In vitro and *In vivo* Validation of Folk Lore Claims of *Thymus serpyllum*

Dissertation Submitted for the Award of the Degree of Masters of Philosophy in Biochemistry

By

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CERTIFICATE

Certified that the work embodied in the dissertation entitled **"*In vitro* and *In vivo* validation of folk lore claims of *Thymus serpyllum*"** is the bonafide work of Ms. Farrukh Rana Mufti and has been carried out under our guidance and supervision in the Department of Biochemistry, University of Kashmir, Srinagar and Indian Institute of Integrative Medicine, Jammu. The work is suitable for the award of M.Phil degree in Biochemistry.

It is further certified that no work under this heading has previously been submitted to the University of Kashmir for the award of any degree or diploma, to the best of our belief.

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DECLARATION

I, Farrukh Rana Mufti, declare that the work embodied in this dissertation entitled **"*In vitro* and *in vivo* validation of folk lore claims of *Thymus serpyllum*"** has been carried out by me in the Department of Biochemistry, University of Kashmir, Srinagar and the Indian Institute of Integrated Medicine, Jammu and is original. The work embodies the results of my observations which are advancement to the previous knowledge in the subject.

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Farrukh Rana Mufti

Abbreviations

%	Percent
µg	Microgram
hr	Hour
Kg	Kilogram
g	Gram
mg	Milligram
mm	Milli meter
nm	Nano meter
ml	Milli litre
µl	Micro litre
min	Minute
µM	Micromolar
mM	Milli molar
M	Molar
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reacting species
MIC	Minimum inhibitory concentration
CFU	Colony forming units
MDA	Malondialdehyde
MOPS	3-(N-morpholino) propanesulfonic acid
RPMI	Rosewell Park Memorial Institute medium
EDTA	Ethylene diamine tetra acetate

DMSO	Dimethyl sulpho oxide
DPPH	1,1- diphenyl- 2- picrylhydrazyl
LPO	Lipid peroxidation
COX	Cyclooxygenase
e-NOS	Endothelial Nitric Oxide Synthase
NF-kB	Nuclear Factor kB
AP 1	Activator Protein 1
rpm	revolutions per minute
^{o}C	Degree celcius

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ABSTARCT

Traditional medicinal plants have a long history of therapeutic use. The beneficial health effects of medicinal plants is often attributed to their potent antioxidant activities due to the presence of secondary metabolites like the polyphenols, since diets rich in antioxidants are epidemiologically associated with a decreased incidence of age-related diseases in humans.

Thymus serpyllum commonly known as Jawand in Kashmir is used as a culinary herb, as well as for aromatizing and traditional medicinal purposes. In the present study, the hexane, ethyl-acetate, ethanol, methanol and the aqueous extracts of *Thymus serpyllum* were studied for the antioxidant, antimicrobial and the anti inflammatory activities.

The antioxidant activity was checked by four different methods- DPPH assay, lipid peroxidation assay of liver microsomes, lipid peroxidation assay and hydroxyl radical scavenging assay. The ethyl-acetate, butanol, ethanol, methnol and the aqueous extracts of *Thymus serpyllum* showed good antioxidant activities, but the methanolic extract being the most active one causing 89.84%, 57.32%, 66.8% and 77.14% inhibition of the radical activity in DPPH assay, lipid peroxidation assay of liver microsomes, lipid peroxidation assay and the hydroxyl radical scavenging assay respectively. The antimicrobial activity was checked by broth micro dilution method. The ethyl-acetate and the methanolic extract were found to be active against bacteria (both gram positive and gram negative) and fungi with MIC values ranging from 2000 to 4000µg/ml. The anti inflammatory activity was assessed by using the model of carregeenen induced edema in rats. The butanolic extract was found to beneficial against inflammation, causing a reduction of 25.23% and 56.07% of edema at doses 250mg/Kg body weight and 500mg/Kg body weight respectively.

INTRODUCTION

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Herbal drugs have gained lot of acceptance in the recent years because they have a relatively higher therapeutic window, less serious side effects, and are economical. They have been extensively studied in many diseases such as cancer, liver diseases, and infectious diseases as well as in neurological disorders like stroke with promising results.

With the associated side effects of western medicine, herbal preparations are gaining a lot of importance and are now being studied to find the scientific basis of their therapeutic actions (Gupta et al., 2010).

Plants have been used as a source of medicine throughout history and continue to serve as the basis for many pharmaceuticals used today. Most botanical therapeutics are derived from medicinal plants that have been cultivated for increased yields of bioactive components. Plants continue to serve as a valuable source of therapeutic compounds because of their vast biosynthetic capacity. A primary advantage of botanicals is their complex composition consisting of collections of related compounds having multiple activities that interact for a greater total activity (Schmidt et al., 2008). Historically, natural products have provided an endless source of medicine. Plant-derived products have dominated the human pharmacopoeia for thousands of years almost unchallenged (Raskin et al., 2004). In 1897, Arthur Eichengrün and Felix Hoffmann, working at Friedrich Bayer, created the first synthetic drug, aspirin. Aspirin (acetylsalicylic acid) was synthesized from salicylic acid, an active ingredient of analgesic herbal remedies (Schmidt et al., 2008).

Ecological relations between co-existing organisms have provided the most obvious demonstration of nature's principles for people living in the wild. In addition to their essential function as the most available food source, plants, which have been waging a co-evolutionary war against herbivores for 300 million years by means of various secondary metabolites, were certainly the main source of medicinal treatment. These powerful sources of metabolites with their biological activities, alongside the different practices in terms of preparing and applying herbal remedies that have been developed by certain ethnic groups throughout the centuries, have become the most useful database for the evaluation of new pharmaceuticals. The incorporation of local knowledge concerning ecological relations into biological and ecological studies strengthens the links between man and the environment, leading to the global conservation of biodiversity. At the same time, this guides scientific research in a new

direction in the sense of determining lesser known biological material from the wild (Jaric´ et al., 2007).

In India, the history of health care goes back to 5000 years B.C., when health care needs and diseases were noted in ancient literatures like 'Rig-Veda' and 'Atharva-Veda'. Later, the texts like 'Charak Samhita' and 'Sushruta Samhita' were documented in about 1000 years B.C., where use of plants and polyherbal formulations was highlighted for health care. Evolution of Ayurveda and plant-based remedies for health care through day-to-day life experiences is a part of cultural heritage of India. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies on traditional medicine for their primary health care needs. In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their backbone. Indian Materia Medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices (Narayana et al., 1998). It is difficult to get reliable figures for the total number of medicinal plants on earth; according to some estimation, around 35,000–70,000 plant species are being used worldwide in health care systems (Farnsworth and Soejarto, 1991). According to WHO estimations the populations in developing countries like India (70%), Ruwanda (70%), Uganda (60%), Tanzania (60%), Benin (80%) and Ethiopia (90%) extensively use traditional and alternative medicines for health care. Plants and plant-based products are an integrated part of most of the traditional and alternative systems of medicines world wide. In developed countries like Belgium (31%), USA (42%), Australia (48%), France (49%), Canada (70%), a significant percentage of the population has used traditional and alternative remedies at least once for health care (WHO, 2002). The global market of trade related to medicinal plants is estimated around US $60 billions per year and is growing at the rate of 7% annually with varying shares (Fig. 1) of developed and developing countries (Dev, 1999; Laird and Pierce, 2002; Raskin et al., 2002). A study reveals that about 42% of the best selling pharmaceutical products in 1997 were biologicals or natural products or chemical entities derived from natural resources, worth of US $17.5 billion (Laird and Kate, 2002).

Ayurveda and other Indian systems of medicine have been developing since the first human civilizations in the Indian subcontinent. These systems are based on experience and interaction with nature and natural resources. Scientific evidence to prove the rationale of using these formulations in health care is essential to develop and to preserve the cultural heritage. Many important modern drugs are plant-based or derived directly or indirectly from

the plants. But only 6% of all therapeutically important species, which are noted in ancient literature, have been analyzed phytochemically for their therapeutic potential (Choudhary, 2002).

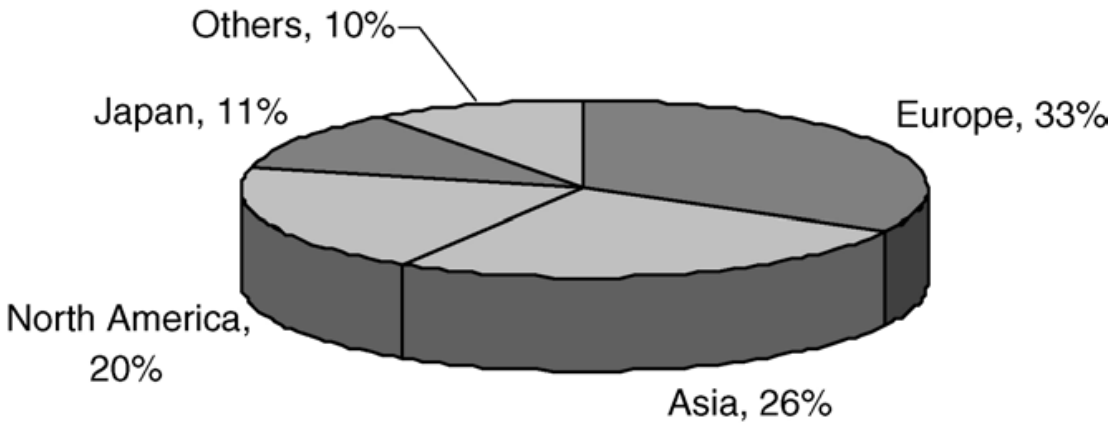

Figure 1.1. World market of herbal remedies

Source: Mukherjee et al., 2006.

REVIEW OF LITERATURE

Recent archeological records suggest that modern agriculture started in the Near East 10 000 to 11 000 years ago with the domestication of figs, cereals, and legumes (Abbo et al., 2003, Kisler et al., 2006). At that time, early Neolithic farmers maintained a subsistence strategy, collecting wild plants for food and medicine while simultaneously domesticating early crops. This point in time marked the beginning of the divergence between medicinal plants and food plants.

Plants must maintain and protect themselves through diverse arrays of complex natural products that they make from the inorganic components of air, soil, and water because they lack the flight response. Remarkably, the oldest known living eukaryotic organism, turning 4772 years old in 2007, is a specimen of a bristlecone pine, *Pinus longaeva*, growing in the White Mountains of Inyo County, California (Flanary et al., 2005) There are many plants that can live hundreds of years without succumbing to diseases or predation. So it should be possible that some of the compounds that have enabled plants to survive may also be used to maintain the health and well-being of humans (Schmidt et al., 2008).

Medicinal plants commonly consumed worldwide contain different chemical substances that display a broad spectrum of biological activities, enabling the induction of positive effects in treatment of many human diseases. The use of plant extracts as functional ingredients in various food, beverage and cosmetic applications is gaining growing interest among scientists, as well as among consumers and food manufacturers. In the last few decades, numerous screening studies of various plant materials have been performed in order to find naturally occurring antioxidants for use in food or medicinal preparations, as replacements for potentially harmful synthetic additives (Reische et al., 1998). The dominant majority of biologically active plant compounds with antioxidative properties are flavonoids and other phenolics. These low molecular weight secondary plant metabolites exhibit excellent antioxidant properties. However, their particular mechanisms of action vary depending both on the structure and environment. Besides phenolic compounds, medicinal plants are reported to contain other natural antioxidants such as vitamins (A, B6, C, E) and some other phytochemicals like co-enzyme ubiquinone (Q10), carotenoids, selenium and zinc (Atoui et al., 2005).

