Chapter 1

Polyphenols and its source *C. terminalis* (L.) kunth and *Myristica fragrans* Houtt.

1.1. INTRODUCTION

Chronic diseases constitute a major challenge for medicine and basic biology and will certainly remain so for the next decades. We have seen the emergence, in epidemic proportions, of modern chronic diseases in the latter part of the 20th century, a process that is still in progress (Wilks et al., 1998). In developing countries, this process is part of what is known as an epidemiological transition (Vio & Albala, 2000). Characteristically, infectious diseases are replaced by chronic or non-communicable diseases as the primary cause of morbidity and mortality. This situation is associated with changes in diet and lifestyle that contribute to the development of chronic diseases. Among the risk behaviors characteristic of the transition are excessive dietary fat intake, low intake of fruits and vegetables, sedentary life style, smoking, and environmental contamination.

A primary focus of preventive medicine is the detection and treatment of individuals at risk, and molecular tools are increasingly used to recognize risk. Today, chronic diseases are at the interface of molecular genetics and preventive medicine. Strikingly, there are some common risk factors and pathophysiological conditions that affect most diseases grouped into the category of modern chronic diseases: cardiovascular disease, hypertension, diabetes mellitus, and some forms of cancer. Oxidative stress is a central risk factor for chronic diseases.

Oxidative stress, the consequence of an imbalance of prooxidants and antioxidants in the organism, is rapidly gaining recognition as a key phenomenon in chronic diseases. It is directly involved in the pathogenic mechanism of risk factors and in the protection exerted by various environmental factors. And the quantification of oxidative stress in populations appears to be a possible indicator for the magnitude of environmental risk factors. Diet plays a major role in the environmental control of
oxidative stress: fruits, vegetables and red wine decrease oxidative stress (Leighton et al., 1999). Compelling evidence has led to the conclusion that diet is a key environmental factor and a potential tool for the control of chronic diseases.

People have used plants for millennia and vast information of the medicinal uses of plants has therefore accumulated especially in the tropical parts of the world. According to the World Health Organization (WHO), about 80 % of the people in developing countries rely primarily on medicinal plants for their primary health care (Wood-Sheldon et al., 1997). Polyphenols are present in a variety of plants utilized as important components of both human and animal diets (Crozier et al., 2000). The economic implications of polyphenolic compounds are substantial as literature reports that polyphenols are able to reduce the inflammation by inhibition of the edema; prevents the development of tumors; present proapoptotic and anti-angiogenic actions; modulate the immune system; prevent the osseous disturbances incriminated in the osteoporosis; increase the capillary resistance by acting on the constituents of blood vessels; protect the cardiovascular system; protect the retina; limit weight gain (Munin & Edwards-Levy, 2011). They are also used in numerous sectors of the food-processing industry as natural additives (natural coloring agents, conservative agents, natural antioxidants, nutritional additives). However, it is probably in the field of human health that the economic implication of polyphenols is the most important. Actually, many plant extracts rich in phenolic molecules of interest are used as food complements or can be integrated into cosmetic or pharmaceutical formulations (Munin & Edwards-Levy, 2011).

Diets containing an abundance of fruit and vegetables are protective against a variety of diseases, particularly cardiovascular disease and cancer. The primary nutrients thought to provide the protection afforded by fruit and vegetables are the antioxidants (Eastwood, 1999). Potter (1997) reviewed 200 epidemiological studies, the majority of which showed a protective effect of increased fruit and vegetable intake. Thus, fruits and vegetables provide the best polypharmacy against the development of a chronic disease, considering that they contain a vast array of antioxidant components such as polyphenols (Urquiaga & Leighton, 2000).
1.2. POLYPHENOLS AND ANTIOXIDANTS

1.2.1 DEFINITION

Polyphenols are polyhydroxylated phytochemicals which are composed of one aromatic ring (A ring) is connected to the second aromatic ring (B ring) by a carbon bridge which consists of three carbon atoms. When the three carbon chain is connected to a hydroxyl group from A, the formed structure become cyclic (C ring), as a 6-membered ring. Most flavonoids bear this type of phenylbenzopyrane structure, which have further been subdivided into various subclasses, based on the position of the B ring relative to the C ring, as well as the functional groups (ketones, hydroxyls) and also by the presence or absence of double bond in the C ring.

![Basic structure of polyphenols](www.sacs.ucsf.edu)

FIGURE: 1.1 Basic structure of polyphenols (Courtesy: www.sacs.ucsf.edu)

1.2.2. CLASSIFICATION OF POLYPHENOLS

Polyphenols are classified on the basis of the number of phenol rings that they contain and of the structural elements that bind phenolic rings to one another. They are broadly divided in four classes; Phenolic acids, flavonoids, stilbenes and lignans.

(a) Phenolic Acids

Phenolic acids are found abundantly in foods and divided into two classes: derivatives of benzoic acid and derivatives of cinnamic acid. Phenolic acids are further
divided into hydroxyl benzoic and hydroxyl cinnamic acids (fig 1.2). The hydroxybenzoic acid content of edible plants is generally low, with the exception of certain red fruits, black radish and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight (Shahidi and Naczk, 1995). The hydroxycinnamic acids are more common than hydroxybenzoic acids and consist chiefly of \( p \)-coumaric, caffeic, ferulic and sinapic acids. Phenolic acids account for about a third of the polyphenolic compounds in our diet and are found in all plant material, but are particularly abundant in acidic-tasting fruits. Caffeic acid, gallic acid, ferulic acid are some common phenolic acids. Stilbenes contain two phenyl moieties connected by a two carbon methylene bridge.

![Chemical structures of sub-classes of flavonoids: Phenolic acids. (Courtesy: Kanti Bhooshan Pandey and Syed Ibrahim Rizvi, (2009)).](image)

**FIGURE 1.2** Chemical structures of sub-classes of flavonoids: Phenolic acids. (Courtesy: Kanti Bhooshan Pandey and Syed Ibrahim Rizvi, (2009)).

