance, spinach leaves have a high content of flavonoids, or onions, particularly their outer scales, which are usually rejected, have high amounts of quercetin and Protocatechuic acid [28-30]. Some of the major source of natural antioxidants is summarized in Table 1.1.

**Table 1.1 Major sources of natural antioxidants in the human diet**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sources</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cereals</td>
<td>Whole wheat products, rice, bran, minor millets, sorghum</td>
</tr>
<tr>
<td>2</td>
<td>Vegetables</td>
<td>Leaf vegetables, potatoes</td>
</tr>
<tr>
<td>3</td>
<td>Fruits</td>
<td>Apples, bananas, berries, olives</td>
</tr>
<tr>
<td>4</td>
<td>Legumes</td>
<td>Beans, peanuts, soybeans</td>
</tr>
<tr>
<td>5</td>
<td>Beverages</td>
<td>Tea, coffee, red wine, beer, fruit juices</td>
</tr>
<tr>
<td>6</td>
<td>Cocoa products</td>
<td>Chocolate</td>
</tr>
<tr>
<td>7</td>
<td>Herbs and spices</td>
<td>Labiatae plants (rosemary, sage, oregano, savory)</td>
</tr>
<tr>
<td>8</td>
<td>Oilseeds</td>
<td>Sesame seeds, hazelnuts, almonds</td>
</tr>
</tbody>
</table>
Vegetables including potatoes acquired second position in terms of contribution of antioxidants in daily food intake. Vegetables of the *Brassicaceae* family belong to one of the most important representatives of vegetables in Europe and they are main source of phenolic antioxidants among vegetables. Organic vegetables, having higher contents of phenolics, acquire second position too. Wild green vegetables are better sources of phenolics than cultivated plants, as expected, because they grow under difficult conditions. The disadvantage of wild green vegetables is more difficult to collect and their growing conditions [31-33].

The third important source of antioxidants in the human diet is fruits. The content of phenolics in fruits shows sufficient intake of antioxidants per serving. Particularly berries such as blueberries, blackberries, seabuckthorn or sweet rowan berries should be mentioned as non-important source of antioxidants [34].

Apples are important, in spite of a lower content of phenolics, because of regularly used in diet. Fruit wastes can be used for the production of extracts possessing antioxidant activity, *e.g.* wastes from wine grape processing. Bioorganic fruits such as oranges also grow under unfavorable conditions compared to those cultivated according to traditional pathway; therefore, they also contain more phenolics. [35].

Olives and olive oil are particularly rich sources of phenolic antioxidants, which have good free radical scavenging and chelating properties, but olives are consumed in higher amounts only in some Mediterranean countries. Antioxidants in olives and olive oil have been intensively studied in the Mediterranean countries. The content of phenolics is high, but depends very much on the variety and the degree of ripening at the time of collection. Spanish commercial virgin olive oils contained 330–500 mg/kg polyphenols. Oil seeds are also good sources of antioxidants, but their consumption is low, except for nuts where toco-pherols are the most important antioxidants, *e.g.* in hazelnuts. Almonds are also rich in various antioxidants. Sesame seeds contain efficient lignan antioxidants [36].
Tea leaves contain high levels of catechins, but the phenolics are partially destroyed by fermentation of the fresh tea leaves used for the production of black tea. The content of total phenolics decreased from 205 mg/gm in green tea to 149 mg/gm in black tea. It would be unfair not to mention that black tea pigments, produced by the oxidation of green tea catechins, also possess free radical scavenging activity. However, it is surprising that semi fermented oolong (Chinese tea) and pu-erh (Chinese tea) teas had higher antioxidant activity (contrary to total phenolics) than either green or black teas. The activity of roasted tea was between those of green tea and oolong tea. Not only tea phenolics but also terpenic volatile substances and benzyl alcohol had antioxidant activity [37]. Green tea consumption of two cups daily increased the plasma antioxidant activity and decreased the oxidative damage of DNA and LDL cholesterol.

The maximum concentration of synthetic antioxidants allowed in the lipidic fraction is 0.02%, i.e. 200 mg/kg. If the daily lipid consumption is about 100 gm (the upper limit advisable), and the whole lipid fraction has been stabilized with synthetic antioxidants, the total daily consumption of synthetic antioxidants would be equal to 20 mg. Usually, only a small part of the dietary lipids contains synthetic antioxidants [38]. Even an intake 100 times higher than that of the permitted concentration it would still harmless, according to the safety tests. This means that even the intake of 2000 mg daily should be considered as safe. The average daily intake of native antioxidants present in the diet consisting of normal food is about 1000 mg, about 50–200 mg polyphenols per meal. The contributions of various food sources were estimated as follows: 44 mg from cereals, 79 mg from potatoes, 45 mg from nuts, 162 mg from vegetables, 290 mg from fruits and juices, and 420 mg from beverages. The daily contribution of fruits and vegetables was calculated as 450 mg/GAE. In countries with lower consumption of vegetables, fruits and red wine, such as Central Europe, it could be lower. Safety tests are not available for most natural
food components and their phenolics, but we assume and sincerely hope that such a concentration does not substantially exceed the safety limits.