Epidemiological evidence demonstrates that diets rich in fruit and vegetables promote health, and attenuate, or delay, the onset of various diseases, including cardiovascular disease, diabetes, certain cancers, and several other age-related degenerative disorders. The

chemical components and the physiological and molecular mechanisms by which fruit and vegetables reduce the risk for these pathophysiological conditions are matters of intense investigation. Regarding plant components, polyphenols are a group of phytochemicals that are gaining acceptance as responsible for the health benefits offered by fruit and vegetables. Because of their chemical structure, plant polyphenols are able to scavenge free radicals and inactivate other pro-oxidants. The connection of these chemical properties to a physiological antioxidant action has triggered extensive research aimed to relate the consumption of plant polyphenols with human health. Although significant progress has been made, there are still some critical areas that need to be elucidated to arrive at definitive conclusions on the mechanisms linking plant polyphenol consumption, reduction in oxidative damage, and health improvement (Fraga, 2007).

Oxidation reactions are an essential part of normal metabolism as oxygen is the ultimate electron acceptor in the electron flow system that produces ATP (Davis et al., 1993). Problems may arise when electron flow and energy production become uncoupled so that oxygen free radicals, that is, reactive oxygen species (ROS), are produced (Nohl et al., 2005). Actually, ROS are continuously produced within the cell as a result of mitochondrial electron transfer processes or as bioproducts of the enzymes xantine oxidase, lipoxygenases and cycloxygenases (Szocs et al., 2004). Furthermore, ROS can be generated as a consequence of the intracellular metabolism of foreign compounds, toxins or drugs by cytochrome P450, monoxygenases,or because of exposure to environmental factors such as excessive iron salts or UV irradiation (Ichihashi et al ., 2003). Other sources of ROS are macrophages and neutrophils that contain enzymes, such as NADPH oxidase complex, able to generate superoxide radicals and hydrogen peroxide (Rosen et al., 1995). Reactive oxygen species thus play different positive roles in vivo, being involved in energy production, phagocytosis, cell growth and intercellular signalling regulation. Reactive oxygen species may be also highly damaging, as they can attack biological macromolecules, namely, lipids, proteins and DNA, induce oxidation and cause membrane damage, enzyme inactivation and DNA damage (Halliwell et al., 1999; Valko et al., 2004). However, when the level of ROS exceeds the antioxidant capacity of the cell, the

intracellular redox homeostasis is altered and oxidative stress ensues (Halliwell, 1999). Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative diseases, such as atherosclerosis, cardiovascular disease, type 2 diabetes and cancer (Gutteridge, 1993; Kehrer, 1993; Storz, 2005). In order to cope with an excess of free

radicals produced upon oxidative stress, humans have developed sophisticated mechanisms in order to maintain redox homeostasis. These protective mechanisms either scavenge or detoxify ROS, block their production, or sequester transition metals that are the source of free radicals, and include enzymatic and nonenzymatic antioxidant defenses produced in the body, namely, endogenous (Hayes et al., 1999; Seis, 1999), and others supplied with the diet, namely, exogenous (Benzie, 1999; Yao et al., 2004; Porrini et al ., 2005). Among these, natural polyphenol compounds have been largely studied for their strong antioxidant capacities and, recently, for additional properties by which cell activities are regulated (Masella et al., 2005).

Many compounds in plants and vegetables have the ability of reacting with free radicals without generating further radicals, therefore, quenching chain reactions. Other compounds scavenge ROS and in so doing they become oxidized and need to be regenerated for further use. Antioxidant compounds react directly with radicals reducing oxidative stress and exerting their protective effects against cellular damage (Halliwell, 1997; Gactke et al., 2003; Gawreih et al., 2004).

Polyphenols comprise a wide variety of compounds that occur in fruits and vegetables, wine and tea, chocolate and other cocoa products (Manach et al., 2004). More than 8,000 polyphenolic compounds have been identified in various plant species. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linkage with other phenol is also common (Kondratyuk et al., 2004).

Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans (Spencer et al., 2008). Figure 2 illustrates the different groups of polyphenols and their chemical structures.

Epidemiological studies showed that increased intake of polyphenols was associated with reduced risk of cardiovascular diseases, cancer and neurodegenerative disorders (Hertog et al., 1996a, 1996b; Sesso et al., 1999; Yochum et al., 1999; Huxley et al., 2003; Arts et al.,

2005). The beneficial effects of polyphenols are mainly ascribed to their capacity to counteract conditions of oxidative stress that accompany these pathologies. Several polyphenols have been demonstrated to have clear antioxidant properties in vitro as they can act as chain breakers or radicals scavengers depending on their chemical structures, which also influence their antioxidant power (Hu et al., 1995; Rice-Evans, 1995,2001; Rice-Evans et al., 1996; Nijveldt et al., 2001). A hierarchy has been established for the different polyphenolic compounds within each class on the basis of their capability to protect lipids, proteins or DNA against oxidative injury (Aviram et al., 1998; Masella et al., 1999, 2001; Coni et al., 2000; Heijnen et al., 2002; Szeto et al., 2002). As a consequence, many of their biological actions have been attributed to those antioxidant properties (Luximon-Ramma et al., 2002). This concept, however, appears now to be a simplistic way to conceive their activity (Azzi et al., 2004). On the other hand, accumulating evidence indicates that polyphenols exhibit several additional properties in complex biological systems, but which are as yet poorly understood (Middleton et al., 2000; Spencer et al., 2001; Williams et al., 2004). Experimental data are available about the multiple potential biological activities of polyphenols:

(i) inhibition or reduction of different enzymes such as telomerase (Naasani et al., 2003), cycloxygenases (Laughton et al., 1991; O'Leary et al., 2004; Hussain et al., 2005), lipoxygenases (Schewe et al., 2001; Sadik et al., 2003), xanthine oxidase (Van Hoom et al., 2002), metalloproteinase (Isemura et al., 1999; Oak et al., 2004), angiotensin-converting enzyme (Actis-Goretta et al., 2003), protein kinases (Agullo et al., 1997; Gamet-Payrastre et al., 1999);

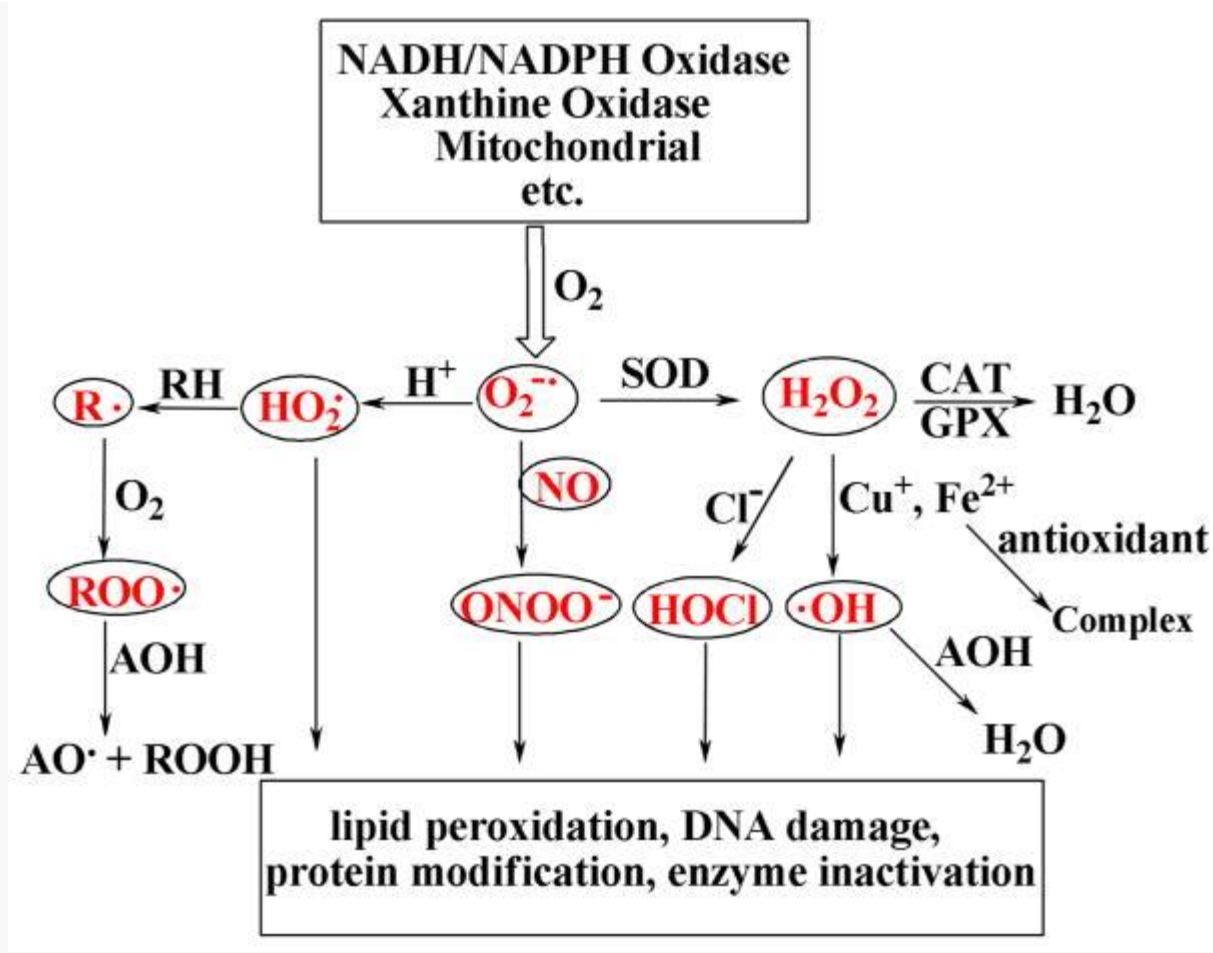

Figure 2.1. ***Summary of ROS types and sources, and action point of antioxidants.*** **$O_2^{-\bullet}$, superoxide anion; $HO_2^{\cdot}$, perhydroxyl radical; ·OH, hydroxyl radical; H_2O_2, hydrogen peroxide; NO, nitric oxide; HOCl, hypochlorous acid; $ONOO^-$, peroxynitrite; R·, lipid alkyl radical; RH, lipid; ROO·, lipid peroxyl radical; ROOH, lipid hydroperoxide; SOD, superoxide dismutase; CAT, catalase; and GPX, glutathione peroxidase.**

Source: Lü et al, 2010.

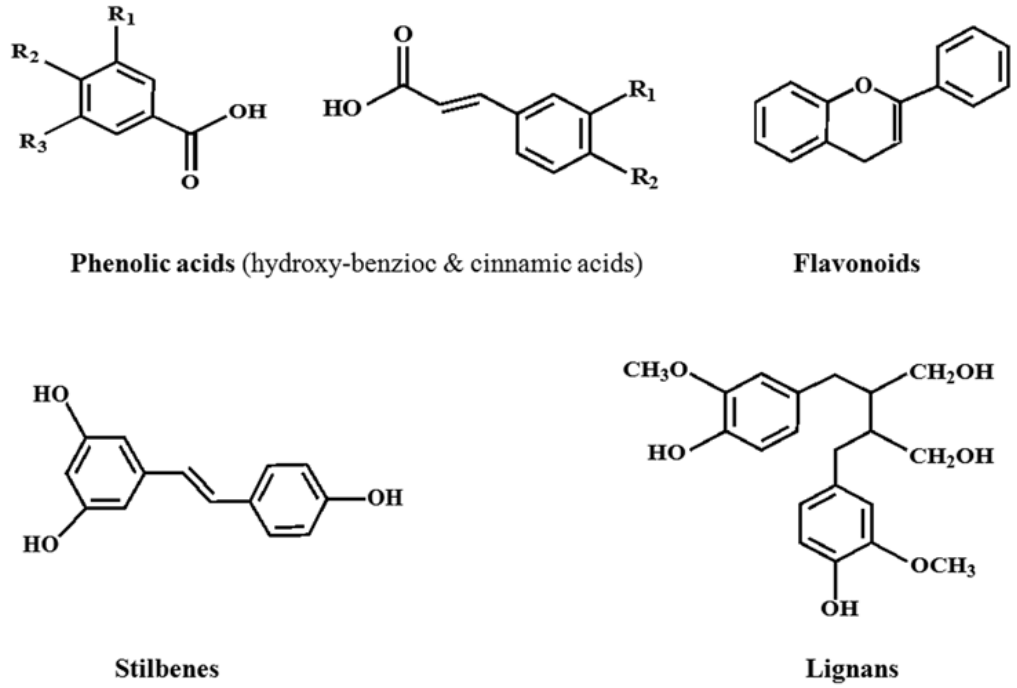

Figure 2.2. ***Chemical structures of the different classes of polyphenols.***

Polyphenols are classified on the basis of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. They are broadly dived in four classes; Phenolic acids, flavonoids, stilbenes and lignans. Phenolic acids are further divided into hydroxyl benzoic and hydroxyl cinnamic 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. 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. Biogenetically, one ring usually arises from a molecule of resorcinol, and other ring is derived from the shikimate pathway. Stilbenes contain two phenyl moieties connected by a twocarbon methylene bridge. Most stilbenes in plants act as antifungal phytoalexins, compounds that are synthesized only in response to infection or injury. The most extensively studied stilbene is resveratrol. Lignans are diphenolic compounds that contain a 2,3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues.