(b) Flavonoids

Flavonoids are most abundant polyphenols in human diet and share a common basic structure consist of two aromatic rings, which are bound together by three carbon atoms that form an oxygenated heterocycle (Fig. 1.3). Biogenetically, one ring usually arises from a molecule of resorcinol, and other ring is derived from the shikimate pathway. More than 4,000 varieties of flavonoids have been identified, many of which are responsible for the attractive colours of the flowers, fruits and leaves (de Groot and Rauen, 1998). Based on the variation in the type of heterocycle involved, flavonoids are divided into six subclasses: flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones (Fig. 1.3). Individual differences within each group arise from the variation in number and arrangement of the hydroxyl groups and their extent of alkylation and/or glycosylation. Flavonols (such as quercetin and kaempferol), have a 3-hydroxy pyran-4-
one group on the C ring. Flavanones (such as naringenin and taxifolin), have an unsaturated carbon-carbon bond in the C ring. Flavanols (such as the catechins), lack both a 3-hydroxyl group and the 4-one structure in the C ring. Flavones (such as luteolin), lack a hydroxyl group in the 3-position on the C ring. Anthocyanins (such as cyanidin), are characterized by the presence of an oxonium ion on the C ring and are highly coloured as a consequence and in isoflavones (such as genistein), the B ring is attached to the C ring in the 3-position, rather than the 2-position as is the case with the other flavonoids.

![Chemical structures of sub-classes of flavonoids](image)

**FIGURE 1.3** Chemical structures of sub-classes of flavonoids (Courtesy: Kanti Bhooshan Pandey and Syed Ibrahim Rizvi, 2009).

(c) Stilbenes

Stilbenes contain two phenyl moieties connected by a two-carbon methylene bridge (Fig. 1.4). Occurrence of stilbenes in the human diet is quite low. Most stilbenes in plants act as antifungal phytoalexins, compounds that are synthesized only in response to infection or injury. One of the best studied, naturally occurring polyphenol stilbene is
resveratrol (3, 4', 5-trihydroxystilbene), found largely in grapes. A product of grapes, red wine also contains significant amount of resveratrol.

(d) Lignans

Lignans are diphenolic compounds that contain a 2, 3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues (Fig. 1.4). Several lignans, such as secoisolariciresinol, are considered to be phytoestrogens. The richest dietary source is linseed, which contains high amounts of secoisolariciresinol and low quantities of matairesinol (Mazur, 1997).

(a). Stilbenes

(b). Lignans

FIGURE 1.4 Chemical structures of the different classes of polyphenols (a) Stilbens, (b) Lignins. (Courtesy: Kanti Bhooshan Pandey and Syed Ibrahim Rizvi, (2009)).

1.2.3. PLANT POLYPHENOLS AS NATURAL ANTIOXIDANTS

There are several thousand plant-derived compounds of biological interest that have more than one phenolic hydroxyl group attached to one or more benzene rings, thus named as polyphenols.

In recent years, polyphenols have gained a lot of attention because of their potential use as therapeutic agents in many diseases, and much work has been done by many scientific groups which focus on their antioxidant effects. They constitute a large group of phytochemicals with more than 8000 identified compounds (Table 1). Traditionally, herbal medicines with antioxidant properties have been used for various purposes. Plant polyphenols have been studied with intention to find compounds which
are protecting against a number of diseases related to oxidative stress and free radical-induced damage, such as cardiovascular and neurodegenerative diseases, cancer, diabetes, autoimmune disorders and some inflammatory diseases. In order to evaluate the efficacy of polyphenols as antioxidants as well as to elucidate their action mode, researchers today are using a wide range of experimental models, from the simplest chemical antioxidant assays through the biologically more relevant cellular-based assays to the most accurate animal models, and ultimately clinical studies in humans. The latest scientific knowledge offers a more detailed understanding of the biological effects of polyphenols and their role in human health promotion and disease prevention. The majority of the polyphenols are from plants (Sanda Vladimir-Knezevic et al., (2012)). Thus the plant polyphenols act as the natural antioxidants.

**Table 1** Classification of natural polyphenols.

<table>
<thead>
<tr>
<th>Polyphenols</th>
<th>Falvonoids</th>
<th>Anthocyanins</th>
<th>Flavonols</th>
<th>Flavones</th>
<th>Flavanones</th>
<th>Isoflavonoids</th>
<th>Isoflavones</th>
<th>Isoflavanes</th>
<th>Flavanols</th>
<th>Monomers</th>
<th>Oligomers and polymers</th>
<th>Non-flavonoids</th>
<th>Phenolic acids</th>
<th>Derivatives of cinnamic acid</th>
<th>Derivatives of benzoic acid</th>
<th>Lignans</th>
<th>Stilbenes</th>
</tr>
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<tr>
<td>E.g. aurantinidin, cyanidin, delphinidin, europinidin, luteolinidin, pelargonidin, malvidin, peonidin, petunidin, rosinidin, etc.</td>
<td>E.g. 3-hydroxyflavone, azaleatin, fisetin, galangin, gossypetin, kaempferide, kaempferol, isorhamnetin, morin, myricetin, natsudaidain, pachypodol, quercetin, rhamnazin, rhamnetin, etc.</td>
<td>E.g. apigenin, luteolin, tangeritin, chrysirn, 6-hydroxyflavone, baicalein, scutellarein, wogonin, diosmin, flavoxate, etc.</td>
<td>E.g. butin, eriodictyol, hesperetin, hesperidin, homoeriodictyol, sosakuranetin, naringenin, naringin, pinocembrin, poncirin, sakuranetin, sakuranin, sterubin, etc.</td>
<td>E.g. genistein, daidzein, lonchocarpane, laxiflorane, etc.</td>
<td>E.g. equol, etc.</td>
<td>E.g. catechin, epicatechin (EC), epigallocatechin (EGC), Epicatechin, gallate (ECG), epigallocatechin gallate (EGCG), epiafzelechin, fisetinidol, guibourtinidol, mesquitol, robinetindol, etc.</td>
<td>E.g. theaflavins, thearubigins, condensed tannins, proanthocyanidins</td>
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(Adapted from Ebrahimi, & Schluesener / Ageing Research Reviews 11 (2012))
1.2.4. APPLICATIONS OF PLANT POLYPHENOLS AS ANTIOXIDANTS

The following are the applications of plant polyphenols as antioxidants:

(i) Polyphenols as antioxidants

Epidemiological studies have repeatedly proved that an inverse association between the risk of chronic human diseases and the consumption of polyphenolic rich diet (Scalbert et al., 2005), (Arts and Hollman, 2005). The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxy radicals, thereby disrupting chain oxidation reactions in cellular components (Clifford, 2000). It is well known that polyphenol-rich foods and beverages may increase plasma antioxidant capacity. This increases in the antioxidative capacity of plasma by consumption of polyphenol-rich food.