If 100 g lipids in the diet would be stabilized by natural antioxidants at the level of 0.05–0.10% on the fat basis, the daily consumption of natural phenolics would increase from about 1000 mg to about 1050–1100 mg, which is not substantial and may be neglected [39-42].

Many extracts of natural plant materials were studied, their antioxidant activity was observed, and they have been proposed as natural antioxidants. Commercially available natural antioxidants are tocopherols, phospho-lipids, ascorbic acid and their esters, rosemary resins, sage resins, and tea leaf extracts. Leaves of *Vitis vinifera* and grape skins are also potential commercial sources. Various other substances may be obtained and used in minor amounts. Of course, an addition of common food components rich in phenolics, and readily available, may be still used instead of extracts [43].

1.4 Free radicals and our body

1.4.1 Free radical generation: Role of oxidation process in body

To help our body, protect itself from the rigors of oxidation, Mother Nature provides thousands of different antioxidants in various amounts in fruits, vegetables, whole grains, cereals, millets, minor millets, nuts and legumes. When our body need to put up its best defence, especially true in today's environment, antioxidants are crucial to our health. As oxygen interacts with cells of any type - an apple slice or, in our body, the cells lining our lungs or in a cut on our skin - oxidation occurs [44]. This produces some type of change in those cells. They may die, such as with rotting fruit. In the case of cut skin, dead cells are replaced in time by fresh, new cells, resulting in a healed cut [45].

This birth and death of cells in the body goes on continuously, 24 hours a day. A process is necessary to keep the body healthy. Oxidation is a natural process that happens during normal cellular functions. Yet, there is a downside, while the body metabolizes oxygen
very efficiently, 1% or 2% of cells will get damaged in the process and turn into free radicals [46].

"Free radicals" is a term often used to describe damaged cells that can be problematic. They are "free" because they are missing a critical molecule, which sends them on a rampage to pair with another molecule. These molecules will rob any molecule to quench that need.

1.4.2 Types of free radicals

Most free radicals are coming from oxygen atoms and are called Reactive Oxygen Species (ROS), such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen.

Superoxide ion (or reactive oxygen species) is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria, DNA and other biomolecules. Superoxide radical is produced in vivo, and the superoxide dismutase enzymes are an important protective antioxidant defence [47, 48].

Scheme 1.1 represents the free radical mechanism of oxygen. Dismutation occurs spontaneously at a rate that decreases with a rise in pH, but the rate can be accelerated enormously by addition of superoxide dismutase (SOD) enzymes, which are present in essentially all body tissues and in most uncooked food foods [49].

Scheme 1.1 Free radical mechanism of oxygen

Fortunately, neither \( \cdot \text{O}_2^- \) nor \( \text{H}_2\text{O}_2 \) is very reactive. \( \text{H}_2\text{O}_2 \) can cross membranes readily, which \( \cdot \text{O}_2^- \) is unable to do; the charged natured of \( \cdot \text{O}_2^- \) renders it membrane impermeable unless there is a
transmembrane channel through, which it can move, such as the anion channel in erythrocytes [50].

**Hydroxyl radical** is formed by the reduction of an oxygen molecule in the electron transport chain as shown in scheme 1.1. It is a neutral (not charged) form of the hydroxide ion. Hydroxyl radicals are highly reactive and form an important part of radical biochemistry [55]. Unlike superoxide, the hydroxyl radical cannot be eliminated by an enzymatic reaction. It has a very short half-life and will only react with molecules within its vicinity. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins. **Singlet oxygen** is formed by immune system. Singlet oxygen causes oxidation of LDL cholesterol.

### 1.4.3 The danger of free radicals

When free radicals are on the attack, they don’t just kill cells to acquire their missing molecule. If free radicals simply killed a cell, it wouldn’t be so bad; the body could just regenerate another one. The problem is that free radicals often injure the cell, damaging the DNA, which creates the seed for disease. When a cell’s DNA changes, the cell becomes mutated and it grows abnormally and reproduces quickly [51].

Normal cell functions produce a small percentage of free radicals, much like a car engine that emits fumes. However, those free radicals are generally not a big problem. They are kept under control by **antioxidants** that the body produces naturally. When a free radical oxidizes a fatty acid, it changes that fatty acid into a free radical, which then damages another fatty acid, and so on.