Source: Kanti Bhooshan Pandey, 2009.

(ii) interaction with signal transduction pathways (Kong et al., 2000; Wiseman et al., 2001; Spencer, 2003);

(iii) interaction with cell receptors (Rosenkranz et al., 2002; Muller et al., 2004).

Polyphenols may also interact with caspase-dependent pathways (Monasterio et al.,2004; Sergeev et al., 2004; Way et al., 2005); interfere with cyclin-dependent regulation of the cell cycle (Fischer et al., 2000); induce detoxifying enzymes (Brit et al., 2001); enhance the production of vasodilating factors such as nitric oxide (Aldini et al., 2003; Wallerath et al., 2003); affect the platelet function (Murphy et al., 2003); compete with glucose for transmembrane transport (Vera et al., 1996). It is mainly by virtue of these properties that polyphenols exert their protective effects and receive more and more attention as therapeutic agents against cancer and cardiovascular diseases (Brit et al., 2001; Kris-Etherton et al., 2002). Experimental data indicate that they may also offer an indirect protection by activating endogenous defense systems (Masella et al., 2005).

Antioxidants As Free Radical Scavengers And Metal Chelators

The presence of phenolic groups confers antioxidant characteristics to plant polyphenols, flavonoids, and flavan-3-ols. These characteristics are due to the hydrogen of the phenoxyl groups that is prone to be donated to a radical, and by the ensuing structure that is chemically stabilized by resonance. Bors (Bors et al., 1997) postulated three criteria that should define the best free radical scavenging activity for flavonoids:

(i) the presence of two hydroxy groups in the 3',4' position on the B ring resulting in stability to the radical formed mainly in the 3’ position;

(ii) a double bond in the 2,3 position providing higher conjugation with other double bonds; and

(iii) 3- and 5-hydroxyl groups with a 4-oxo function.

Quercetin is a flavonoid (flavonol) that fulfills these criteria and flavan-3-ols partially accomplish them. However, no definitive structure-activity can be established. The flavanoid conjugation in plants or metabolization after consumption can alter the free radical scavenging capacity of the parent compound. For example, the glucuronidation or sulfation of quercetin reduces its free radical scavenging activity (Morand et al., 1998), but the presence of a gallate group in the EC molecule increases its free radical scavenging activity (Salah et al ., 1995). The oligomerization of EC to form procyanidins can decrease or enhance antioxidant capacity depending whether the activity was evaluated in synthetic

membranes oxidized in the presence of free radical generating molecules or ferrous iron (Lotito et al., 2000). As an alternative antioxidant property, some polyphenols with dihydroxy groups can conjugate metals, preventing metal-catalyzed free radical formation (Guo et al., 1996). Essentially it is accepted that iron, as well as other redox active metals (e.g., copper and manganese) catalyze the decomposition of hydrogen peroxide into hydroxyl radicals which is one of the most powerful oxidant species, being able to initiate free radical chain reactions by abstracting hydrogen from almost any molecule. In theory, these two antioxidant actions can result in a reduction in the steady state concentration of free radicals and oxidant species, diminishing the subsequent oxidation of target molecules such as lipids, proteins and nucleic acids. In addition to the oxidation of these physiologically important macromolecules, another relevant detrimental effect of oxygen radicals include the reaction of NO with superoxide anion to form peroxynitrite (Radi, 2004). Thus, trapping of radicals could be a key mechanism to maintain NO-mediated actions. Based on these potential capacities to prevent free radical mediated reactions, there is an extensive literature demonstrating that flavonoids in general, and flavan-3-ols, in particular, have free radical scavenging activity in a myriad of biochemical and *ex vivo* systems.

Following the observed antioxidant actions *in vitro,* flavonoids and flavan-3-ols have been also widely evaluated in vivo in laboratory animals. Also, it has been shown that catechin administration prevented ethanol-induced liver oxidation in rats (Videla et al., 1983) and carbon tetrachloride-induced oxidative damage in mice as determined by *in situ* liver chemiluminescence (Fraga et al., 1987).

Subsequently a large number of studies with rodents demonstrated clear associations between the presence of the administered flavonoid and an antioxidant protection. Studies in experimental animals have included feeds with not only purified flavonoids, but also flavonoid-rich foods (Osakabe et al., 2000; Orozco et al., 2003). In summary, the evidence from *in vitro* systems as well as from experimental animals consistently supports the antioxidant effects of flavan-3-ols, either as monomeric units or procyanidins, as well as other flavonoids and polyphenols (Fraga, 2007).

Antioxidants and Membrane Interactions

Flavonoids and procyanidins have multiple phenolic hydroxyls that favour their interaction with biological membranes that can occur via the formation of hydrogen bonds. Further, the presence of both, hydrophobic and hydrophilic residues within the flavan-3-ol molecule, allow these compounds to interact with phospholipid head groups and be adsorbed onto the surface of membranes. These interactions can result in changes in a number of membrane properties leading to alterations in the regulation of membrane- bound enzymes and receptors (Conseil et al., 1998; Rosenkranz et al., 2002; Lancon et al., 2004; Tachibana et al., 2004). Using purified procyanidins, it has been observed that these compounds interact with synthetic membranes protecting them against both, chemically- induced oxidation and detergent-induced membrane disruption (Verstraeten et al., 2003, 2004, 2005; Erlejman et al., 2004). Furthermore, procyanidins can induce changes in membrane surface potential regulating the transport of molecules across the membrane as reported in Caco-2 cells (Erlejman et al., 2006). In all these studies it was observed that the effects on membrane properties were strongly dependent on the type and size of the flavonoid studied, underscoring the effect of 4 – 6 units procyanidins (Oteiza et al., 2005).

Antioxidants and Protein Interactions

Flavan-3-ols polymerize forming tannins, which have an astringent taste and occur in wine, chocolate, and other flavan- 3-ol-containing foods. This astringency is related to tannins precipitating saliva proline-rich proteins (Luck et al., 1994). Polyphenol protein interactions have been described that are similar to antigen-antibody interactions in that a binding agent and a ligand associate through multiple moieties to form a complex. For polyphenol-protein interactions, the chemical characteristics of such ‘multivalent’’ associations are mainly related to:

(i) the hydrophobicity of the aromatic nuclei of polyphenols and

(ii) the availability of multiple phenolic hydroxyls allowing hydrogen bonding (Hagerman et al., 1981). As mentioned, proline-rich proteins are preferential targets of polyphenol interactions. It has also been reported that a significant number of enzyme activities are inhibited by polyphenols, flavonoids, and flavan- 3-ols (Middleton et al., 2000). Some of these enzymes are directly associated with oxidant metabolism, such as 5-lipoxygenase (Schewe et al., 2001, 2002), cyclooxynase- 2 (Kim et al., 2004), and metalloproteinases (Oak et al., 2004). Additionally, it has also been characterized the inhibition of the angiotensin converting enzyme (ACE) by flavan-3-ols and procyanidins from cacao, wine and tea (Actis-

Goretta et al., 2003, 2006; Ottaviani et al., 2005). It is worth noting that flavan-3-ol consumption leads to an increase in vascular NO levels which has been shown to be associated with the redox-sensitive phosphorylation of endothelial NO synthase (NOS) (Ndiaye et al., 2003). However, the concentrations of flavan-3-ols that are required to inhibit or activate the enzymes are significantly lower than the concentrations necessary to inhibit oxidation by free radical scavenging or metal chelation. The interaction of polyphenols and proteins can also lead to changes in the modulation of gene expression. A direct interaction between nucleic acids and polyphenols is thermodynamically feasible (Ottaviani et al., 2002), but the possibility that these compounds reach the DNA and achieve mechanistically relevant concentrations is rather low. The modulation of signaling pathways by polyphenols has been extensively addressed (Khan et al., 2006). More specifically, the effects of flavan-3-ol and procyanidins on the oxidant-regulated NF-kB activation pathway have received special attention. EGCG, the major polyphenol in tea, prevented NF-kB activation being triggered by different stimuli, in several cell types, and by acting at different steps in the activation cascade (Yang et al., 1998; Afaq et al., 2003; Wheeler et al., 2004). The molecular mechanisms associated with EGCG go beyond the control of the cellular levels of oxidants, including for example the inactivation of IKK (Yang et al., 2001; Chen et al., 2002), and the activation of caspases (Gupta et al., 2004). In Jurkat T cells, it was demonstrated that EC and CT, and a dimer fraction isolated from cocoa inhibited phorbol mirystate acetate (PMA)-induced IL-2 production, and interfered with several steps of the NF-kB activation cascade (Mackenzie et al., 2004). Essentially, it should be considered that monomers and dimers (and their metabolites) can be transported into the cells and then could act by:

(i) attenuating the increase of oxidants associated with select stimuli, and the subsequent activation of NF-kB (antioxidant effect); and/or

(ii) interacting with specific proteins, resulting in the inhibition of the phosphorylation and/or degradation of the inhibitory protein I-kB, the transport of active NF-kB from the cytosol into the nucleus, and/or the binding of NF-kB to kB DNA (Mackenzie et al., 2004,2006). Large procyanidins (with 3 or more units), that are mostly affecting cells from outside, could regulate NF-kB activation by modulating the binding of the ligand (stimuli) to its receptor (Fraga, 2007).

Antioxidants and Human Diseases

Epidemiological studies have repeatedly shown an inverse association between the risk of chronic human diseases and the consumption of antioxidant rich diet (Arts et al., 2005; Scalbert et al., 2005). The phenolic groups in polyphenols can plasma following accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components (Clifford, 2000). It is well established that polyphenol-rich foods and beverages may increase plasma antioxidant capacity. This increase in the antioxidative capacity of the consumption of polyphenol-rich food may be explained either by the presence of reducing polyphenols and their metabolites in plasma, by their effects upon concentrations of other reducing agents (sparing effects of polyphenols on other endogenous antioxidants), or by their effect on the absorption of pro-oxidative food components, such as iron (Scalbert et al., 2005). Consumption of antioxidants has been associated with reduced levels of oxidative damage to lymphocytic DNA. Similar observations have been made with plyphenol-rich food and beverages indicating the protective effects of polyphenols (Vitrac et al., 2002). There are increasing evidences 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 et al., 2006; Pandey et al., 2009a, 2009b).