![Diagram of applications of polyphenols in different areas](image)

**FIGURE 1.4** Applications of polyphenols in different areas (Adapted from: Kanti Bhooshan Pandey and Syed Ibrahim Rizvi, (2009)).

Consumption of antioxidants has been associated with reduced levels of oxidative damage to lymphocytic DNA. Similar observations have been made with polyphenol-rich food and beverages indicating the protective effects of polyphenols (Vitrac *et al.*, 2002). There are number of evidences which says that as antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various
Degenerative diseases associated with oxidative stress (Luqman and Rizvi, 2006; Pandey et al., 2009).

(ii) Plant polyphenols in protection of cardiovascular diseases (C.V.Ds):

Many studies have proved that consumption of polyphenols limits the incidence of coronary heart diseases (Renaud and de Lorgeril, 1992) (Dubick and Omaye, 2001) (Nardini et al., 2007). Atherosclerosis is a chronic inflammatory disease that develops in lesion-prone regions of medium-sized arteries.

Polyphenols are potent inhibitors of LDL oxidation and this type of oxidation is considered to be a key mechanism in development of atherosclerosis (Aviram et al., 2000). Other mechanisms by which polyphenols may be protective against cardiovascular diseases are antioxidant, anti-platelet, anti-inflammatory effects as well as increasing HDL, and improving endothelial function (García-Lafuente et al., 2009).

Quercetin, the abundant polyphenol in onion has been shown to be inversely associated with mortality from coronary heart disease by inhibiting the expression of metalloproteinase 1 (MMP1), and the disruption of atherosclerotic plaques (García-Lafuente et al., 2009).

Tea catechins have been shown to inhibit the invasion and proliferation of the smooth muscle cells in the arterial wall, a mechanism that may contribute to slow down the formation of the atheromatous lesion (Maeda et al., 2003).

Polyphenols may also exert antithrombotic effects by means of inhibiting platelet aggregation. Consumption of grapes juice (red wine) or non-alcoholic wine reduces bleeding time and platelet aggregation. Thrombosis induced by stenosis of coronary artery is inhibited when grape juice (red wine) or grape juice is administrated (Demrow et al., 1995).
Polyphenols can improve endothelial dysfunction associated with different risk factors for atherosclerosis before the formation of plaque; its use as a prognostic tool for coronary heart diseases has also been proposed (Schachinger et al., 2002).

A higher consumption of tea and therefore higher excretion of 4OMGA were associated with lower blood pressure (BP). Tea polyphenols may be the components responsible for the lowering of BP. The effect may be due to antioxidant activity as well as improvement of endothelial function or estrogen like activity (García-Lafuente et al., 2009). Resveratrol, the wine polyphenol prevents the platelet aggregation via preferential inhibition of cyclooxygenase 1 (COX 1) activity, which synthesizes thromboxane A2, an inducer of the platelet aggregation and vasoconstrictor (Pirola and Frojdo, 2008). In addition to this, resveratrol is capable of relaxing the isolated arteries and rat aortic rings.

Association between polyphenol intake or the consumption of polyphenol-rich foods and incident of cardiovascular diseases were also examined in several epidemiological studies and it was found that consumption of polyphenol rich diet have been associated to a lower risk of myocardial infarction in both case-control and cohort studies (Peters et al., 2001).

(iii) Plant polyphenols in Anti-Cancer Effect

Cancer effects have been observed at various sites, including mouth, stomach, duodenum, colon, liver, lung, mammary gland or skin. Many polyphenols, such as quercetin, catechins, isoflavones, lignans, flavanones, ellagic acid, red wine polyphenols, resveratrol and curcumin have been tested; all of them showed protective effects in cell line models although their mechanisms of action were found to be different (Johnson et al., 1994).

Several mechanisms of action have been identified for chemoprevention effect of polyphenols, these include estrogenic/antiestrogenic activity, antiproliferation, induction of cell cycle arrest or apoptosis, prevention of oxidation, induction of detoxification enzymes, regulation of the host immune system, anti-inflammatory activity and changes
in cellular signaling (García-Lafuente et al., 2009). Effect of polyphenols on human cancer cell lines, is most often protective and induce a reduction of the number of tumors and their growth (Yang et al., 2001).

Khan and Mukhtar (2008) reported that tea catechins in the form of capsules when given to men with high-grade prostate intraepithelial neoplasia (PIN) demonstrated cancer preventive activity by inhibiting the conversion of high grade PIN lesions to cancer. Theaflavins and thearubigins, the abundant polyphenols in black tea have also been shown to possess strong anticancer property. Black tea polyphenols were found to inhibit proliferation and increase apoptosis in Du 145 prostate carcinoma cells (Sharma and Rao, 2009).

Polyphenols like Quercetin has also been reported to possess anticancer property against benzo(a)pyrene induced lung carcinogenesis in mice, an effect attributed to its free radical scavenging activity (Kamaraj et al., 2007). Resveratrol prevents all stages of development of cancer and has been found to be effective in most types of cancer including lung, skin, breast, prostate, gastric and colorectal cancer. It has also been shown to suppress angiogenesis and metastasis. Extensive data in human cell cultures indicate that resveratrol can modulate multiple pathways involved in cell growth, apoptosis and inflammation. The anti-carcinogenic effect of resveratrol appears to be closely associated with its antioxidant activity (Athar et al., 2007).

