3. REVIEW OF LITERATURE

3.1 Status of medicinal plants in India:

India is one of the 12 mega biodiversity centres having 45,000 plant species; its diversity is unrivaled due to the 16 different agroclimatic zones, 10 vegetative zones and 15 biotic provinces it has. The country has a rich floral diversity. Various indigenous systems of medicines such as Ayurveda, Siddha, Unani and Allopathy use several plant species to treat different ailments. In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases. Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. About 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments, which also forms a rich source of knowledge. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.

Market potential of phytomedicine: The estimation of sale of total phytomedicine reported was about US$ 4 billion in 1991 and $ 6 billion in 1996. The present global market is said to be US $ 14 billion\(^3\). In India the sale of total herbal products is estimated at $ 1 billion (Table 2) and the export of herbal crude extract is about $80
million, of which 50% is contributed by Ayurvedic classical preparations (Table 3). Though we have a rich treasury of medicinal plants, we are not able to compete the global market due to lack of high quality of our medicinal plants, in terms of its microbial safety, medicinal quality and other aspects. And especially in case of Ayurvedic products, drugs are used as such or in the form of aqueous extract, where in microbial safety of the product becomes a major concern.

**Table 2. Market size of phytomedicine and their sale in US Dollar.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Country</th>
<th>Years</th>
<th>Drugs sales in US $ (billion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Europe</td>
<td>1991</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>France</td>
<td></td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Italy</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>Europe</td>
<td>1996</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>USA</td>
<td>1996</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>India</td>
<td>1996</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>Other countries</td>
<td>1996</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>All countries</td>
<td>1998</td>
<td>30.0 - 60.0</td>
</tr>
</tbody>
</table>

**Table 3. Percentage of herbal drugs imported by various countries for drug preparation**

<table>
<thead>
<tr>
<th>Country</th>
<th>Percentage of herbal drugs imported</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>45%</td>
</tr>
<tr>
<td>USA</td>
<td>15.6%</td>
</tr>
<tr>
<td>Australia</td>
<td>10.5%</td>
</tr>
<tr>
<td>India</td>
<td>3.7%</td>
</tr>
<tr>
<td>South Korea</td>
<td>1.4%</td>
</tr>
<tr>
<td>Taiwan</td>
<td>1.7%</td>
</tr>
<tr>
<td>Indonesia</td>
<td>8.1%</td>
</tr>
</tbody>
</table>
The two most widely used medicinal plants are Ashwagandha and Kalmegh. They have a wide range of pharmacological activity. There is an average demand of 7,000 tons a year, but the quantity cultivated in India is only around 1,200 tons. It is very important to ensure the quality of the cultivated plants. These two plants also find their use as Anti-cancer drugs, which are mostly given to immunocompromised patients. Hence, there must be no compromise in microbial and medicinal quality of these two plants and their products.

3.1.1 ASHWAGANDHA

*Withania somnifera* Dunal belonging to family Solanaceae, commonly known as Ashwagandha is an important cultivated medicinal crop of India. The plant grows throughout the country. “Ashwa” means horse and “gandha” means smell, hence the name Ashwagandha comes from smell of horses. The plant is commonly known as “Indian Ginseng”. Ashwagandha is mentioned as an important drug in ancient Ayurvedic literature and in Unani system of medicine. Mention of the drug is made in ancient literatures like Charaka samhita, Sushruta samhita, Bhava Prakasha etc. Ashwagandha is what is known in herbal medicine as an "adaptogen", which means it can help restore overall health, as well as help the body acclimate to stress.

**Ashwagandha - Plant Profile**

*Family:* Solanaceae

*English name:* Winter Cherry

*Indian name:* Sanskrit: Asvagandha, Varahakarni

Kannada: Viremaddinagaddi, Kiremallinagida

Hindi: Asgandh, Punir

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*Department of Pharmacognosy, KLE University’s College of Pharmacy, Bangalore*
**Distribution:** It is indigenous to India. It is found to grow in Madhya Pradesh, Punjab, Himachal Pradesh, Uttar Pradesh, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala and other regions.