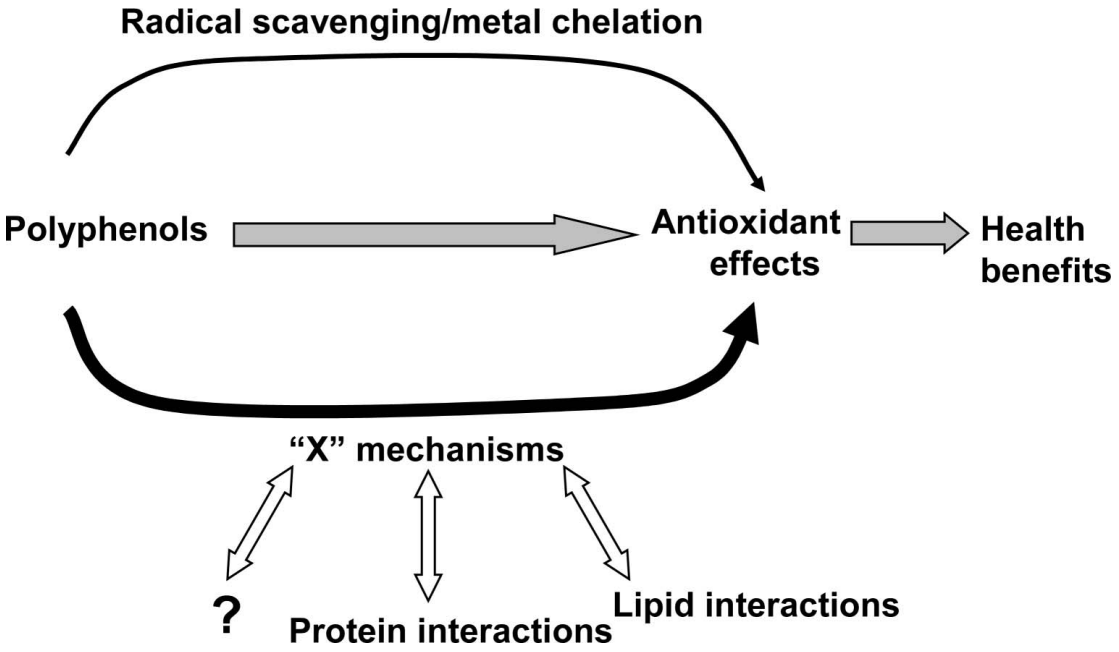

Figure 4. Scheme relating polyphenols with health benefits, through their observed antioxidant effects. The body of the black arrows indicates the 'x' mechanism with free radical scavenging or metal chelating capacity of polyphenols.

Source: Fraga, 2007.

Cardio-Protective Effect

Number of studies has demonstrated that consumption of polyphenols limits the incidence of coronary heart diseases (Renaud et al., 1992; Dubick et al., 2001; Nardini et al., 2007). Atherosclerosis is a chronic inflammatory disease that develops in lesion-prone regions of medium-sized arteries. Atherosclerotic lesions may be present and clinically silent for decades before becoming active and producing pathological conditions such as acute myocardial infarction, unstable angina or sudden cardiac death (Vita, 2005). 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 (Garcia-Lafuente et al., 2009). Polyphenols may also contribute to stabilization of the atheroma plaque. Polyphenols may also exert antithrombotic effects by means of inhibiting platelet aggregation. Consumption of red wine or non-alcoholic wine reduces bleeding time and platelet aggregation. Thrombosis induced by stenosis of coronary artery is inhibited when 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. 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 (Garcia-Lafuente et al., 2009). 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).

Anti-Cancer Effect

Effect of polyphenols on human cancer cell lines, is most often protective and induce a reduction of the number of tumors or of their growth (Yang et al., 2001). These 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 some models although their mechanisms of action were found to be different (Johnson et al., 1994). Development of cancer or carcinogenesis is a multistage and microevolutionary process. Into the three major stages of carcinogenesis: initiation, promotion and progression. Initiation is a heritable aberration of a cell. Cells so initiated can undergo transformation to malignancy if promotion and progression follow. Promotion, on the other hand, is affected by factors that do not alter DNA sequences and involves the selection and clonal expansion of initiated cells. 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 signalling (Garcia-Lafuente et al., 2009). Polyphenols influence the metabolism of pro-carcinogens by modulating the expression of cytochrome P450 enzymes involved in their activation to carcinogens. They may also facilitate their excretion by increasing the expression of phase II conjugating enzymes. This induction of phase II enzymes may have its origin in the toxicity of polyphenols (Scalbert et al., 2005). Polyphenols can form potentially toxic quinones in the body that are, themselves, substrates of these enzymes. The intake of polyphenols could then activate these enzymes for their own detoxication and, thus, induce a general boosting of our defenses against toxic xenobiotics (Talalay et al., 1988). 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. These and other in vitro and in vivo studies provide a rationale in support of the use of dietary polyphenols in human cancer chemoprevention, in a combinatorial approach with either chemotherapeutic drugs or cytotoxic factors for efficient treatment of drug refractory tumor cells (Pandey et al., 2009).

Anti-Diabetic Effect

Impairment in glucose metabolism leads to physiological imbalance with the onset of the hyperglycemia and subsequently diabetes mellitus. There are two main categories of diabetes; type-1 and type-2. Studies have shown that several physiological parameters of the body get altered in the diabetic conditions [(Rizvi et al., 2001a, 2005b). Numerous studies report the antidiabetic effects of polyphenols. Tea catechins have been investigated for their anti-diabetic potential (Rizvi et al., 2001b, 2005a).

Polyphenols may affect glycemia through different mechanisms, including the inhibition of glucose absorption in the gut or of its uptake by peripheral tissues. The inhibition of intestinal glycosidases and glucose transporter by polyphenols has been studied (Matsui et al., 2001). Individual polyphenols, such as (+)catechin, (-)epicatechin, (-)epigallocatechin, epicatechin gallate, isoflavones from soyabeans, tannic acid, glycyrrhizin from licorice root, chlorogenic acid and saponins also decrease S-Glut-1 mediated intestinal transport of glucose. Saponins additionally delay the transfer of glucose from stomach to the small intestine (Dembiuska-Kiec et al., 2008). Many mechanisms have been proposed to explain the anti-diabetic action of this stilbene, modulation of SIRT1 is one of them which improves whole-body glucose homeostasis and insulin sensitivity in diabetic rats (Milne et al., 2007; Harikumar et al., 2008).

Onion polyphenols, especially quercetin is known to possess strong 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 et al., 2009). Ferulic acid (FA) is another polyphenol very abundant in vegetables and maize bran. Several lines of evidence 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 a significantly increased plasma insulin and a negative correlation between blood glucose and plasma insulin (Jung et al., 2007; Barone et al., 2009).

Anti-Aging Effect

Aging is the accumulation process of diverse detrimental changes in the cells and tissues with advancing age, resulting in an increase in the risks of disease and death. Among many theories purposed 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 et al., 2007a, 2007b). Antioxidant capacity of the plasma is related to dietary intake of antioxidants; it has been found that the intake of antioxidant rich diet is effective in reducing the deleterious effects of aging and behavior. Several researches suggest that the combination of antioxidant/anti-inflammatory polyphenolic compounds found in fruits and vegetables may show efficacy as anti-aging compounds (Cao et al., 1998; Joseph et al., 2005).

Fruit and vegetable extracts that have high levels of flavonoids also display high total antioxidant activity such as spinach, strawberries and blueberries. A recent 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 et al., 2008).

Polyphenols are also beneficial in ameliorating the adverse effects of the aging on nervous system or brain. Paramount importance for the relevance of food polyphenols in the protection of the aging brain is the ability of these compounds to cross the blood-brain barrier (BBB), which tightly controls the influx in the brain of metabolites and nutrients as well as of drugs. Resveratrol, the grape polyphenol has been found to consistently prolong the life span; its action is linked to an event called caloric restriction or partial food deprivation (Harikumar et al., 2008).

Neuro-Protective Effects

Oxidative stress and damage to brain macromolecules is an important process in neurodegenerative diseases. Alzheimer's disease is one of the most common occurring neurodisorder affecting up to 18 million people worldwide. Because polyphenols are highly antioxidative in nature, their consumption may provide protection in neurological diseases (Letenneur et al., 2007).

It was found 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). Polyphenols from fruits and vegetables seem to be invaluable potential agents in neuroprotection by virtue of their ability to influence and modulate several cellular processes such as signaling, proliferation, apoptosis, redox balance and differentiation (Singh et al., 2008).

Recently it was reported that administration of polyphenols provide protective effects against Parkinson's disease, a neurological disorder (Aquilano et al.,2008), characterized by degeneration of dopaminergic neurons in the *substantia nigra zona compacta.* Nutritional studies have linked the consumption of green tea to the reduced risk of developing Parkinson's disease. EGCG may also protect neurons by activating several signaling pathways, involving MAP kinases which are fundamental for cell survival (Rossi et al., 2008). The therapeutic role of catechins in Parkinson's disease is also due to their ability to chelate iron. This property contributes to their antioxidant activity by preventing redox-active

transition metal from catalyzing free radicals formation. Moreover, the antioxidant function is also related to the induction of the expression of antioxidant and detoxifying enzymes particularly in the brain, which is not sufficiently endowed of a well-organized antioxidant defense system (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).

Other Benefits

Except above explained pathological events, polyphenols show several other health beneficial effects. Dietary polyphenols exert preventive effects in treatment of asthma. In asthma the airways react by narrowing or obstructing when they become irritated. This makes it difficult for the air to move in and out. This narrowing or obstruction can cause one or a combination of symptoms such as wheezing, coughing, shortness of breath and chest tightness. Epidemiological evidence that polyphenols might protect against obstructive lung disease come from studies that have reported negative associations of apple intake with prevalence and incidence of asthma, and a positive association with lung function (Tabak et al., 2001; Woods et al., 2003). 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. Theaflavins present in black tea were found to have anti HIV-1 activity. These polyphenols inhibited the entry of HIV-1 cells into the target cells. HIV-1 entry into the target cell involves fusion of glycoprotein (GP) and envelope of the virus with the cell membrane of the host cells. Haptad repeat units present at N and C terminals of GP41 (membrane protein) on the viral envelope, fuse to form the fusion active GP41 core, which is a six-helical bundle. Theaflavins were found to block the formation of this six-helix bundle required for entry of the virus into the host (58, Kanti Bhooshan Pandey, 2009). Theaflavin 3 3' digallate, and theaflavin 3' gallate were found to inhibit Severe Acute Respiratory Syndrome (SARS) corona virus. This antiviral activity was due to inhibition of

the chymotrypsin like protease (3CL Pro) which is involved in the proteolytic processing during viral multiplication (Sharma et al., 2009) 58, Kanti Bhooshan Pandey, 2009].

Antioxidants and Inflammation

In the classic literature, inflammation is described as the principal response of the body invoked to deal with injuries and its hallmarks include swelling, redness, pain and fever (tumor, rubor, dolor and calor) (Larsen et al., 1983). Inflammation is a reaction of the microcirculation that is characterized by the movement of serum proteins and leukocytes (neutrophils, eosinophils and macrophages) from the blood to the extra-vascular tissue.

There are many mediators, such as vasoactive amines: histamine and 5 hydroxytryptamin (5-HT); adhesion molecules: intercellular adhesion molecule 1 (ICA M 1), vascular adhesion molecule 1 (VCA M 1), selectins; lipid-derived eicosanoids: prostaglandin E2 (PGE2), prostaglandin I2 (PGI2), leukotriene B4 (LTB4), leukotriene C4 (LTC 4); cytokines: tumour necrosis factor α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10) and chemokines: interleukin-8 (IL-8), monocyte-chemoattractant protein-1 (MCP- 1), macrophage inflammatory molecule 1α (MIP1α), that coordinate the events of acute inflammation, regulate vascular changes and inflammatory cell recruitment (Larsen et al., 1983; Lawrence et al., 2002, 2007; Bengmark, 2004).