(iv) Plant polyphenols as anti-diabetic effect

Enormous studies reported the antidiabetic effects of polyphenols. Tea catechins have been investigated for their anti-diabetic potential (Rizvi et al., 2005; Rizvi and Zaid, 2001). Polyphenols may affect glycemia through different mechanisms, including the inhibition of glucose absorption in the gut or of its uptake by peripheral tissues.

Matsui et al., (2001) reported that the inhibition of intestinal glycosidases and glucose transporter by polyphenols has been studied. Individual polyphenols, such as
(+)-catechin, (-) epicatechin, (-)-epigallocatechin, epicatechin gallate, isoflavones from soyabean, tannic acid, glycyrrhizin from licorice root, chlorogenic acid and saponins also decrease S-Glutathionase-1 mediated intestinal transport of glucose. Polyphenols like saponins additionally delay the transfer of glucose from stomach to the small intestine (Dembinska-Kiec et al., 2008).

Resveratrol, a polyphenol has also been reported to act as an anti-diabetic agent. Many mechanisms have been proposed to explain the anti-diabetic action of this stilbene, modulation of SIRT1 is one of them which improve whole-body glucose homeostasis and insulin sensitivity in diabetic rats (Harikumar and Aggarwal, 2008) (Milne et al., 2007). Treatment with resveratrol also decreased insulin secretion and delayed the onset of insulin resistance (Chen et al., 2007).

Onion polyphenols, especially quercetin is known to possess anti diabetic activity. A recent study shows that quercetin has ability to protect the alterations in diabetic patients during oxidative stress. Quercetin significantly protected the lipid peroxidation and inhibition antioxidant system in diabetics (Rizvi and Mishra, 2009).

A study reported as by Lee et al., (2009) showed that polyphenols present in the extracts from Hibiscus sabdariffa attenuate diabetic nephropathy including pathology, serum lipid profile and oxidative markers in kidney.

Ferulic acid (FA) is another polyphenol very abundant in vegetables and maize bran. Several studies have shown that FA acts as a potent anti-diabetic agent by acting at many levels. It was demonstrated that FA lowered blood glucose followed by significantly increased plasma insulin and a negative correlation between blood glucose and plasma insulin (Barone et al., 2009) (Jung et al., 2007).

(v) Plant polyphenols as Anti-Aging agents

Several researchers suggest that the combination of antioxidant/anti-inflammatory polyphenolic compounds found in fruits and vegetables may show effectiveness as anti-aging compounds (Cao et al., 1998) (Joseph et al., 2005).
Among many theories proposed for the explaining the mechanism of aging, free radical/oxidative stress theory is one of the most accepted one (Harman 2006). A certain amount of oxidative damage takes place even under normal conditions; however, the rate of this damage increases during the aging process as the efficiency of antioxidative and repair mechanisms decrease (Rizvi and Maurya, 2007a; 2007 b).

Antioxidant capacity of the plasma is related to dietary intake of antioxidants; it has been found that the intake of antioxidant rich diet is valuable in reducing the harmful effects of aging. Fruit and vegetable extracts that have high levels of flavonoids also display high total antioxidant activity such as spinach, strawberries and blueberries. It is reported that the dietary supplementations (for 8 weeks) with spinach, strawberry or blueberry extracts in a control diet were also effective in reversing age-related deficits in brain and behavioral function in aged rats (Shukitt-Hale et al., 2008).

Another study demonstrates that the tea catechins carry strong anti-aging activity and consuming green-tea rich in these catechins, may delay the onset of aging (Maurya and Rizvi, 2008).

Polyphenols are also helpful in ameliorating the unfavorable effects of the aging on nervous system or brain. Grape polyphenol, resveratrol is very recent entry as an anti-aging agent (Harikumar and Aggarwal, 2008). Recently quercetin has also been reported to exert preventive effect against aging (Belinha et al., 2007).

(vi) Plant polyphenols showing Neuro-Protective Effects

Polyphenols from fruits and vegetables seem to be valuable potential agents in neuroprotection by asset of their ability to influence and modulate several cellular processes such as signaling, proliferation, apoptosis, redox balance and differentiation (Singh et al., 2008). It has been reported that the consumption of fruit and vegetable juices containing high concentrations of polyphenols, at least three times per week, may play an important role in delaying the onset of Alzheimer’s disease (Dai et al., 2006).
Letenneur et al., (2007) reported that polyphenols are highly antioxidative in nature their consumption may provide protection in neurological diseases. It also reported that the people drinking three to four glasses of wine per day had 80% decreased incidence of dementia and Alzheimer’s disease compared to those who drank less or did not drink at all (Scarmeas et al., 2007).

Resveratrol a polyphenol which scavenges O$_2$ - and OH $\cdots$ free radicals in vitro, as well as lipid hydroperoxyl free radicals and shows antioxidant activity is involved in the beneficial effect of the moderate consume of red wine against dementia in the elderly. Resveratrol inhibits nuclear factor κB signaling and thus gives protection against toxicity in a model of Alzheimer’s disease (Markus and Morris, 2008).

Recently Aquilano et al., (2008) reported that administration of polyphenols provide protective effects against Parkinson’s disease. Nutritional studies have linked the consumption of green tea to the reduced risk of developing Parkinson’s disease. In animal models epigallocatechin gallate (EGCG) has been shown to exert a protective role against the neurotoxin MPTP (N-methyl-4- phenyl-1, 2, 3, 6-tetrahydropyridine), an inducer in Parkinson’s disease. EGCG may also protect neurons by activating several signaling pathways (Rossi et al., 2008). The therapeutic role of catechins in Parkinson’s disease is also due to their ability to chelate iron (Aquilano et al., 2008). Maize bran polyphenol, ferulic acid is also reported to be beneficial in Alzheimer’s disease. This effect is due to its antioxidant and anti-inflammatory properties (Barone et al., 2009).