**Parts used:** Roots, leaves, stem bases, fruits and seeds.

**Botanical description:** An erect branching under shrub reaching about 150cm in height, usually clothed with minutely stellate tomentum; leaves ovate upto 10cm long; flowers are greenish or lurid yellow in axillary fascicles; fruits globose berries which are orange coloured when mature, enclosed in a persistent calyx. The fleshy roots when dry are cylindrical, gradually tapering down with a brownish white surface and pure white inside when broken.

**Chemical constituents:**


Alkaloids include anaferine, isopallatierine, isopallatierine HCl, tropine, pseudotropine, 3α-trigloylrotropane, 3-tropyltigloate, cuscohygrine, anahygrine and hygrine. The pyrazole alkaloid-withasomnine has also been reported from the roots.

The structure of Withaferin A is,

![Structure of Withaferin A](image-url)
Chemistry

The chemistry of Ashwagandha has been extensively studied. More than 35 chemical constituents have been identified, extracted and isolated. Pharmacological activity was mainly due to alkaloids, Withanolides and sitoindosides.

Alkaloids: Roots of Ashwagandha is considered to have a significant pharmacological property. The alkaloidal constituent of *Withania somnifera* was studied and six alkaloids were isolated and confirmed as somniferine, somniferinine, withanine, withanonine and withaninine. About 0.2% of total alkaloids are present. Withanolides are compounds with C-28 steroids on an ergostane-type skeleton in which C-22 and C-26 are appropriately oxidized to form a δ-lactone ring. They are characterized by 9C atoms side chain with a 6-membered ring lactone occurring in plants of Solanaceae family. About 35 withanolides have been isolated till now.

Ashwagandha is characterized by the presence of steroidal lactones, alkaloids and flavonoids. Steroidal lactones were named as Withanolides.

Three new withanolides were isolated from *Withania somnifera* (Solanaceae) of which the major steroidal components were Withaferin A and Withanolide D. Structures were assigned based on spectral evidence (NMR, IR and UV), analysis of their fragmentation under electron impact, as well as on chemical degradation of known compounds.

Methanolic extraction of the leaves of *Withania somnifera* yielded Withanolides. The most important one was reported as Withaferin A that possessed antibiotic and antitumour activity and it fairly exhibits anti-arthritic and anti-inflammatory activities.

Pharmacological activity of Ashwagandha is mainly due to the presence of steroidal lactones like Withaferin A and alkaloids. The total alkaloidal content in the plant is
found to vary widely. Total alkaloids were reported to be present in the range of 0.1 – 0.33%. Reports\textsuperscript{4,42} have shown that the amount of Total Withanolides were present in the range of 0.5-1.5% and glycowithanolides were present in the range of 0.3-2.5% in roots of Ashwagandha. Standardization of Ashwagandha and its extracts have been reported in many standard books\textsuperscript{3,4,42}. Withaferin A is used as one of the marker compounds to standardize Ashwagandha\textsuperscript{3,4}.

USES:

1. **Traditional and modern use:**

   Ashwagandha holds a prominent place in Ayurveda and Unani. The plant finds its use as a rejuvenative herb. It is used as health care food supplement\textsuperscript{39}. The fresh berries are used as emetic, sedative and anti-asthmatic. Dried fruits and roots are sedative, carminative, diuretic, anti-inflammatory and used for curing general and sexual weakness in human beings, goiter, fainting, blood disorders, leucoderma, chronic liver complications, skin diseases, bronchitis and ulcers. In Rajasthan, roots are used for curing rheumatism and dyspepsia; in Punjab they are used to relieve loin pain and in Sind for abortion. Leaves are used as anthelmintic, insecticide, febrifuge and tonic. It is used to cure inflammation of tubercular glands, piles, sore eyes, boils and swelling of hand and foot. In some areas, warm leaves are used to provide comfort during eye diseases. The plant is used as abortifacient, antiasthmatic, bactericide, contraceptive, diuretic, sedative, tonic, anti-inflammatory and in treatment of cold, dropsy, headaches, convulsions, sleeplessness, anemia, fever, hypertension and lumbago\textsuperscript{43-50}.