The inflammatory response is a complex self-limiting process precisely regulated to prevent extensive damage to the host. When the self-limiting nature of this protective mechanism is inappropriately regulated, it is transformed to a detrimental, chronic state of inflammation. All chronic diseases are interrelated as they contain an element of increased inflammatory response, often observed long before the disease is clinically documented (Bengmark, 2004). The increase in inflammatory *tonus* is mainly the result of lifestyle and nutritional habits, making the increase controllable (Bengmark, 2006). During the past several decades, the incidence of obesity has significantly raised worldwide (Park et al., 2007). Obesity is associated with a state of chronic, low-grade inflammation, particularly in white adipose tissue (Wellen et al., 2005) demonstrating a close link between metabolism and immunity. The integration of metabolism and immunity, under normal condition can be viewed as a central homeostatic mechanism, but whose dysfunction (described as meta-flammation) can lead to a cluster of chronic metabolic disorders, particularly obesity, type 2 diabetes and cardiovascular diseases (Hotamisligil, 2006; Semenkovich, 2006). It is safe to suggest that the link between inflammatory and metabolic signalling is a delicate balance

(Hotamisligil, 2006). It is clear that chronic excess of nutrients engages common or overlapping pathways regulating both metabolic and immune functions through common key regulatory molecules and signalling systems. It has been shown that phenolic compounds can exert modulatory action in cell by interacting with a wide spectrum of molecular targets central to the cell signalling machinery.

The molecular mechanisms involved in the anti-inflammatory activities of polyphenols have also been suggested to include:

i) the inhibition of pro-inflammatory enzymes, such as cyclooxygenase (COX-2), lipoxygenase (LOX) and inducible nitric oxide synthase (iNOS), through the activation of peroxisome proliferatorsactivated receptor gamma (PPAR γ)

ii) the inhibition of phosphoinositide 3-kinase (PI 3-kinase), tyrosine kinases, nuclear factor-kappa B (NF-κB), c-JUN

iii) the activation of phase II antioxidant detoxifying enzymes, mitogen-activated protein kinase (MAPK), protein kinase C (PKC), serin/threonin protein kinase Akt/PKB and

iv) the modulation of several cell survival/cell-cycle genes (Kim et al., 2004; Williams et al., 2004; Yoon et al., 2005; Stangl et al., 2007).

Anti-microbial activity of Plants

Despite the development of antibiotics, bacterial and fungal infections are still a major issue in medicine, and the presence of numerous drug-resistant strains poses a new challenge. Herbal drugs have been extensively used in this field for many centuries. Recently, there has been a growing interest in natural products due to their availability, fewer side effects or toxicity as well as better biodegrability as compared to the available antibiotics and preservatives.

The need to counter bacterial resistance is obvious and pressing. The strengths of phytochemicals in this area are less obvious but certainly compelling;

i. Firstly, in terms of plant chemical ecology, it is logical that plants produce antibacterial metabolites as part of their chemical defence strategy to protect themselves against microbes in their environment and this includes many

Gram-positive bacteria. Soil is rich in bacteria, fungi and viruses and it is likely that plants contain latent antimicrobials or synthesise them *de novo* as part of a phytoalexic response on microbial invasion.

ii. Secondly there are countless examples of plants which are used topically and systemically to treat bacterial infections in the ethnobotanical setting (Magassouba et al., 2007).

iii. Thirdly it is the extensive functional group chemistry, chirality and ultimately chemical diversity of phytochemicals, and natural products in general, which mark them out as a valuable pool of bioactive molecules.

iv. Finally, phytochemicals are structurally distinct from microbially derived antibiotic natural products such as the tetracyclines and macrolides. It is likely that this chemical uniqueness will give rise to classes of antibacterial which have modes of action which are distinct from existing compounds, e. g., protein synthesis inhibition (Tasdemir et al., 2007).

In this regard, plant essential oils may offer a great potential and hope. Therefore, their composition and antimicrobial activities have been thoroughly and systematically studied. Essential oils are complex natural mixtures of volatile secondary metabolites The main constituents of essential oils – mono- and sesquiterpenes including carbohydrates, alcohols, ethers, aldehydes and ketones – are responsible for the fragrant and biological properties of aromatic and medicinal plants. Due to these properties, since ancient times spices and herbs have been added to food, not only as flavouring agents but also as preservatives. Essential oils cover a broad spectrum of activities. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant and anticancerogenic properties. Others are biocides against a broad range of organisms such as bacteria, fungi, viruses, protozoa, insects and plants. (Koedam,1977; Janssen 1987; Deans, 1991; Cole, 1994).

The plant under study

The word thyme is a general name for more than three hundred Thymus species, hybrids, varieties and ecotypes, all of which are small perennial herbs native to Europe and Asia. The word Thymus has two possible derivations: from the Greek word *thyo* meaning *scent*, cleanse or fumigate, or from the word *thymon* for *courage*.

There are many traditions related to the tonic character of these plants. The Egyptians used it in embalmment.The Roman soldiers used to take a bath in water with thyme to provide vigour. Thyme sprigs were thought to offer protection against the plague, and were also burned indoors to cleanse the air. Thyme oil was used as an antiseptic during World War I. Still today, thyme is used in the embalmment liquids, protects paper from mould, and is used to preserve anatomy and botany specimens (Kruger, 1992; Morales, 2002; Zarzuelo et al., 2002).

Teas from several species of thyme are a traditional remedy for gastro-intestinal complaints and the oils were once taken to expel intestinal parasites, particularly hookworm.

Thyme also has antispasmodic properties, which make it an effective remedy for the sore throats, irritable coughs, and bronchitis. Thyme mouthwashes are also used against gum infections. Externally it is applied to clean the skin against acne. Thyme from several origins is traditionally included in sausages, meat loaf, terrines and stuffing mixtures, both for its preservative qualities as well as its savoury taste. Due to its wide applications, from poultry, shellfish, hunt meat, meat, fish, fruit salads to sweets, it is an important herb in southern French, Greek, Creole and Cajun cuisines. The honey from thyme flowers is largely appreciated for its delicacy (Simon et al.,1984; Kruger, 1992). In addition to the plant applications, thyme oils are also used in flavour and food industries, mainly in the manufacture of perfumes and cosmetics, or for flavouring chocolates, toothpastes, mouthwashes, and cough medicines (Kruger, 1992; Morales, 2002; Zarzuelo et al., 2002).

MATERIALS & METHODS

3.1. Chemicals

Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Chemicals	Source
α- Tocopherol, Hydrogen peroxide	**SRL India**
Ethanol	**Bengal chemicals**
Hexane, Butanol, Ethyl-acetate	**Rankem**
Ascorbic acid, Calcium chloride	**E. Merk**
Trichloroacetic acid,	**E. Merk**
Sodium chloride, Sucrose	**E. Merk**
2, 2 diphenyl picryl hydrazyl (DPPH)	**E. Merk**
Methanol, BHT,	**SRL India**
Hydrochloric acid, Ferric chloride	**Qualigens Pvt Ltd**
Potassium dihydrogen phosphate	**Qualigens Pvt Ltd**,
Linoleic acid	**Sigma Chemical Co, USA**
Thiobarbituric acid	**CDH India**
Trypticase soy agar Potato dextrose agar	**TSA; Becton–Dickinson Difco Laboratories, Detroit, Mich.**
Mueller–Hinton broth	**MHB; Becton–Dickinson, Cockeysville, MD, USA**
Potassium chloride	**Hi-media**

Sodium dihydrogen monophosphate	**Hi-media**
Ethylene diamine tetra acetate	**Hi-media**
Ferric nitrate	**Hi-media**
RPMI 1640 medium	**Sigma Aldrich**
MOPS	**Sigma Aldrich**

3.2. Plant Material Collection

The plant *Thymus serpyllum* was collected from Aharbal and Kongdoori area of Gulmarg in the month of May-June and was identified and authenticated by the courtesy of Centre of Plant Taxonomy, Department of Botany, University of Kashmir. The voucher specimen has been retained in the herbarium of Taxonomy, Department of Botany, University of Kashmir for future reference under herbarium no: (KASH-639).

3.3. Extraction Procedure

The authentically identified plant material was shade dried under room temperature at 30 ± 2^0C. The dried material was grind into powder using mortar and pestle and sieved with a sieve of 0.3mm aperture size. The powder obtained was successively extracted in hexane, ethyl acetate, absolute ethanol, methanol and distilled water by using Soxhlet extractor (60^0C -80^0C). The powdered plant material was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a flask containing the extraction solvent. The Soxhlet was then equipped with a condenser. The solvent was heated to reflux. The solvent vapours moved up the distillation arm and drop back into the chamber containing the extract. The condenser ensures that the solvent vapours cool down and drip back down into the chamber containing the extract. The chamber containing the extract gets slowly filled with warm solvent. Some of the desired compound gets dissolved in the warm solvent. When the Soxhlet chamber is almost full, the chamber gets automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was repeated many times, for about four days. During each cycle, a portion of the non-volatile compound gets dissolved in the solvent. After many cycles the desired compound gets concentrated in the distillation flask.

The extracts were then concentrated with the help of rotary evaporator under reduced pressure and the solid extracts were stored in refrigerator for further use.

3.4. Antioxidant activity assays

3.4.1. Preparation of liver microsomes

The liver microsomes were prepared from rat liver using calcium precipitation method (Johnson *et al*., 2002). Liver from the freshly killed rats were perfused and kept in an ice cold normal saline 0.9% NaCl and extraneous material was removed. All operations were performed at 4ºC. Tissue was blotted between the folds of a filter paper and weighed. 20% (w/v) homogenate was prepared in 0.25 M sucrose. The homogenate was filtered through a muslin cloth and centrifuged at 12000g for 20 minutes at 4^0C to separate nuclear debris .The supernatant so obtained was diluted 1:5 with 0.125 M sucrose containing 8mM $CaCl_2$ and kept on ice for 50 minutes with constant stirring. The pellet obtained after centrifugation at 12000g for 10 minutes was washed with the washing solution containing 0.15M KCl, 1mM EDTA and 0.01M NaH_2PO_4 and was again centrifuged at 12000g for 10 minutes to get the microsomal pellet.

The microsomal pellet was then resuspended in a minimum volume of 0.25M sucrose and stored at -80^0C for experimental use.

3.4.2. Lipid peroxidation Assay (Liver Microsomes)

The assay of lipid peroxidation was done using the method of Tasduq *et al*. (2008). Liver microsomes were incubated for 5 minutes in presence and absence of plant extract (50µ-1000µg) prior to addition of 100µM $FeSO_4$ and 50µM H_2O_2 and then incubated for 20 minutes (37^0C) in 0.15M NaCl (pH 7). Control incubation received vehicle only and the induced incubation contained vehicle plus liver microsomes but had no addition of plant extract. The reaction was terminated by the addition of TCA-TBA reagent (5% w/v) and the lipid peroxidation content of the samples was determined as malondialdehyde (MDA) formed per mg of protein. Percentage inhibition was calculated using the formula;

$$\% \text{ inhibition} = 100 - [\text{Induced-Treated/Induced-Control}] \times 100$$

3.4.3. DPPH radical scavenging activity

The DPPH assay was performed by using the method of Braca, *et al.* (2001). Various concentrations of plant extracts (100-1000 µg/ml) were added to 1ml of the 20 mg% methanol solution of DPPH, and the mixture was vortexed vigorously. The

tubes were then incubated at room temperature for 30 minutes in dark, and the absorbance was taken at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. α-tocopherol was taken as positive control. The percentage inhibition activity was calculated by using the formula;

% Inhibition = [Absorbance of control-Absorbance of sample /Absorbance of control] × 100

3.4.4. Lipid peroxidation (LPO):

LPO was performed according to the method of Wright, *et al. (*1981). The reaction mixture in a total volume of 2.0 ml, contained 1 ml of linoleic acid (3%), 0.2 ml ferric nitrate (20 mM), 0.2 ml of ascorbic acid (200 mM) and 0.2 ml of H_2O_2 (30mM) and different concentrations of plant extracts (50-250 µg/ml).this was followed by incubation at 37^0C in a water bath for 1h. The reaction was stopped by the addition of 1.0 ml TCA (10% w/v). Following which 1.0 ml of TBA (1%w/v) was added and all the tubes were placed in a boiling water bath for 20 mins. The tubes were then centrifuged at 5000 rpm for 10 mins. The amount of malondialdehyde formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm against a reagent blank containing 1ml of linoleic acid and 1ml of distilled water.