(vii) The other beneficial effects of polyphenols as antioxidants

Polyphenols also show several other health beneficial effects. Dietary polyphenols exert preventive effects in treatment of asthma. Polyphenols like isoflavone, genistein, were associated with better lung function in asthmatic patients (Smith et al., 2004). Intake of polyphenols is also reported as beneficial in osteoporosis. Supplementation of diet with genistein, daidzein or their glycosides for several weeks prevents the loss of bone
mineral density and trabecular volume caused by the ovariectomy (Nakajima et al., 2001).

Polyphenols also protect skin damages induced from sunlight. Study on animals provide evidence that polyphenols present in the tea, when applied orally or topically, ameliorate adverse skin reactions following UV exposure, including skin damage, erythema and lipid peroxidation (Kim et al., 2001).

Black tea polyphenols are reported to be helpful in mineral absorption in intestine as well as to possess antiviral activity. Polyphenols like theaflavins present in black tea were found to have anti human immune virus (HIV-1) activity. These polyphenols inhibited the entry of HIV-1 cells into the target cells. Theaflavin 3'3' digallate, and theaflavin 3' gallate were found to inhibit Severe Acute Respiratory Syndrome (SARS) corona virus (Sharma and Rao, 2009).

1.2.5. RATIONAL FOR CHOOSING C. TERMINALIS AND MYRISTICA FRAGRANS

Main objective of the study is the polyphenols identification and stability of the polyphenols encapsulated in casein beads for a period of one year. Polyphenols play major role in human health.

Based on the local availability we have chosen two plants for our studies, the above two plants are (C. terminalis (L.) kunth and Myristica fragrans Houtt). C. terminalis (L.) kunth also known as goodluck plant belongs to the Agavaceae family. The subfamily has previously been treated as a separate family Laxmanniaceae. Myristica fragrans Houtt. also known as nutmeg belongs to the Myristicaceae family as in the literature have been reported that these plants are rich in polyphenols. The reasons for choosing these plants are:

- These are easily available in this geographical zone.
- Economic.
1.3. *CORDYLINE TERMINALIS* (L.) KUNTH

*Cordyline terminalis* (L.) kunth commonly called as Good luck plant. *Cordyline’s* are known to the tropical world by many names & are crowned as “king of tropical foliage” with a vast rampout range of colors and sizes; it is reaching height approximately 10 among gardens, land scapes and collections alike.

It is the most popular *Cordyline* species as an indoor potted plant and is used extensively by florists as cut green foliage for flower arrangements and decorative displays. It is also used as a food wrapping (Kent Kobayashi *et al.*, 2007).

*C. terminalis* (L.) kunth (*C. terminalis*) formerly treated in the families Agavaceae and Laxmanniaceae (now both subfamilies of the Asparagaceae in the Angiogerm phylogeny group (APG III system). Botanists had previously placed it in Lily family (Wong, 2007).

1.3.1. SCIENTIFIC CLASSIFICATION

<table>
<thead>
<tr>
<th>Kingdom –</th>
<th>Plantae</th>
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<tr>
<td>Subkingdom –</td>
<td>Tracheobionta</td>
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<td>Species -</td>
<td><em>C. terminalis</em> L.Kunth</td>
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1.3.2. BOTANICAL DESCRIPTION

*C. terminalis* is an upright evergreen shrub with slender single or branched stems, growing up to 10 feet high. Its spread is 3–4 feet. The growth rate is moderate to moderately fast. The taproot is long, thick, white, and sweet and becomes large in older plants.

Leaves: Leaf clusters are arranged in close spirals at the branch tips. Leaf type is simple. Leaf margin is entire, shape is linear, venation is parallel, leaf type and persistence is evergreen. The large, narrow-oblong leaves (1–2 feet long and about 4 inches wide) are smooth, flexible, and glossy, with deeply channeled petioles 2–6 inches long. Some varieties have very small leaves, only 8 inches long and 2 inches wide. Leaf blades are lanceolate to broadly elliptic with a prominent midrib on the underside. Foliage may be green or variegated with various combinations of red, pink, purple, maroon, rose, yellow, and orange. Seedlings and propagated cuttings show their true color when they begin to produce mature leaves. Older basal leaves turn yellow and drop from the stems, leaving rings of leaf scars.

Flowers: Many sessile (stalkless) or shortly stalked flowers 1⁄3–1⁄2 inch long are borne on a panicle (drooping branched stem) about 1 foot long that grows from the tip of the plant in the spring. The flowers come in several colors white, pink, lavender, or yellow. As the six petals open, they expose six yellow stamens and one white pistil. Florets are star-shaped. Flowering is in spring season.

Fruits: The fruits, which are not uncommon, are round berries less than 1⁄2 inch in diameter containing many seeds. The fruit cover is fleshy and may be green, yellow, red, or scarlet. Some cultivars have red flowers and large scarlet berries.

Trunk and Branches: Trunk/bark/branches are not particularly showy; typically multi-trunked or clumping stems. The Current year stem/twig color is reddish and Current year
stem/twig is thick. The plant has outstanding ornamental features and could be planted more (Kent Kobayashi et al., 2007).

Botanical Synonyms: The name Cordyline comes from "kordyle," the Greek word for club. The epithet "terminalis" describes the flowers resting at the terminal end of the leaf cluster. The term "cabbage tree" refers to the fact that the sailors of Captain Cook ate the young shoots of Cordyline like cabbage. Cordyline fruticosa, Convallaria fruticosa, Dracaena terminalis, Terminalis fruticosa, Taetsia fruticosa (Native of Eastern Asia).

Vernacular names: Vernacular names of C. terminalis are Boundary marsh, Cabbage tree, Dracaena palm, Lily palm, Hawaiian good-luck plant, Green ti, ti tree, good-luck plant, tree of kings, Common dracaena, Dracaena, Miracle plant.