These external attacks can overwhelm the body’s natural free-radical defence system. In time, and with repeated free radical attacks that the body cannot stop, that damage can lead to a host of chronic diseases, including cancer, heart disease, Alzheimer’s disease, and Parkinson’s disease and so on[52].
1.5 Antioxidants

In the 21st century, people need to get more antioxidants in their diet to offset all these assaults. These toxins are ubiquitous in the environment. If you live in a city, we breathe the air. The oxidative burden (on the body) is much, much, much higher than it was 200 years ago. It is a fact of modern life, so we have to consider those facts.

Antioxidants work to stop this damaging and disease-causing chain reaction that free radicals have started. Each type of antioxidant works either to prevent the chain reaction or to stop it after it is started. For example, the role of vitamin-C is to stop the chain reaction before it starts. It captures the free radical and neutralizes it. Vitamin-E is a chain-breaking antioxidant. Increasing interest in the role of antioxidants in food preservation, nutraceuticals, herbal medicines, and health maintenance is observed now a day due to its importance in diet [53-56].

Polyphenols constitute one of the largest categories of phytochemicals, most widely distributed among the plant kingdom, and an integral part of the human diet. Dietary phenolic include phenolic acids, phenolic polymers (commonly known as tannins), and flavonoids. Polyphenols and flavonoids are powerful antioxidants, and their activity is related to their chemical structures [57, 58].

1.5.1 Sources for Antioxidants

We have clear science about antioxidants, that our bodies need a Natural Antioxidant Defence Network (NADN), for lack of a better term. Such antioxidants can be transferred by two different ways, either in form of medicines or in form of daily food intake using natural products like fruits, vegetables and whole grains (cereals, millets and minor millets). These natural food products contain antioxidants in the structure.

Millions of people are using natural products like vegetable, grains, and cereals still from those days when synthetic drugs were not
available and these natural products are great source of antioxidants [59].

1.5.2 Medicinally synthesised antioxidants in drugs
There are number of drugs available in the market like metformin [60], chloropropamide, gliplzide, phenformin and buformin are more potent than metformin that provides antioxidants to body and they initially enjoyed greater acclaim and use, but their association with lactic acid is led to discontinuation in most countries by the end of the 1970s. These drugs were synthesized in laboratory and had several disadvantages. Each drug has its own side effects, which gets effects to patient according to his/her health condition [61].

1.5.3 Naturally occurring antioxidants from natural products
The body needs a mixture of vitamins and minerals, such as vitamins A, C, E, and beta-carotene, to neutralize free radical assault. Multivitamins and vitamin supplements can provide the body with an antioxidant boost. Yet getting too much of some supplements, like vitamin E, can be harmful. Fruits, whole grains, cereals, legumes and vegetables contain complex mixes of antioxidants and lay the benefit of eating a variety of healthy foods [62].

For that, we need to eat many different foods. Each type works in different tissues of the body, in different parts of cells. Some are good at quenching some free radicals, some are better at quenching others. When we have appropriate amounts of different antioxidants, we are doing what we can do to protect ourselves. Nevertheless, to eat various food or supplement is not possible for everybody with different economic and health backgrounds. Therefore, it is necessary to use such diet, which contains all kind of antioxidants such as flavonoids and phenolic acids [63].

Mother Nature has provided us a great source of such antioxidants called “Minor Millets”, which contains all that antioxidants that our body needs. Minor millets could be a powerful source of antioxidants like flavonoids, phenolic acids [64].
1.6 Potential source of Antioxidant: Minor Millets

Minor millets (also referred to as small millets) have received far less attention than sorghum in terms of cultivation and utilization. They include finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*), common or prove millet (*Panicum miliaceum*), little millet (*Panicum sumatrense*) and barnyard or sawa millet (*Echinochloa crus-galli, Echinochloa corona*). India is the largest producer of many kinds of millets called coarse cereals. Thus, they are not important in terms of world food production, but they are essential as food crops in their respective agro-ecosystems. They are mostly grown in marginal areas or under agricultural conditions where major cereals fail to give sustainable yields. Detailed descriptions of these millets are given by Purseglove [65]. General classification of minor millet is depicted in figure 1.1.

1.6.1 *Echinochloa crus-galli*

*Echinochloa colonola* L. (Barnyard, Japanese barnyard or sawa millet) is the fastest growing of all millets and produces a crop in six weeks. It is grown in India, Japan and China as a substitute for rice when the paddy fails. It is grown as a forage crop in the United States and can
produce as many as eight harvests per year. The plant has attracted some attention as a fodder in the United States and Japan [66]. The Tamil name of various minor millet shown in figure 1.2.

![Figure 1.2 Various Minor millet with their Tamil names](image)

1.6.2 *Panicum sumatrense*

*Panicum sumatrense* (Little millet) is grown throughout in India to limited extent up to altitudes of 2100 m but is of little importance elsewhere. It has received comparatively little attention from plant breeders [67].