3.4.5. Assessment of Hydroxyl radical scavenging activity:

Hydroxyl radical, generated from the Fe^{3+}-Ascorbate-H_2O_2 (Fenton reaction), was evaluated by degradation of deoxyribose that produced thiobarbituric acid reactive species (TBARS) (Halliwell, *et al*., 1987). The reaction mixture containing 25mM deoxyribose, 10mM Ferric chloride, 100mM ascorbic acid, 2.8 mM H_2O_2 in 10mM KH_2PO_4 (pH 7.4) and various concentrations of *Thymus serpyllum* extracts. The reaction mixture was incubated at 37^0C for 1h. Then 1 ml of 1% thiobarbituric acid and 1 ml of 3% trichloroacetic acid were added and heated at 100^0C for 20 min. The TBARS was measured spectrophotometrically at 532 nm. The results were expressed as percentage inhibition of deoxyribose oxidation, as determined by the following formula.

$$\text{Percentage inhibition} = [(A-B)/A] \times 100$$

Where A was the malondialdehyde produced by Fenton reaction treated alone, and B was the malondialdehyde produced in the presence of *Thymus serpyllum* extracts and α-tocopherol, the known antioxidants.

3.5. Antimicrobial activity

3.5.1. Microbial strains

Eight reference strains of the following species were used for their susceptibility to *Thymus serpyllum* in this study: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Enterococcus coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Aspergillus fumigatus* MTCC 1811 and *Aspergillus niger* ATCC 16404. These strains were procured from the American Type Culture Collection (ATCC, Manassas, VA, USA) and Microbial Type Culture Collection (MTCC, Chandigarh, India).

3.5.2. Determination of antimicrobial activity

The antibacterial and antifungal activities of the *Thymus serpyllum* were performed by broth micro dilution methods as per the guidelines of Clinical and Laboratory Standard Institute (formerly, the National Committee for Clinical Laboratory Standards). Mueller–Hinton broth was supplemented with calcium (25 mg/L) and magnesium (12.5 mg/L) for bacterial strains. The RPMI 1640 medium buffered to a pH of 7.0 with 0.165 M MOPS was used for fungal strains. The minimum inhibitory concentration (MIC) was determined by serial two–fold dilution of the test sample in the respective test medium in amounts of 100 µl per well in 96–well U–bottom microtiter plates (Tarsons, Kolkata, India). The stock inoculum suspensions of the bacteria, were prepared in sterile normal saline (0.85%) containing 0.05 % polysorbate 20 from the overnight cultures grown on Trypticase soy agar and the stock inoculum suspensions of the fungi were prepared in sterile normal saline (0.85%) containing 0.05 % polysorbate 20 from the overnight (7–day for *Aspergillus* species) cultures grown on potato dextrose agar. Inocula were verified for each assay by plating onto agar plates for colony enumeration.

These suspensions were further diluted in the respective mediums and a 100 µl volume of this diluted inoculum was added to each well of the plate, resulting in a

final inoculum concentration of 5×10^5 CFU/ml for bacteria, 0.5×10^4 to 2.5×10^3 CFU/ml for *Candida* species while as 0.4×10^4 to 5×10^4 CFU/ml for *Aspergillus* species (Wayne, 2008a, 2008b, 2008c). The controls comprised of the bacterial and the fungal cells or spores suspended in sterile medium and the sterile medium only. The microtiter plates were incubated at 35°C for 24 hrs for bacterial cultures and 48 hrs for fungal cultures. The plates were read visually and the MIC was defined as the lowest concentration of test sample that prevented visible growth with respect to the growth control.

3.6. Anti-inflammatory method

3.6.1. Animals and feed

Pathogen free adult Wistar strain of rats (130-140g body weight) for acute study were procured and kept in the Central Animal House Facility of Indian Institute of Integrative Medicine Jammu in an environmentally controlled room with a 12 hr. light-dark cycle at constant room temperature (24 ± 2°C) and relative humidity (60 ± 15 %). Animals were acclimatized for one week before starting the experiment. At a maximum four rats were kept in each polypropylene cage. Animals had free access to pellet diet and water ad libitum.

3.6.2. Carrageenen-induced edema in rats:

This test was performed by the most and widely and commonly used technique of Winter et al., (1962). Edema was induced in rats in groups of three by injecting 100µl of carrageenen (1%w/v) solution in normal saline into the sub plantar region of the left hind paw after 45 minutes of drug administration. Paw volume was measured immediately and after every hour for the first 4 hours and then after 24 hours of carrageenen injection.

3.6.3. Determination of Acute Anti- Inflammatory Activity.

In acute tests, doses of the test material were administered orally 45 minutes before the induction of inflammation by injecting 100ul of 1% of λ-carrageenen. One group of animals was kept as vehicle control in experiment and given only normal saline, whilst one group of animals received Carrageenen only and was referred as control group and one group received the standard drug for comparison of the activity and authenticity of the experiment. Standard drug and vehicle were also given as per

the scheme of test drug administration. Paw volume was measured at different time intervals depending upon the nature of experiment with the help of volume differential meter model 7101 (Ugo Basile Italy).

Mean increase in the paw volume and standard error of the mean of each group was calculated and the results were expressed as percentage inhibition of edema as compared to control group.

Percentage inhibition calculated by the following formula:

% Inhibition = [Mean of control – Mean of treated / Mean of control] × 100

3.7. Stastistical Analysis

Each of the experiment was performed in triplets and the statistical analysis of the data was performed using the software “Primer”. The results were expressed as mean ± standard error.

RESULTS

4.1. LPO Assay (Liver Microsomes)

The LPO assay with liver microsomes was performed with the hexane, ethyl-acetate, ethanol, methanol and the aqueous extracts extracts of *Thymus serpyllum*. The hexane, ethyl-acetate, ethanol and the aqueous extracts did not show any significant activity. However, the methanolic and the butanolic extracts of *Thymus serpyllum* showed the potent activity, the methanolic extract being slightly more active. The results are given in Table 4.1. Butylhydroxy toulene (BHT) was taken as a reference standard to compare the activity of the plant extracts (Figure 4.1).

4.2. DPPH Radical Scavenging Assay

The extracts of *Thymus serpyllum* were checked for DPPH radical scavenging activity. All the extracts of *Thymus serpyllum* except the hexane extract showed appreciable DPPH radical scavenging activity, the methanolic extract showing the highest activity. The results are shown in Table 4.2. α- tocopherol was taken as a reference standard to compare the activity of the extracts (Figure 4.2).

4.3. LPO Assay

The LPO assay of the hexane, ethyl-acetate, ethanol, methanol and the aqueous extracts extracts extracts of *Thymus serpyllum* was performed. The hexane, ethyl-acetate, ethanol and the aqueous extracts of *Thymus serpyllum* were not found to have any significant amount of activity. However, the methanolic and the butanolic extracts of *Thymus serpyllum* were found to be active, the methanolic extract showing better activity. The results are tabulated in Table 4.3. The activity of the extracts of *Thymus serpyllum* was compared with butylated hydroxyl toluene (BHT), which was taken as reference standard during the experiment (Figure 4.3).

Table 4.1. Effect of methanolic and butanolic extracts of *Thymus serpyllum* on lipid peroxidation of liver microsomes.

Concentration (µg/ml)	% Inhibition of Lipid peroxidation		
	BHT	**Methanolic extract**	**Butanolic extract**
50	33.73 ± 2.71*	17.86 ± 3.41*	14.73 ± 1.68*
75	50.24 ± 2.56*	25.63 ± 1.84*	23.91 ± 2.59*
100	62.44 ± 1.97*	30.85 ± 1.73*	27.64 ± 1.96*
500	93.54 ± 1.58*	37.06 ± 1.98*	35.12 ± 2.57*
1000	97.84 ± 2.48*	57.32 ± 2.09*	54.47 ± 1.86*

The results are expressed as mean ± standard error.

*P<0.05 are statistically significant.

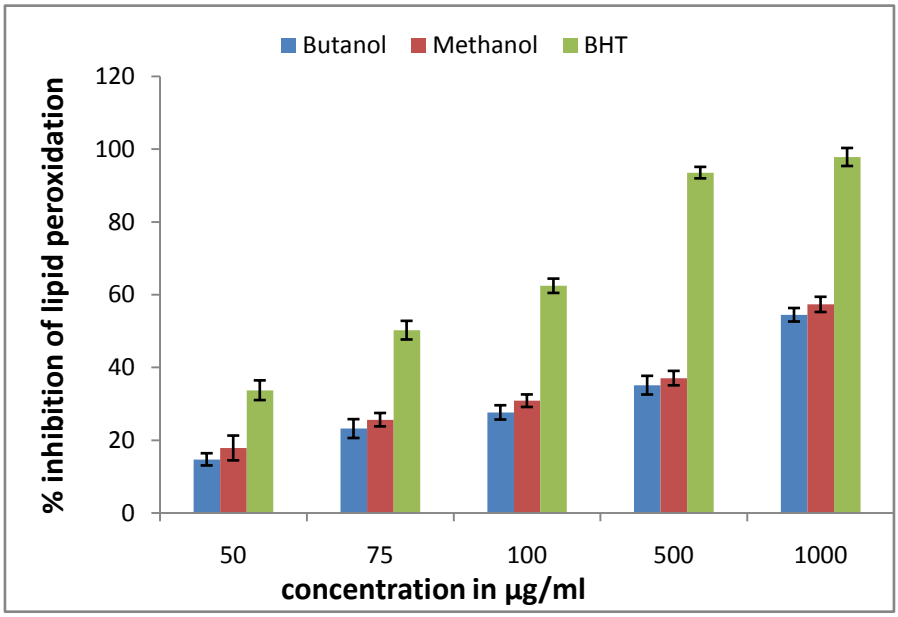

Figure 4. 1. Effect of butanolic and methanolic extracts of *Thymus serpyllum* on lipid peroxidation of liver microsomes compared to control BHT.

Table 4.2. Effect of the extracts of *Thymus serpyllum* on DPPH radical scavenging activity.

Concentration (µg/ml)	% Inhibition of DPPH Radical					
	α-tocopherol	Ethyl-acetate extract	Butanolic extract	Ethanolic extract	Methanolic extract	Aqueous extract
100	43.62 ±2.14*	13.82 ± 1.59*	34.45 ± 2.00*	35.01 ± 2.00*	37.31 ± 1.78*	17.89 ± 1.78*
200	79.28 ±3.87*	26.59 ± 2.68*	62.63 ± 1.38*	65.75 ± 1.41*	75.36 ± 1.89*	28.73 ± 1.12*
300	81.65 ±3.05*	39.29 ± 1.81*	72.28 ± 1.72*	75.69 ± 1.48*	77.78 ± 1.92*	31.87 ± 1.41*
400	86.45 ±2.99*	48.69 ± 2.5*	82.85 ± 1.73*	83.49 ± 1.39*	84.53 ± 1.87*	43.75 ± 1.98*
500	88.26 ±1.62*	56.79 ± 1.79*	84.09 ± 1.91*	86.39 ± 1.61*	87.35 ± 1.50*	46.89 ± 1.35*
600	89.95 ±1.71*	69.92 ± 1.78*	85.39 ± 1.56*	87.90 ± 1.59*	88.67 ± 1.95*	47.85 ± 1.74*
700	90.49 ±1.89*	73.24 ± 2.35*	86.94 ± 1.78*	88.26 ± 1.51*	89.84 ± 1.22*	52.36 ± 1.29*

The results are expressed as mean ± standard error.