Plant material of interest:
Leaves are linear in shape, leaf venation is parallel and leaf type is evergreen. The large, narrow-oblong leaves (1–2 feet long and about 4 inches wide) are smooth, flexible, and glossy, with deeply channeled petioles 2–6 inches long. Some varieties have very small leaves, only 8 inches long and 2 inches wide. Leaf blades are lanceolate to broadly elliptic with a prominent midrib on the underside. Foliage may be green or variegated with various combinations of red, pink, purple, maroon, rose, yellow, and orange (Edward F. Gilma, 1999).
FIGURE 1.6. *C. terminalis*: a) Whole plant (b) leaves; (c) fruits and (d) flowers

1.3.2. GEOGRAPHICAL DISTRIBUTION:

*C. terminalis* originated from the tropical and sub tropical regions which is found throughout tropical Asia, Australia, and the Pacific islands. *C. terminalis* belongs to Agavaceae family and are with about 20 species is found growing throughout East
Himalayas, China, Malaysia and North Australia (Saifullah Khan, 2004). Among them *C. terminalis* and *Cordyline fruticosa* are being the most popular.

Generally *C. terminalis* grows in moist, semi-shaded areas in wet valleys and forests and the temperature between 65 and 95°F and is hardy to about 30°F. Leaf color is more intense during the cooler weather in the winter and spring (Kent Kobayashi et al., 2007).

1.3.3. CONSTITUENTS

*C. terminalis* leaves contain a range of biologically active compounds. The various constituents found in *C. terminalis* includes Smilagenin, sarsapogenin, imidazole alkaloids, linoleic acid, tyramine, sterols, tyramine, saponins, glycosidic compounds, Quinic acid, shikimic acid, linoleic acid, sarsasapogenin, smilagenin, tyramine, sterols, imidiazole alkaloids and a compound closely related to cinchophen are reported in *C. terminalis* (Yoshida et al., 1975; Weiner, 1971).

1.3.4. APPLICATIONS / USES

The important therapeutic uses of the *C. terminalis* described in folk medicine and in traditional systems of medicine, the list of different illnesses and conditions, aided by use of *C. terminalis* is indeed impressive, covering everything includes from antiseptic, aphrodisiac, depurative, diuretic, febrifuge, hemostatic, laxative, purgative, and tonic. Clear heat, cool the blood. Bland, sweet, cool. However, the young raw leaf shoots are sweet and edible.

(1) As cosmetics
- *C. terminalis* plant roots are used to treat for stopping hair loss and Baldness

(2) As dermatological
- *C. terminalis* plant juice is used for Skin ailments and for dermatosis in Malaya.
- *C. terminalis* leaf juice is used for Eczema.
(3) *C. terminalis* plant as digestive

- The *C. terminalis* plant parts are used for indigestion and for stomach problems in Caroline Islands.
- The leaf juice of *C. terminalis* is used for abdominal disease, pain and gastritis and the leaf bud is used for lower chest pain.
- The *C. terminalis* plant parts are used for diarrhea, enteritis, dysentery, severe constipation and lump in the stomach”.
- Unfurled juice of *C. terminalis* terminal leaves stirred in water is used for eye wash and forehead, throat, Eye problems, eyestrain, inflammation and the normal leaf juice is used for eye infection. For Nasal polyps the flowers are juiced and snorted.
- *C. terminalis* plants has been used for the treating the Liver cancer in China and for malarial fevers in Malaya.

(4) As Chinese traditional medicine

- Cools the blood and stops bleeding.
- Clears heat quells fire.
- Reduces swelling.
- Clears the lungs and opens the chest.
- Clears lower burner damp heat.
- *C. terminalis* plant parts are used in China for bleeding hemorrhoids.
- *C. terminalis* plant is used for fevers in Trinidad and for smallpox in Philippines.

(5) Use as food

The *C. terminalis* roots were boiled or baked and made into food, a sweet candy, and as a fermented drink. The leaves were used in Hawai'i to preserve breadfruit. Also used for cooking in underground ovens (called an 'imu) in Hawai'i, and as a sweetener in Fiji. While the leaves of native New Zealand Cordyline, reportedly have been eaten by the Maori (Cambie and Ferguson, 2003).
(6) Other commercial uses

*C. terminalis* with its beautiful and attractive red decorative foliage is one of the most economically important ornamental houseplant.

*C. terminalis* leaf skirts are worn for the hula. Leaves have been used as thatchs for houses, rain capes, sandals, plates, food for horses and cattle, fishnets for hukilau, whistles, and as sleds to slide down slopes. Dry leaves were fastened to fishing nets to drive fish into shallow water in hukilau fishing. Fresh leaves are used as a wrapper for foods such as taro, pork, and fish before they are steamed or roasted in an imu.


Seeds and berries: The seeds of *C. australis* are one of the richest sources of linoleic acid (Cambie and Ferguson, 2003).

1.4. *MYRISTICA FRAGRANS* HOUTT.

*Myristica fragrans* (*M. fragrans*) is commonly known as Nut meg, a tropical evergreen dioecious tree with narrow range of distribution is the source of the high value medicinal spices, nutmeg (endosperm) and mace (the reddish aril) has immense phytochemical diversity and is a horticultural crop of great importance (Latha *et al.*, 2005). The *M. fragrans* tree is any of several species of trees in genus Myristica. The important commercial species is *M. fragrans*. The *M. fragrans* tree is important for two species divided from fruit: nutmeg and mace.

Nutmeg is known to have been used as a spice and medicine in India and the Middle East as early as 700 B.C., (Kalbhen, 1971), while its therapeutic applications have been recorded by Arab physicians since the seventh century (Weil, 1967). did not appear
in Europe until the Middle Ages and reports conflict regarding whether it was introduced by Arab traders or by returning crusaders, although it was probably a little of both. While introduced to Europe in the middle Ages, *M. fragrans* was likely a rare commodity until the sixteenth century when the Portuguese discovered that the Banda Islands were the hitherto concealed source of *M. fragrans* (Stein *et al*., 2001).

1.4.1. SCIENTIFIC CLASSIFICATION

Angiogerm phylogeny group (APG III classification) of *M. fragrans*.