1.6.3 *Panicum miliaceum L.*

*Panicum miliaceum L.* (Common, Proso millet) is also known as prove millet, hog millet, broom-corn millet, Russian millet and brown corn. This millet is of ancient cultivation. It is the milium of the Romans and the true millet of history. It was cultivated by the early Lake Dwellers in Europe. It is believed to have been domesticated in central and eastern Asia and because of its ability to mature quickly was often grown by nomads [68].

1.6.4 *Eleusine coracana L.*

*Eleusine coracana L.* (Finger millet) is also known as African millet, koracan, ragi (India), wimbi (Swahili), bulo (Uganda) and telebun (the Sudan. It is an important staple food in parts of eastern, central Africa.
and India. It is the principal cereal grain in northern and parts of western Uganda and north-eastern Zambia. The grains are malted for making beer. *Eleusine coracana* L. can be stored for long periods without insect damage and thus it can be important during famine. Its nutritional importance is well recognised because of its higher content of Calcium (Ca, 0.38 %) and dietary fibres (18 %) compared to the continental cereals such as barley, rice, maize and wheat. It is consumed as a whole, thereby retaining the fibre, phenolics, minerals and vitamins present in the outer layer of the grain, which are nutritionally beneficial [69].

### 1.6.5 *Setaria italica* L.

*Setaria italica* L. (Foxtail millet) is also known as Italian, German Hungarian or Siberian millet. It is generally considered to have been domesticated in eastern Asia, where it has been cultivated since ancient times. The main production area is China, but *Setaria italica* L. is the most important millet in Japan and is widely cultivated in India. It is believed to have one of the five sacred plants of ancient China (from 2700 B.C.). Because of its short duration it is a suitable crop for growing by nomads and it was probably brought to Europe in this way during the Stone Age, as seeds abound in the Lake Dwellings in Europe [70].

### 1.6.6 *Paspalum scrobiculatum* L.

*Paspalum scrobiculatum* L. (Kodo millet) is a minor grain crop in India but is of great importance in the Deccan Plateau. Its cultivation in India is generally confined to Gujarat, Karnataka and parts of Tamil Nadu. It is classified into the groups Haria, Choudharia, Kodra and Haria-Choudharia depending on panicle characters. Kodo is an annual tufted grass that grows to 90 cm high [71].

### 1.7 Phenolics

#### 1.7.1 Flavonoids

The flavonoids, derivatives of 1,3-diphenylpropane, are a large group of natural products, which are widespread in higher plants but also found in some lower plants, including algae. Most flavonoids are
yellow compounds, and contribute to the yellow colour of the flowers and fruits, where they are usually present as glycosides. Flavonoids are the largest group of phenols in the plant kingdom and are metabolites with a benzopyran nucleus having an aromatic substituent at carbon number 2 (C-2) of the C ring. Figure 1.3 shows the basic flavonoid ring structure.

The most common types of flavonoids are flavanones, flavonols, flavones and flavans. As shown in figure 1.3, characteristic features of these flavonoids are a carbonyl at C-4 (flavanones), carbonyl at C-4, double bond between C-2 and C-3, and hydroxyl at C-3 (flavonols), carbonyl at C-4 and a double bond between C-2 and C-3 (flavones) and no carbonyl at C-4 but with a hydroxyl at C-3 for the flavans [72].

**Figure 1.3 Basic flavonoid ring structure**

Among the types of flavonoids that exist in nature, flavans are the major group found in millet/sorghum and the major flavans are leucoanthocyanidins (hydroxyl at C-3 and C-4), (+)-catechin (hydroxyl at C-3) and anthocyanidin (hydroxyl at C-3, double bond between C-3 and C-4). Anthocyanidins such as luteolinidin, cyanidin and apigeninidin have been reported in sorghum. Awika, Rooney and Waniska identified apigeninidin and luteolinidin as the major 3-deoxyanthocyanidins (anthocyanidins that lack a hydroxyl group at the C-3 position) in black and brown sorghums. Blessin, Van Etten and Dimler found an anthocyanidin, fisetidin, in sorghum, and Yasumatsu, Nakayama and Chichester reported pelargonidin. Strumeyer and Malin however did not find any anthocyanidins in Leoti and Georgia 615 sorghums [73-76].
Figure 1.4 presents basic skeleton of flavonoids. The anthocyanin content of sorghum brans ranges from 1.6-9.8 mg/g. Other flavonoids reported in sorghum include naringenin and eriodictoyl (flavanones), taxifolin (flavonol) apiforol (flavan-4-ol) and luteolin (flavone). This shows that there is a wide variation in the flavonoid composition among sorghums. Flavonoids may undergo modification such as loss of oxygen substituents, methylation, glycosylation, dimerization or polymerization, which may affect their properties [77].