*P values < 0.05 are statistically significant.

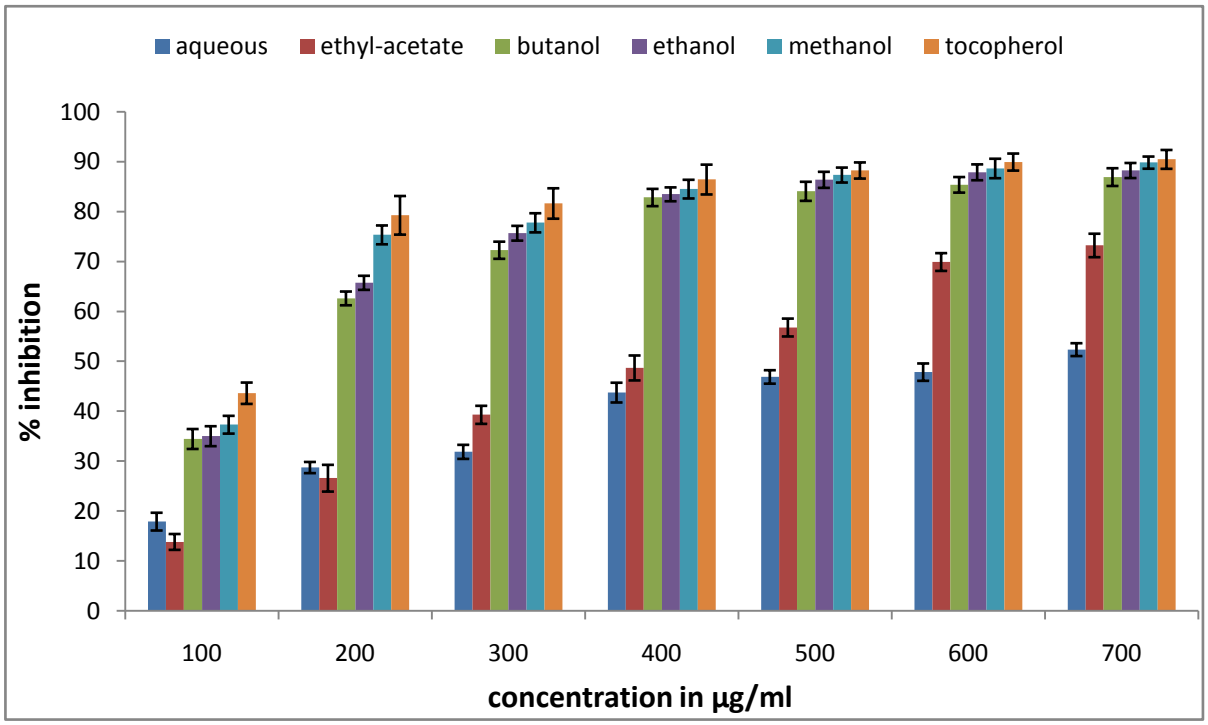

Figure 4.2. DPPH radical scavenging activity of extracts of *Thymus serpyllum* compared to control α-tocopherol.

Table 4.3. Effect of the methanolic and the butanolic extracts of *Thymus serpyllum* on lipid peroxidation.

Concentration (µg/ml)	% inhibition of Lipid peroxidation		
	BHT	**Methanolic extract**	**Butanolic extract**
20	47.00 ± 1.79*	32.5 ± 2.79*	30.62 ± 1.52*
40	54.10 ± 1.35*	39.7 ± 1.95*	35.64 ± 1.60*
60	60.16 ± 1.54*	41.58 ± 1.53*	39.95 ± 1.72*
80	85.73 ± 1.77*	46.9 ± 2.29*	44.01 ± 1.57*
100	98.23 ± 1.67*	66.8 ± 4.01*	60.62 ± 1.86*

The results are expressed as mean ± standard error.

*$P<0.05$ are statistically significant.

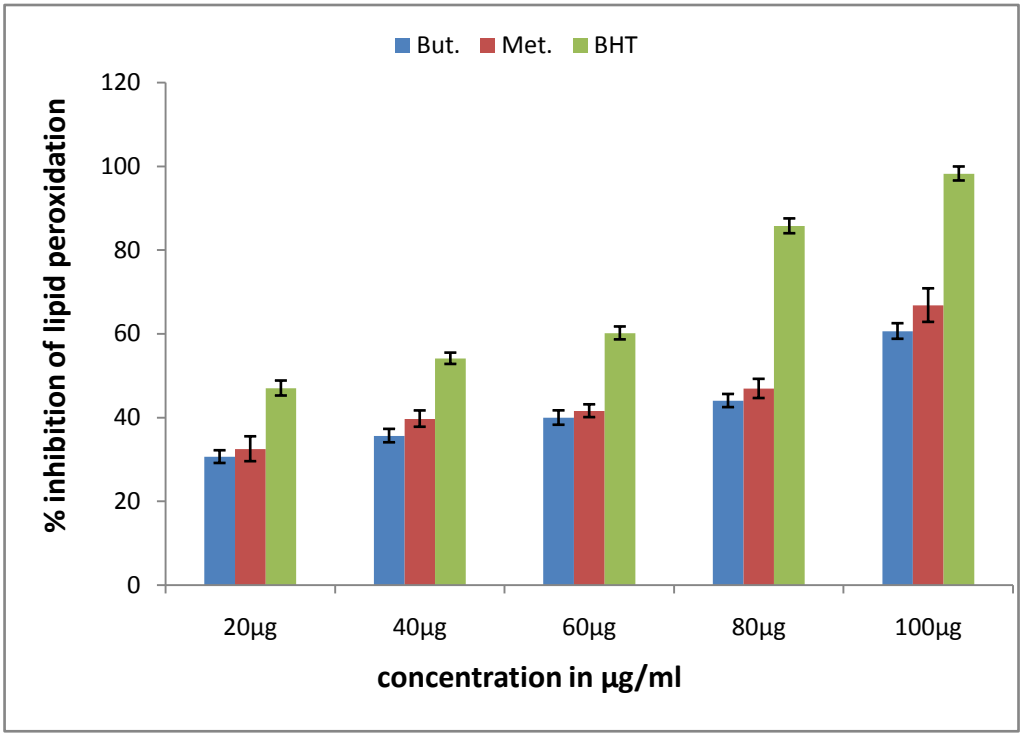

Figure 4.3. Effect of butanolic and methanolic extracts of *Thymus serpyllum* on lipid peroxidation compared to control BHT.

4.4. Hydroxyl Radical Scavenging Assay

The extracts of *Thymus serpyllum* were checked for the hydroxyl radical scavenging activity. The hexane, ethyl-acetate, butanol and the aqueous extracts of *Thymus serpyllum* were devoid of any hydroxyl radical scavenging activity. The ethanolic and the methanolic extracts showed the appreciable activity. The results are tabulated in Table 4.4. The activity of the extracts of *Thymus serpyllum* was compared to α-tcocopherol which was taken as a positive control (Figure 4.4).

4.5. Antimicrobial Activity

The antibacterial and antifungal activities of the extracts of *Thymus serpyllum* were performed by broth micro dilution methods. The minimum inhibitory concentration (MICs) of the plant extracts against eight microorganisms were tested and the results are tabulated in Table 4.5 and Table 4.6.

It was observed that all tested bacterial (gram positive as well as gram negative) and fungal species were susceptible to the ethyl-acetate and the methanolic extracts of *Thymus serpyllum* and exhibited the MICs ranging from 1000 to 4000 µg/ml, however the hexane, butanol, ethanol and the aqueous extracts were not found to be significantly active.

4.6. Anti inflammatory Activity

The anti inflammatory activity of extracts of *Thymus serpyllum* was checked using the carrageenen induced paw edema model. The hexane, ethyl-acetate, ethanol, methanol and the aqueous extracts of *Thymus serpyllum* were not found to have any activity, only the butanolic extract was found to be active. The results are tabulated in Table 4.7. The activity of the extracts was compared with a standard drug ibuprofen, which was taken as a reference control during the experiment (Figure 4.5).

Table 4.4. Effect of the methanolic and the ethanolic extracts of *Thymus serpyllum* on hydroxyl radical scavenging activity.

Concentration (µg/ml)	% Inhibition of Hydroxyl Radical		
	α- tocopherol	**Methanolic extract**	**Ethanolic extract**
20	58.01 ± 3.14*	47.76 ± 2.13*	32.69 ± 1.52*
40	73.23 ± 1.83*	69.43 ± 1.95*	55.72 ± 1.60*
60	82.26 ± 2.37*	72.38 ± 1.53*	59.70 ± 1.72*
80	90.24 ± 1.98*	75.24 ± 2.29*	60.69 ± 1.57
100	97.44 ± 2.78*	77.14 ± 1.11*	64.18 ± 1.86

The results are expressed as mean ± standard error.

*P values < 0.05 are statistically significant.

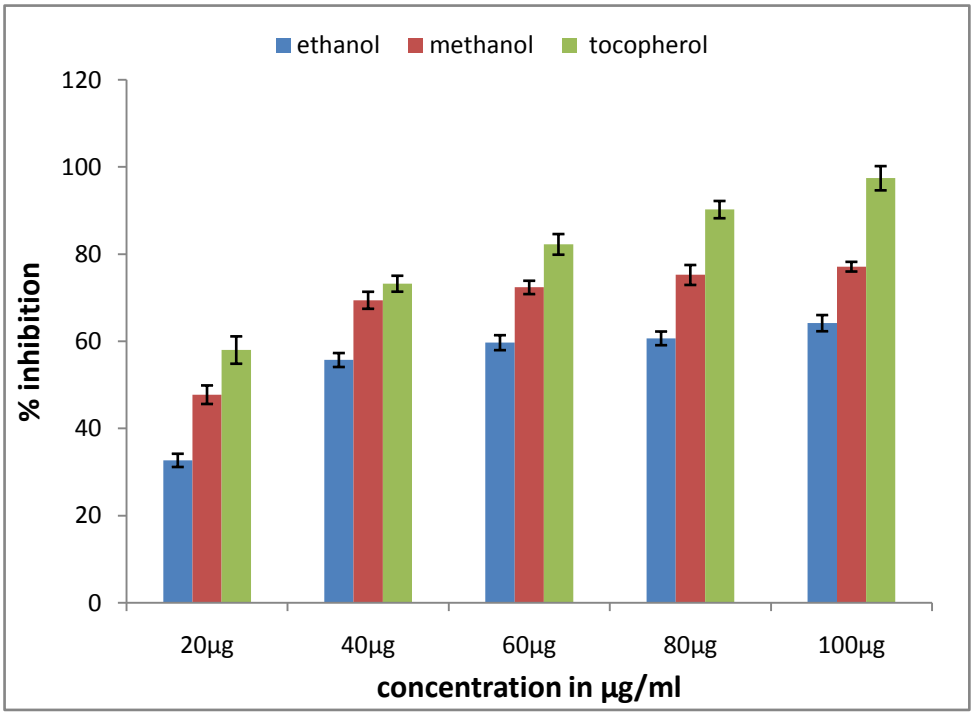

Figure 4. 4. Hydroxyl radical scavenging activity of ethanolic and methanolic extracts of *Thymus serpyllum* compared to control α-tocopherol.

Table 4.5. The inhibition of the microbial growth in presence of the ethyl-acetate extract of *Thymus serpyllum*.