2. **Ethnoveterinary use:**

   The plant is used in cough, dropsy, rheumatism, scabies and sores. The roots are used to promote milk flow in ruminants\textsuperscript{51}. 
3. Medicinal and pharmacological use:

a) Adaptogenic and anti-stress activity:

**Stress**

**Introduction:** Stress is a reaction people have when excessive pressure or demands are placed upon them and arises when an individual believes they are unable to cope.

In prehistoric times, the physical changes in response to stress were an essential adaptation for meeting natural threats. Even in the modern world, the stress response can be an asset for raising levels of performance during critical events such as a sports activity, an important meeting, or in situations of actual danger or crisis.

If stress becomes persistent, all parts of the body's organs (the brain, heart, lungs, vessels, and muscles) become chronically over or under-activated. This may produce physical or psychologic damage over time. Acute stress can also be harmful in certain situations.

**Psychologic Effects of Stress**

Studies suggest that the inability to adapt to stress is associated with the onset of depression or anxiety. Certainly, on a more obvious level, stress diminishes the quality of life by reducing feelings of pleasure and accomplishment, and relationships are often threatened.

**Heart Disease**

The effects of mental stress on heart disease are controversial. Stress can certainly influence the activity of the heart when it activates the sympathetic nervous system. Such actions and others could negatively affect the heart in several ways. Emotional
effects of stress alter the heart rhythms, which could pose a risk for serious arrhythmias.

**Effect on the Immune System**

Chronic stress affects the immune system in complex ways, which may have various effects.

*Susceptibility to Infections.* Chronic stress appears to blunt the immune response's response to infections and may even impair a person's response to immunizations.

*Inflammatory Response.* Some evidence suggests that chronic stress triggers an over-production of certain immune factors called cytokines, which in excess levels can cause very damaging effects.

**Cancer**

Some animal studies suggest that increased stress had negative effects on immune function and contributed to tumor growth. Although stress reduction techniques have no effect on survival rates, studies show that they are very helpful in improving a cancer patient's quality of life.

**Gastrointestinal Problems**

Prolonged stress can disrupt the digestive system, irritating the large intestine and causing diarrhea, constipation, cramping and bloating. Irritable bowel syndrome (or spastic colon) is strongly related to stress.
Diabetes

Chronic stress has been associated with the development of insulin-resistance, a condition in which the body is unable to use insulin effectively to regulate glucose (blood sugar). Insulin-resistance is a primary factor in diabetes. Stress can also exacerbate existing diabetes by impairing the patient's ability to manage the disease effectively.

Pain

*Muscular and Joint Pain.* Chronic pain caused by arthritis and other conditions may be intensified by stress.

*Headaches.* Tension-type headache episodes are highly associated with stress and stressful events.

Sleep Disturbances

The tensions of unresolved stress frequently cause insomnia, generally keeping the stressed person awake or causing awakening in the middle of the night or early morning. In fact, evidence suggests that stress hormones can increase during sleep in anticipation of a specific waking time.

Memory, Concentration, and Learning

Stress affects the brain, particularly memory, but the effects differ significantly depending on whether the stress is acute or chronic.

*Effect of Acute Stress on Memory and Concentration.* Studies indicate that the immediate effect of acute stress impairs short-term memory, particularly verbal memory.
Effect of Chronic Stress on Memory. If stress becomes chronic, sufferers often experience loss of concentration at work and at home, and they may become inefficient and accident-prone. In children, the physiologic responses to chronic stress can clearly inhibit learning. Chronic stress in older people may play an even more important role in memory loss than the aging process.