![Flavonoid structures](image)

**Figure 1.4 Basic structures of common flavonoid types**

Most flavonoids are potent antioxidant compounds. Several flavonoids possess anti-inflammatory, antihepatotoxic, antitumour, antimicrobial and antiviral properties. Many traditional medicines and medicinal plants contain flavonoids as the bioactive compounds. The antioxidant properties of flavonoids present in fresh fruits, vegetables and whole grains (cereals, millets and minor millets) are thought to contribute to their preventative effect against cancer and heart diseases. *Apigenin*, a flavonol found in many cereals, like finger millet and pearl millet. Another flavonol, *Luteolin* from finger millet, is also included in a number of dietary supplements, and claimed to have a beneficial effect for the treatment of diabetes (to maintain sugar level) [78].
1.7.2 Phenolic acids

Phenolic compounds are substances that possess an aromatic ring bearing one or more hydroxyl (-OH) substituent. As mentioned earlier, sorghum contains phenolic compounds that have antioxidant properties and provide a protective role to the sorghum plant. M Blummel et al. reported antioxidant activities of sorghums and millets, their brans, baked and extruded products due to phenolic compounds. These phenolic compounds in grain may be classified very broadly, into phenolic acids, flavonoids and condensed tannins [79]. Phenolic acids are the simplest phenolic compounds and are derivatives of benzoic or cinnamic acids. They contain hydroxyl or methoxyl groups substituted at various positions on the aromatic ring. Some of references suggest that protocatechuic, gentisic, caffeic, p-coumaric, salicylic, ferulic, sinapic and cinnamic acids as the major phenolic acids are present in sorghum grain and millet, and p-hydroxybenzoic, vanillic and syringic acid as minor phenolic acids. On the other hand, J Martína et al. separated and identified, by reverse phase high performance liquid chromatography (HPLC), eight main phenolic acids in sorghum grain extracts in both white and brown sorghum and millet. (gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, p-coumaric, ferulic and cinnamic acid) [80]. These phenolic acids are found in both the free and bound form, with brown sorghum containing the highest amount of free phenolic acids. Bound phenolic acids are those that are complexed or form conjugates with sugars, proteins or cell wall polysaccharides. Figure 1.6 and 1.7 represents derivatives of benzoic acid and cinnamic acid respectively. Table 1.2 and 1.3 are given for the convenience to identify the derivatives of benzoic and cinnamic acid respectively, according to the position of functional group attached with basic skeleton. [81].
Quercetin  Apigenin  Apigenidin

Cyanidin  Luteoloidin

Luteolin  Pelargonidin

Figure 1.5 Some major flavonoids reported in Minor Millet
Possible chemical structure present in minor millet, which we would like to extract isolate and characterized are given below

<table>
<thead>
<tr>
<th>Name of acid</th>
<th>Functional group position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>H  H  H</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>H  -OH  -OH</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>H  -OH  -OH</td>
</tr>
<tr>
<td>p-hydroxy benzoic acid</td>
<td>H  H  -OH</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>-OH  H  H</td>
</tr>
</tbody>
</table>

Table 1.2 Position of functional groups with respect to R₁, R₂ & R₃ in Benzoic acid
Table 1.3 Position of functional groups with respect to $R_1$, $R_2$ & $R_3$ in Cinnamic acid

<table>
<thead>
<tr>
<th>Name of acid</th>
<th>Functional group position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamic acid</td>
<td>-OCH$_3$ -OH -OCH$_3$</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>-OH -OH H</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>-OCH$_3$ -OH H</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>H -OH H</td>
</tr>
</tbody>
</table>
Superiority of natural product over synthesized drugs

Phytochemicals with biological activity has great utility as pharmaceuticals and pest-management agents. Through the 19th century and into the first half of the 20th century, the primary strategy for discovery of plant compounds with these uses was determining the active ingredients of plants with reported medicinal or pesticidal properties. Pharmaceuticals, including salicylic acid, morphine, and pesticides, such as the pyrethroids and rotenone, are examples of the fruits of this strategy \[82\]. Although this historical approach is still used, it reached the point of diminishing returns decades ago. Considering the large number of plant-derived products, different human populations co-distributed across the globe with different flora have probably discovered very few of the potential uses of plant products in medicine and agriculture. In the latter half of this century, industries depending upon medicinal and agricultural chemistry have become increasingly reliant on purely synthetic approaches to product discovery thus, reducing their interest in natural products, despite the virtually untapped biological and chemical potential of natural products. Molecular design around a molecular target site is a commonly used in synthetic chemistry approach that has been somewhat successful in pharmaceuticals, but much less productive in pesticide discovery. Combinatorial Chemistry, in combination with high throughput screening, has further expanded the potential of the solely synthetic approach. This strategy appears to be currently favoured by many companies. However, within the past few years, a resurgence of
interest in botanical sources of new medicines, nutraceuticals, and other bioactive compounds has emerged.