Dilution Factor	Bacterial Strains				Fungal Strains			
	SA	**SE**	**EC**	**PA**	**CA**	**CP**	**AF**	**AN**
1	3	3	3	3	3	3	3	3
2	3	3	2	2	3	3	2	3
4	2	2	2	2	2	3	2	2
5	1	1	1	1	1	1	1	1

The concentration of the stock was 4mg/ml.

The following scale was used: 3, means full inhibition, no growth; 1, means no inhibition, full growth and 2 is the value in between.

SA (*Staphylococcus aureus* ATCC 29213), SE (*Staphylococcus epidermidis* ATCC 14990), EC (*Enterococcus coli* ATCC 25922), PA (*Pseudomonas aeruginosa* ATCC 27853), CA (*Candida albicans* ATCC 90028), CP (*Candida parapsilosis* ATCC 22019), AF (*Aspergillus fumigatus* MTCC 1811) and AN (*Aspergillus niger* ATCC 16404).

Table 4.6. The inhibition of the microbial growth in presence of the methanolic extract of *Thymus serpyllum*.

Dilution Factor	Bacterial Strains				Fungal Strains			
	SA	**SE**	**EC**	**PA**	**CA**	**CP**	**AF**	**AN**
1	3	3	3	3	3	3	3	3
2	3	3	2	2	3	3	2	3
4	2	3	2	2	3	3	2	2
5	1	1	1	1	1	1	1	1

The concentration of the stock was 4mg/ml.

The following scale was used: 3, means full inhibition, no growth; 1, means no inhibition, full growth and 2 is the value in between.

SA (*Staphylococcus aureus* ATCC 29213), SE (*Staphylococcus epidermidis* ATCC 14990), EC (*Enterococcus coli* ATCC 25922), PA (*Pseudomonas aeruginosa* ATCC 27853), CA (*Candida albicans* ATCC 90028), CP (*Candida parapsilosis* ATCC 22019), AF (*Aspergillus fumigatus* MTCC 1811) and AN (*Aspergillus niger* ATCC 16404).

Table 4.7. Effect of the butanolic extract of *Thymus serpyllum* on carageenen induced edema in rats.

Sample used	% Inhibition of Edema				
	Treatment Dose (mg/kg body wt.)	**1st hour**	**2nd Hour**	**3rd Hour**	**4th Hour**
Butanolic extract	500	28.84 ± 2.21*	56.07 ± 1.59*	45.38 ± 2.35*	35.60 ± 2.29*
Butanolic extract	250	13.46 ± 2.53*	25.23 ± 1.68*	19.86 ± 1.79*	15.15 ± 2.82*
Ibuprofin	100	13.46 ± 1.89*	57.94 ± 1.74*	58.26 ± 2.30*	60.60 ± 2.31*

The results are expressed as mean ± standard error.

*P values < 0.05 are statistically significant.

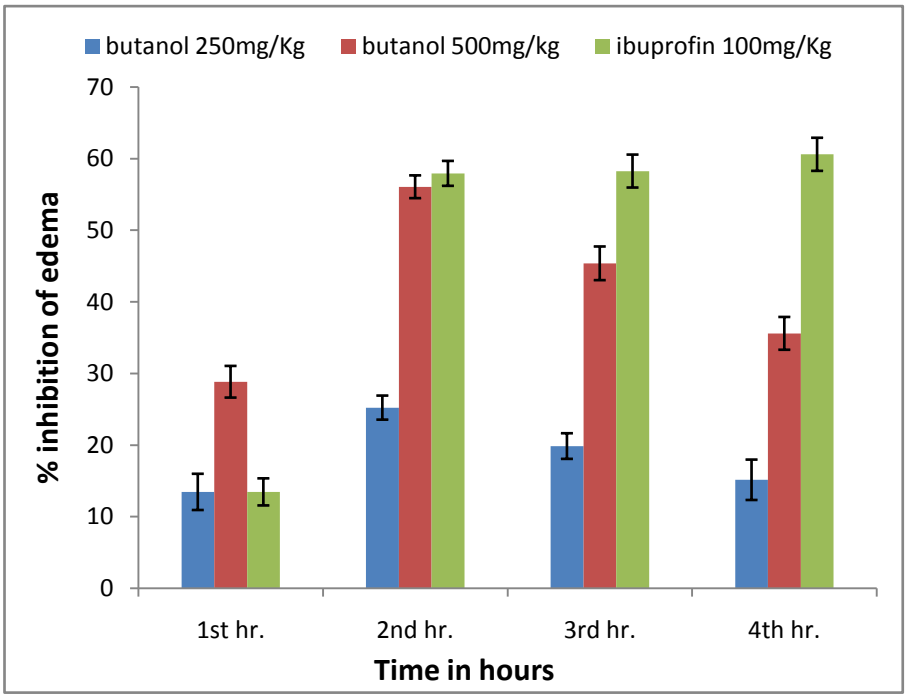

Figure 4.5. Effect of butanolic extract of *Thymus serpyllum* on carrageenen induced paw edema in rats compared to control drug ibuprofin.

DISCUSSION & CONCLUSION

5.1. Antioxidant Activity

For a very long time, plants have played an important role in the treatment of many diseases especially in the East region countries (Fallah-Hoseini et al., 2006). In several chronic diseases, free radicals are by-products of abnormal body metabolism and are important factors for late complications and secondary disease (Bartsch et al., 2004, Fallah-Hoseini et al., 2004, valko et al., 2004). There is increasing evidence that in certain pathologic states the increased production and/or ineffective scavenging of reactive oxygen species (ROS) may play a critical role. High reactivity of ROS determines chemical changes in virtually all cellular components, leading to lipid peroxidation (Abdollahi et al., 2004, 2005, Rahimi et al., 2005, Rezaie et al., 2007). Medicinal plants are a source for a wide variety of natural antioxidants (Bouayed et al., 2007). In the last few decades, numerous screening studies of various plant materials have been performed in order to find naturally occurring antioxidants for use in food or medicinal preparations, as replacements for potentially harmful synthetic additives (Reische *et al*., 1998). The dominant majority of biologically active plant compounds with antioxidative properties are flavonoids and other phenolics. These low molecular weight secondary plant metabolites exhibit excellent antioxidant properties. However, their particular mechanisms of action vary depending both on the structure and environment. Besides phenolic compounds, medicinal plants are reported to contain other natural antioxidants such as vitamins (A, B6, C, E) and some other phytochemicals like co-enzyme ubiquinone (Q10), carotenoids, selenium and zinc (Atoui *et al*., 2005).

In this study, the antioxidant activity of the hexane, ethyl-acetate, butanol, ethanol, methanol, and the aqueous extracts of *Thymus serpyllum* was tested by the DPPH radical scavenging assay, lipid peroxidation assay and hydroxyl radical scavenging assay. The plant *Thymus serpyllum* was found to have appreciable antioxidant activity, but the butanol, ethanol and the methanol extracts comparatively showed better activity. This may be due to the presence of higher concentration of bioactive metabolites in the butanolic, ethanolic and the methanolic extracts. Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in scavenging free radicals, inhibiting lipoxygenase and chelating transitional metals (Decker et al., 1997). The two phenolic compounds thymol and carvacrol are the main components responsible for the antioxidant potential of the

plant extracts (Ruberto Gand Baratta et al., 2000). The essential oil of *Thymus* specials are rich in sources of phenolic monoterpenes such as thymol and carvacrol (Pank et al., 2004). So the antioxidant activity of the extracts of *Thymus serpyllum* can be attributed to the presence of these compounds in plant *Thymus serpyllum*.

5.2. Antimicrobial Activity

In recent years there has been an increasing interest in the use of natural substances, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources (Kalemba et al., 2003). Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids, and other compounds, reported to have in vitro antifungal properties. A series of molecules with antifungal activity against different strains of fungus have been found in plants, which are of great importance to humans. These molecules may be used directly or considered as a precursor for developing better molecules. Plants have an almost limitless ability to synthesize aromatic substances of different functional groups, most of which are phenols or their oxygen-substituted derivatives. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores (Arif et al., 2009).

In the present study the antimicrobial activity of plant *Thymus serpyllum* was evaluated, although the hexane, butanol, ethanol and the aqueous extracts did not show any significant activity, but the ethyl-acetate and the methanolic extracts were found to be active. The antimicrobial activities of many plants can be attributed to the presence of high concentrations of carvacrol, which is known to occur at very high concentrations in many plant oils, including the members of the *Labiatae* family, such as *Thymus, Coridothymus, Satureja* and *Origanum* (Bounatirou et al., 2007, Chorianopoulos et al., 2004, Sokmen et al., 2004). The pharmacological actions of the plant extracts are suggested to parallel to their carvacrol contents (Aydin et al., 2007). Carvacrol is considered to be biocidal, resulting in bacterial membrane perturbations. Furthermore, carvacrol might cross the cell membranes, penetrate the interior of the cell and interact with intracellular sites critical for antibacterial activities (Cristani et al., 2007, Ultee et al., 1999). Another major component of the plant extracts, *p*-cymene, which is precursor of carvacrol, is a very weak antibacterial, but it probably acts synergistically with carvacrol by expanding the membrane, which results in the

destabilisation of the membrane (Ultee et al., 2004). It has also been suggested that minor components interact with the other components, affecting the antimicrobial activities of the oils. It is possible that the activity of the main components is regulated by the other minor molecules (Bounatirou et al., 2007).

5.3. Anti inflammatory Activity

Active oxygen-induced and free radical-mediated oxidation of biomolecules are implicated in various pathological conditions including atherosclerosis, cancer, inflammation, arthritis and regressive changes in ageing (Diplock, 1996, Scott, 1995). Several lines of evidence suggest that ROS play a main role in cellular damage and are implicated in many inflammatory conditions (Laser et al., 1989). Free radicals and ROS can readily react with most biomolecules, starting a chain reaction of free radical formation. Most studies have shown that activated neutrophils (polymorphonuclear cells or PMNs), eosinophils, monocytes, and macrophages produce ROS and lysosomal hydrolytic enzymes at sites of inflammation (Boumann et al., 1994, Morel et al., 1991). These two agents can initiate and maintain the acute phase of the inflammatory response (Morikawa et al., 1996).

In the present study the anti inflammatory activity of plant *Thymus serpyllum* was evaluated, the hexane, ethyl-acetate, ethanol, methanol and the aqueous extracts were found devoid of any activity, whereas the butanolic extract being the only one to be active. This may be due to presence of components such as flavonoids and other phenolic compounds which might act as antioxidants by reacting with free radicals and thus interrupting the propagation of new free radical species, or by chelating metal ions such as Fe^{2+} which catalyze lipid peroxidation. At least 2000 varieties of flavonoids have been isolated from the plants products. Apart from their physiological role in plants, flavonoids are well known to possess anti-inflammatory activities (Marshall, 2000). Flavonoids have been reported to inhibit phospholipase A2 (Lee et al., 1982), COX (Baumann et al., 1980), e-NOS (Chiesi et al., 1995). With regard to inflammatory cytokines, flavonoids were reported to inhibit IL-1b, IL-6 and TNF-a production by LPS-stimulated human blood monocytes (Geng et al., 1993). Flavonoids appear to inhibit the expression of inflammation-related enzymes/proteins partly by suppressing activation of NF-kB and AP-1, an effect potentially mediated by the inhibition of different protein kinases (e.g. mitogen-activated protein kinase;

extracellular signal-regulated kinase 1/2) involved in signal transduction pathway (Calder et al., 2009).

This study confirms that the plant *Thymus serpyllum* possesses significant *in vitro* antioxidant and antimicrobial activity and *in vivo* anti inflammatory activity. Since plant based drugs are more safe to use due to their lesser side effects, these extracts can be further screened and evaluated for the presence of pure compounds with antimicrobial and anti inflammatory activity that can be used for the development of new antibiotics and new anti inflammatory drugs.

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