Kingdom ....................................................... *Plantae* - Plants
Subkingdom ............................................... *Tracheobionta* - Vascular plants
Super division .......................................... *Spermatophyte* - Seed plants
Division ................................................... *Mangoliophyta* - Flowering plants
Class .......................................................... *Mangoliopsida* - Dicotyledons
Subclass .................................................... *Mangoliidae*
Order .......................................................... *Mangoliales*
Family ....................................................... *Myristicaceae*
Genus ....................................................... *Myristica*. - nutmeg
Species ..................................................... *Myristica fragrans* Houtt. - nutmeg

Botanical synonym: The name nutmeg comes from Latin, *nux muscat*, meaning musky nut. Legend has it that when *M. fragrans* sets seed (Krieg., 1964). Nut meg belongs to the family of myristiceae family .this genus consists of over 100 species of myristica plants *Myristica argentea*, *M. fragrans*, *Myristica inutilis*, *Myristica malabarica*, *Myristica macrophylla*, *Myristica otoba*, *Myristica platysperma* etc. Most formularies and reference books regard myristica fragrans as the correct species name, and *M. fragrans* Houtt. as a synonym.
Vernacular names: Vernacular names of *M. fragrans* are *jaiphal* in most parts of India. Hindi: *jaiphal*, Kannada: *jaipha*, Tamil: *jaayi-kaayil/jaipatre*, *jathikai*, Kerala: *jatipatri* and *jathi* seed, Telugu: *jaaji kaaya* and mace is called *jaapathri*.

Plant part of interest: The seeds of *M. fragrans* Houtt. is used. Myristica fragrans is a spice harvested from plants of the Myristica genus. It comes from the plants seed, and its outer red membrane is scrapped to form the spice form.

**FIGURE 1.7.** *M. fragrans* tree showing with (a) leaves, fruits and seed. (b) Dried seeds (Courtesy: [http://www.google.co.in/imgaes](http://www.google.co.in/imgaes); [http://cache.psychotropicicon.info/](http://cache.psychotropicicon.info/); [http://www.thefloweringgarden.com](http://www.thefloweringgarden.com))

**FIGURE 1.8.** *M. fragrans*: (a) Fresh fruit, (b) dried seeds and (c) powdered form ([http://www.thefloweringgarden.com/myristica-fragrans.htm](http://www.thefloweringgarden.com/myristica-fragrans.htm))
Fruit: *M. fragrans* and mace are two important spices derived from the fruit. *M. fragrans* is the actual seed of the tree, roughly egg-shaped and about 20–30 mm long and 15–18 mm wide, and weighing between 5 and 10 grams dried, Mace is within *M. fragrans* fruit, and dried "lacy" reddish covering or arillus of the seed. The most important species commercially is the Common or Fragrant Nutmeg. *M. fragrans*, native to the Banda Islands of Indonesia and Caribbean, especially in Grenada. Other species include Papuan Nutmeg, *Myristica argentea* from New Guinea, and Bombay Nutmeg, *Myristica malabarica* from India; both are used as adulterants of *M. fragrans* products. Several other commercial products are also produced from the trees, including essential oils, extracted oleoresins, and nutmeg butter. Nutmeg is the seed of the tree. It is always used in powdered form. It roughly egg-shaped and size about 20 to 30 mm (0.8 to 1 inch) long and 15 to 18 mm (0.6 to 0.7 inch) wide, and weighing between 5 and 10 g (0.2 and 0.4 oz) dried, while mace is the dried "lacy" reddish covering or aril of the seed.

1.4.2. BOTANICAL DESCRIPTION

*M. fragrans* is a spreading, medium to large sized, aromatic evergreen tree usually growing to around 5-13 m high, occasionally 20 m. Leaves alternate, pointed, dark green 5-15 cm in length and 2-7 cm width arranged along the branches and are borne on leaf stems about 1 cm long, shiny on the upper surface. Flowers dioecious, pale yellow, waxy, fleshy and bell-shaped. Male flowers 5-7 mm long and in groups of 1-10; female flowers up to 1 cm long and in groups of 1-3 occasionally both sexes are found on the same tree. When ripe, husk splits into 2 halves revealing a purplish-brown, shiny seed surrounded by a leathery red or crimson network of tissue. The shiny, brown seed inside, and the kernel of the seed is the Nutmeg. The brown seed has a red cover that makes another spice called Mace. Bark contains watery pink or red sap.

1.4.3. GEOGRAPHICAL DISTRIBUTION

*M. fragrans* is a genus of evergreen trees indigenous and native to the Banda Islands in eastern Indonesia (Moluccas) and is cultivated in the Banda Islands, Grenada,
the Caribbean, South India, Sri Lanka, Malaysia, Sumatra, Brazil, tropical Southeast Asia and Australasia. It also grows in Kerala, a state in southern India. The major producers of *M. fragrans* are Indonesia and Grenada which dominate production and exports of both products with a world market of 75% and 20%, respectively. Other countries that supply *M. fragrans* to the world market include India, Malaysia, Papua New Guinea and Sri Lanka, and other Caribbean countries. The major importers are the European Community, the United States, Japan and India (Abdullah et al., 2010).

1.4.4. CONSTITUENTS

*M. fragrans* consists of 45-60% cellulose and solid matter, 24-40% of fixed oils and 5-15% of volatile oils. The fixed oil (or "butter") of *M. fragrans* is an orange-colored waxy substance. The butter contains 70-85% trimyristin, which also contains myristic acid. Mainly monoterpenes (87.5%), monoterpane alcohols (5.5%), and other aromatics (7%) are present. The main components in *M. fragrans* is essential oil, the main constituents of essential oil is sabinene, α-pinene, myrcene, limonene, 1,8-cineole, terpinen-4-ol, myristicin, γ-terpinene, and safrole has been reported by Pooja et al., (2012). The actual activity of *M. fragrans* extracts has been reported in the volatile (or essential) oils (Nagano I, 2008).