This renewed interest is due to several factors, including the realization that nature has already selected for biological activity, that many botanical compounds have yet to be discovered and that relatively few known compounds have been adequately characterized biologically. Furthermore, modern analytical instrumentation and improved microbioassays have made discovery of these compounds less time consuming and laborious. New strategies other than exploiting anecdotal ethno botanical must be used to more fully explore the plant world for compounds that can be used directly or as molecular leads for pharmaceutical and agricultural products.

Synthetic antioxidants such as 2,6-di-tert-butyl-4-methylphenol (BHT), 2-tert-butyl-4-methoxyphenol (BHA), 2-tert-butylbenzene-1,4 diol (TBHQ) and propyl gallate (Figure 1.8-1.11) are currently widely used in the food industry to increase the oxidative stability of fats and oils [83]. The use of these synthetic antioxidants as food additives has however, faced resistance in recent times due to accumulating evidence that they could be toxic, carcinogenic or mutagenic. Due to these safety concerns, there has been a growing interest in the exploration of natural antioxidants. Naturally, occurring antioxidants are presumed safe since they are found in food that has been used by man for centuries. The natural antioxidants in food may also be beneficial because there is evidence that they prevent cancer development and that they inhibit oxidative reactions in the body system. For instance, the typical Mediterranean diet, which is rich in
fruits and vegetables, has been linked to low occurrence of cardiovascular disease and cancer due in part to the high proportion of bioactive compounds such as flavonoids in the diet [84-86].

1.9 Structure-activity relationship: An overview
1.9.1 Structure-activity relationships between flavonoid compounds and antioxidant activity

Phenolic compounds have both primary and secondary antioxidant activities by acting both as chain breaking antioxidants. It may either donate hydrogen atoms to lipid radicals (radical scavenging) or by preventing initiation reactions, such as chelating metals. Both the chain breaking (radical scavenging) and metal chelating properties are known to be possible due to the structural features of the phenolic compounds. [87]. The key structural features that have been shown to enable phenolic compounds exert radical scavenging activity are as follows:

- **The number of phenolic hydroxyl groups on the phenolic compound molecule:** Studies have shown that generally, the more the number of hydroxyl groups on a phenolic structure, provide more sites for radical scavenging. Hence, the increase in antioxidant activity of compounds with multiple hydroxyl groups is observed.

- **Presence of hydroxyl groups on carbon number 3 and 4 in the B ring (3', 4'-dihydroxyl catechol configuration)** The strong radical scavenging activity by the 3,4'-dihydroxyl group in the B ring is due to its ability to provide highly stable phenoxy radicals after the donation of a hydrogen atom. Some of the sorghum flavonoids i.e. luteolinidin, quercetin, luteolin, pelargonidin, cyaniding, apigenin and apigenidin contain this configuration and therefore, could contribute significantly to the antioxidant activity of sorghum. [88-90].

- **Presence of a hydroxyl group at C-3 in combination with a carbonyl (keto) group on C-4 of the C ring as in taxifolin:** This may be due to maximal radical scavenging potential and strong radical absorption due to hydrogen bonding between the -OH group at C-3 and the carbonyl group at C-4.
The unsaturation of the C ring such as a double bond between C-2 and C-3 in conjunction with the 4-carbonyl group on the C ring (such as in Luteolin), or in combination with a 3-hydroxy group on the C ring. This structural feature allows the delocalization of an electron from phenoxy radicals on the B ring to the C ring. A 2,3 double bond in combination with a 3-hydroxy group on the C ring also results in increased antioxidant activity because the resonance stabilization for electron delocalization across the molecule is increased [91, 92]. On the contrary, methoxylation of hydroxyl groups (introduction of a –OCH₃ group into the phenolic structure, replacing the -OH group) decreases its antioxidant activity. This reduction in the antioxidant activity may be attributed to differences in redox potential of the -OH groups and the substituted groups (such as the –OCH₃ group) on the phenyl ring. Hydroxyl groups have lower redox potential, which makes them good electron-donating groups (i.e. good reducing agents), hence increasing the efficacy of phenolic antioxidants. Substituted groups such as –OCH₃ however increase the redox potential of phenols; hence, reducing their antioxidant potential due to a reduction in their ability to donate electrons. Phenolic compounds, flavonoids in particular, are able to chelate metals due to the presence of hydroxyl groups attached to their ring structures. The complexed oxidized forms of metal are more stable and become more difficult to reduce, resulting in a decrease in its tendency to take an electron [93]. Three possible metal complexing positions on a flavonoid molecule that can interact with metal ions by hydrogen bonding have been proposed:

(a) Between the 3’, 4’-dihydroxy group located on the B ring (as in luteolinidin, fisetinidin and luteolin.
(b) Between the 3-hydroxy and 4-carbonyl group in the C ring.
(c) Between the 5-hydroxy of the A ring and 4-carbonyl group in the C ring as in luteolinidin, luteolin. Various flavonoids found in sorghum may be able to chelate metal ions due to the possible metal complexing positions.
1.9.2 Structure-activity relationships between phenolic acid and antioxidant activity

It is widely accepted that physical and chemical properties of a selected compound are determined by its chemical structure and may be altered by a number of other factors. Structure-antioxidant activity relationships of wheat phenolic acids have been discussed in several previous publications. Wheat phenolic acids are generally either benzoic acid or cinnamic acid derivatives with different number of hydroxyl and/or methoxy substituents at different positions of the phenyl ring. The difference between the benzoic and cinnamic acid skeletons is the extension of the conjugated side chain due to presence of an additional conjugated double bond in cinnamic acid structure. When the hydroxyl derivatives of both cinnamic and benzoic acids act as free radical scavengers, they donate a proton to the radical and terminate the radical chain reactions, and form corresponding phenoxy radicals. As shown in fig. 1.7, the phenoxy radicals from cinnamic acid derivatives may form more possible resonance structures than that of the corresponding hydroxyl benzoic acid derivatives, explaining why hydroxyl cinnamic acid derivatives act as stronger radical scavengers than the corresponding hydroxyl benzoic acid derivatives. This conclusion is supported by the observation that p-coumaric acid had stronger ABTS \( \cdot \) and O\(_2\) \( \cdot \) scavenging activity than p-hydroxybenzoic acid, and ferulic acid had stronger ABTS \( \cdot \), O\(_2\) \( \cdot \), and DPPH (2, 2-Diphenyl-1-Picrylhydrazy) scavenging activity than vanillic acid [94-98].

This conclusion is also supported by the previous observation that cinnamic acid derivatives had higher reactivity against the 2,2'-azobis(amidinopropane) dihydrochloride (AAPH) -generated peroxyl radicals and more effective in suppressing lipid peroxidation in human LDL than their benzoic counterparts. Presence of additional hydroxyl and methoxyl substituents on the phenyl ring may enhance the overall antioxidant properties, including radical scavenging and chelating capacities, of the phenolic acids. Caffeic and proto catechuic acids...
exhibited stronger inhibitory effects against lipid peroxidation in human LDL induced by either Cu$^{2+}$ or AAPH than p-coumaric and p-hydroxybenzoic acids, respectively, indicating that the additional o-hydroxyl groups enhanced the antioxidant property of phenolic acids. Caffeic acid had higher ABTS ·•+ and O$_2$ ·•+ scavenging activity, and stronger inhibitory against Cu$^{2+}$ and AAPH-induced lipid peroxidation in human LDL than ferulic acid, suggesting that o-hydroxyl derivative of cinnamic acid may have greater antioxidant activity than their o-methoxyl counterpart. In contrast, vanillic acid (o-methoxyl p-hydroxybenzoic acid) had stronger ABTS ·•+ scavenging activity than protocatechuic acid (o-hydroxyl p-hydroxybenzoic acid). In addition, ESR measurements showed that ferulic and vanillic acids had significant capacity to interact and form chelating complex with Cu$^{2+}$, but syringic acid, p-coumaric acid, and p-benzoic acids could not provide similar mode of action. These data indicate that presence of the first o-methoxyl substituent is critical to chelating capacity against Cu$^{2+}$ under the experimental conditions. In contrast, addition of the second o-methoxyl substituent on the phenyl ring, syringic acid versus vanillic acid, may eliminate the chelating capacity of the phenolic acid because of the interruption of the plane conjugation system due to steric exclusion of the substituents [99]. Taking together, these previous studies suggest that presence of o-methoxyl and o-hydroxyl may enhance overall antioxidant activity of a selected phenolic acid because of their electron donating potentials. The other factors that may alter the antioxidant activity estimation for phenolic acids may include but are not limited to (1) type of radical systems used, (2) physicochemical properties of the testing system, (3) physicochemical interactions between chemical compounds present in the testing reaction, and (4) the determination method. It has been widely recognized that type of radical systems such as the stable pure DPPH·, ABTS ·•+ generated by a chemical reaction outside of the testing system, ·OH generated by a chemical reaction in the testing system, or O$_2$ ·•+ generated using an enzymatic reaction in the testing system may
significantly alter the radical scavenging property estimation. This may be explained by the fact that individual testing systems may involve different reaction mechanisms and may interact with a selected phenolic acid differently. It is recommended that two or more radical systems can be employed in evaluating the radical scavenging property of a selected phenolic acid. [100, 101].

The physicochemical properties of the testing system may significantly alter the antioxidant activity estimation results. For instance, caffeic acid is a stronger antioxidant than ferulic acid in the bulk methyl linoleate system to inhibit hydroperoxide formation, but ferulic acid is more effective to prevent lipid peroxidation in the ethanol-buffer system. This observation is in good agreement with the “polar paradox,” which is the fact that polar antioxidant is generally more effective in bulk oil (low polarity) system and nonpolar antioxidant is more active in polar system (emulsion). This is because of the partition or distribution of the antioxidant in the testing system and the antioxidant molecules at oil-air or oil-water interface may provide better prevention of lipid oxidation. It is understandable that physicochemical interactions between sample components, reagents and contaminations may also alter the antioxidant activity estimation [102-105].

1.10 Antimicrobial Survey

1.10.1 What is Antimicrobial?

A wide variety of active phytochemicals include the flavones, flavonoids, flavonols, phenolics, polyphenolics, quinones, tannins, coumarins, terpenoids, alkanoids, lectins, and polypeptides are believed to the present in plant parts, and possesses antimicrobial properties. Some volatile essential oils of commonly used culinary herbs, spices, and herbal teas have also exhibited a high level of antimicrobial activity. From the literature and other survey, we can tell that flavonoids are naturally occurring substances possessing several biological properties. The antimicrobial effects of some flavonoids and phenolics are studied by several researchers [106].
Antimicrobial chemotherapy has been an important medical treatment since, the first investigations of antibacterial dyes by Ehrlich in the beginning of the twentieth century. However, by the late 1940s bacteria resistant to antimicrobials were soon recognized as a serious problem in clinical environments such as hospitals and care facilities. Bacterial resistance forces the research community to develop methods of altering structures of antimicrobial compounds to avoid their inactivation yet structural modifications alone are not enough to avert bacterial resistance. The increasing use of household antibacterial products and agricultural antimicrobials fosters resistance to drugs specific for human therapy, and may have huge consequences particularly for children, and elderly persons. Antimicrobials contained in manure, and bio solids may enhance selection of resistant bacteria by entering the aquatic environment through pathways of diffuse pollution. Surface water, and shallow groundwater are commonly used for drinking water, and antimicrobials are now found to pollute many aquatic sources [107, 108]. Antimicrobials are used worldwide in human medicine, food, agriculture, livestock and household products [109]. In many cases, the use of antibiotics is unnecessary or questionable. An overview of antimicrobial pathway used in human medicine is shown in Figure 1.12.

![Figure 1.12 Antimicrobials used in human cell and its biochemical pathway](image-url)
Consumption of antibiotics is linked to bacterial resistance. In hospitals, most common resistant bacteria include methicillin-resistant, Staphylococcus aureus, vancomycin-resistant enterococci, and gram-negative rods, including the Enterobacteriaceae, and Pseudomonas aeruginosa. Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with antimicrobial activity, with possibly new modes of action. [110-111].

This expectation that some naturally occurring plant compounds can kill antibiotic-resistant strains of bacteria such as Bacillus cereus, Escherichia coli, Bacillus megaterium, and Staphylococcus aureus. In these studies one finds a wide range of criteria related to the discovery of antimicrobial compounds in plants. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes or naphthoquinones. After detection of antimicrobial activity in the plant extract, some of these compounds were isolated or obtained by bioassay-guided isolation. A second block of studies focuses on the random screening of natural flora of a specific region or country, and the third relevant group of papers is made up of in-depth studies of the activity of a plant or plant compound against a specific pathological microorganism [112, 113].

1.11 Aims and Significance of work
Minor millets are rich dietary source of many phytochemicals/phytonutrients. According to “Ayurveda” minor millet is having laxative and purgative properties, which is due to the presence of phenolics in its matrix. Literature survey shows that a very less attention is given to this class of cereals.

Major aim of the study lies in separation of phenolic acids and flavonoids. Their characterization using various modern technique is as importance as method development and validation. So the present study is focused on two aspects.
1. Extraction of phenolic acid and flavonoids, their isolation with highest possible purity followed by characterization with modern hyphenated analytical techniques.

2. Possible application study of isolated active ingredients and recommendations based on results from study, especially for type 2 diabetic patients.
References


[40] D E Austin, Fox-Tail Millets (Setaria:Poaceae) Abandoned Food In Two Hemispheres, *Economic Botany*, 60 (2), (2006), 143-158.


[42] O S. Lawal. Starch hydroxyalkylation: Physicochemical properties and enzymatic digestibility of native and hydroxypropylated


[79] M Blummel, E Zerbini, B V S Reddy, C T Hash, F. Bidinger, & D. Ravia. Improving the production and utilization of sorghum and


[88] E. Verzelloni, D. Tagliazucchi, & A. Conte, Relationship between the antioxidant properties and the phenolic and flavonoid...