Twenty eight compounds were identified, accounting for 92.9% of the contents. They are: α-Thujene , α- Pinene , Camphene, Sabinene , β – Pinene, Myrcene , Phellandrene 3-Carene , Terpinene , p-Cymen, Limonene, γ-Terpinene , Terpinolene , Linalool , cis-p-Menth-2-en-1-ol, β-Terpineole , Terpine-4-ol , α-Terpineol, Safrole , α-Cubebene ,α-Terpinyl acetate ,Citronellyl acetate, Eugenol, Geranyl acetate, Methyl eugenol , Myristicin , Vanillin acetate , Elemicin. Depending on its origins, mace has 7% to 14% essential oil and about 30% fixed oil. It contains the same aroma compounds as *M. fragrans* but in different amount (Milan Suhaj, 2006), Siddharthan Surveswaran et al., (2007).
1.4.5. APPLICATIONS/USES

- *M. fragrans* has been used in folk medicine since seventh century. In the 19th century it was used as an abortifacient, which led to numerous recorded cases of *M. fragrans* poisoning, although used as a folk treatment for other ailments.

- *M. fragrans* are popular as an alternative to standard Western allopathic medicine for a variety of problems, including acting as an aphrodisiac, reducing skin problems as well as relieving muscle spasm.

- *M. fragrans* has been used in European medieval cuisine as a flavouring, medicinal, and preservative agent.

- The leaves juice of *M. fragrans* used as tea to relief flatulence and intestinal spasm. The shoots are used medicinally to treat hypertension. The mace was preferred to use as tonic to stomach and for healing headache and migraine (Milanjuki et al., 2006). The mace is also used in folk medicine for curing rheumatism.

- The seed kernel of *M. fragrans* is widely used as spice with possible health beneficial effects such as aphrodisiac, anthelmintic, anticonvulsant and antiseptic and useful in treating inflammation, vomiting, diarrhea, dysentery, asthma, heart disease, liver and spleen disorder, insomnia, colic, menorrhagia (Sharma et al., 2002), flatulence, nausea and dyspepsia.

- A resin obtained from the bark is applied externally to treat polyarthritis and gout.

- *M. fragrans* used as folk medicine

  ✓ Since the time that *M. fragrans* became popular as a spice, it has also been used in medicine. *M. fragrans* has been employed for healing purposes from the Middle East, to India, to China.

  ✓ The essential oil of *M. fragrans* is used externally to treat rheumatic pains, limb pains, general aches, and inflammation (Rudgley 1998).

  ✓ *M. fragrans* has been used for its sedative effect to treat nervous complaints and to promote sleep in Malaysia and India.

  ✓ *M. fragrans* has also been widely used as an analgesic and *M. fragrans* is most widely used to treat stomach complaints.

  ✓ *M. fragrans* has been used in South East Asia, India, the Middle East, and Europe to treat stomach aches and cramps, to aid digestion, and to dispel gas.
- *M. fragrans* used as medicine
  - Indian mixture containing *M. fragrans* used against tuberculosis, smallpox, measles, skin disease, rheumatism, cardiac ailments and as antifungal remedy (Tewary and Mishra, 1997).

- Used as food stuff:
  - In Indian cuisine, *M. fragrans* is used almost exclusively in sweets and in small quantities in garam masala for flavouring many dishes. Where as in the Caribbean *M. fragrans* is often used in Barbados rum punch drinks, just a sprinkle on the top of the drink.
  - *M. fragrans* butter is trimyristin which can be turned into myristic acid, a 14-carbon fatty acid and can be used as replacement for cocoa butter, can be mixed with other fats like cottonseed oil or palm oil, and has applications as an industrial lubricant.
  - *M. fragrans* in traditional folk medicine used as aphrodisiac, skin problems, reducing heartburn, rheumatism, muscle spasm, increasing appetite, relieving diarrhea, in Ayurvedic medicine for premature ejaculation and incontinence. *M. fragrans* has certain therapeutic properties and benefits of using it internally, in the form of an herbal tea (infusion).

- Pharmacology and Toxicity:
  - Myristicin is active at the 5-HT receptors in the brain, and has been shown to have hypotensive, sedative, anti-depressant, anesthetic, hallucinogenic, and serotonergic properties (Sangalli & Chiang, 2000).
  - Several studies on mice suggest that myristicin may reduce and inhibit the growth of tumors. One study showed that myristicin significantly reduced tumor formation in the lungs and forestomachs of mice with benzo (a) pyrene-induced carcinogenicity (Hallstrom & Thuvander, 1997). Myristicin has also been shown to be an inducer of GST (glutathione S transferase), a substance that inhibits tumorigenesis.
  - Myristicin was shown to cause a fourfold increase in GST activity in the liver and a threefold increase in the small intestine (C.S.W.G. 1997).
Elemicin has displayed anti-depressant, hallucinogenic, anti-histamine, hypotensive and anti-serotonergic properties (Sangalli & Chiang, 2000).

1.5. OBJECTIVES OF THE STUDY

The proposed project deals with studying the following objectives.

- To extract and identify the polyphenols and their subclasses from seeds of *Myristica fragrans* and leaves of *Cordyline terminalis*.
- To encapsulate the polyphenols using natural polymers.
- To check the stability of the polyphenols.

Many experiments were conducted to fulfill the above objectives. The experimental conditions and procedures are too extensive to be described in a common chapter on "Materials and Methods". Therefore, for convenience, each chapter describes experiments and will have sections on introduction, materials and methods, results, and discussion.

In this work, attempt has been made to study the polyphenol content and antioxidant activities of *Cordyline terminalis* (L.) kunth and *Myristica fragrans* Houtt. The stability of the polyphenols and antioxidant activities of both the plants with extracts and extracts encapsulated in casein beads for duration of 12 months. To identify the polyphenols present in *Cordyline terminalis* (L.) kunth and *Myristica fragrans* Houtt through RP-HPLC and to confirm through HPLC-ESI-MS. A comparative study for the stability of extracts and extracts encapsulated with casein has been carried out for a period of 12 months.