Other Disorders

Stress has been related to skin allergies, hair loss etc.

i. A 50% ethanolic extract of *Withania somnifera* was evaluated on mouse. Results showed sex and dose dependent righting reflux and extract had close synergy with phenobarbitol in depressing the mouse CNS response\textsuperscript{52}.

ii. Benoit el al\textsuperscript{53} suggests that among all animal models, the forced swimming test (FST) remains one of the most used tools for screening anti-stress agents. Strain is one of the important parameters to consider and Swiss mice can be used to discriminate the actions of drug. They also mention that FST is currently a popular model, due to low cost of the experiments and it is arguably the most reliable model available. Acute drug treatments are effective to the clinical time course of action in case of FST. Moreover it has been reported to be reliable across laboratories.

iii. Cilene et al\textsuperscript{54} has reported that FST or behavioral despair test is useful for screening anti-stress drugs. Swimming, Climbing, Diving, Head shakes and Immobility of the animals are the parameters which have to be recorded.

iv. *Withania somnifera* is an Indian medicinal plant used widely in the treatment of many clinical conditions in India. Its anti-stressor property have been investigated in a study by Archana et al\textsuperscript{55} using adult Wistar strain albino rats and cold water swimming
stress test. The results indicate that the drug treated animals show better stress tolerance.

v. The forced swimming test (FST), also called behavioral despair or the Porsolt test\textsuperscript{56} was first proposed as a simpler variation of the learned helplessness test, and is probably the most widely used screening test for anti-stress potential of novel compounds. Animals are forced to swim in a confined space. They become immobile following a phase of extensive swimming and climbing. Swimming time of the mice increases according to the potentiality of the drug.

vi. Adaptogenic activity of an herbal formulation containing Ashwagandha was investigated in terms of Anti-stress activity. Sub-chronic administration of the formulation containing Ashwagandha for 7 days, in the doses of 5 and 10ml/kg, orally, showed increased swimming endurance under adverse ambient conditions, which clearly indicates that Ashwagandha has significant anti-stress activity\textsuperscript{57}.

vii. Salil Bhattacharya et al\textsuperscript{58} has studied about anti-stress activity of few compounds including Withaferin A from \textit{Withania somnifera}. It was observed that sitoindosides also produced anti-stress activity, which was potentiated by Withaferin-A. It was also observed from preliminary acute toxicity studies that the compounds have a low order of toxicity.

\textbf{b) Memory enhancement:} The sitoindosides VII-X and Withaferin A, isolated from the aqueous extract of the roots were investigated. The active principles of the plant reversed various cognitive deficits and cholinotoxic effects after two weeks of treatment\textsuperscript{59}.

\textbf{c) Immunomodulatory activity:} \textit{Withania somnifera} exhibited non-specific immunostimulatory activity in various models. The Immunomodulatory activity of sitoindosides IX and X was studied in rats and mice. A stastically significant
mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes secreted by the activated macrophages were observed.  

d) Inhibition of morphine tolerance in mice: Treatment with a root extract inhibited the development of tolerance to the analgesic effect of morphine and inhibited withdrawal jumps.  

e) Antihypertensive activity: An extract induced significant decrease in the arterial and diastolic blood pressure in normotensive pentobarbital anaesthetized dogs.  

f) Antiinflammatory activity: *Withania somnifera* extract exhibited significant antiinflammatory activity against carrageenan-induced paw oedema in rats.  

g) Antiviral activity: An extract showed a dose-dependent inhibition of spinach mosaic virus.  

h) Hepatoprotective activity: A marked histopathological improvement in hepatotoxicity induced by antitubercular drugs was observed on administering herbal preparation containing extracts of *Piper longum* and *Withania somnifera*.  

i) Antioxidant activity: Effects of Ashwagandha root powder administered for 15 and 30 days was investigated on lipid peroxidation (LPO, superoxide dismutase (SOD) and catalase (CAT) activities in mice. It showed a significant decrease in LPO and increase in SOD and CAT.  

j) Antitumour activity: Ethanolic extract of dried roots of *Withania somnifera* showed significant anti tumour activity.  

3.1.2 KALMEGH  

*Andrographis paniculata* Nees belonging to family Acanthaceae, commonly known as Kalmegh is an important medicinal herb. The plant is widely distributed in tropical
and sub-tropical parts of India. The plant is commonly known as “King of bitters”. Kalmegh has been used for medicinal purposes for centuries in India and China. It is mentioned as an important drug in ancient Ayurvedic literature. Mention of the drug is made in ancient literatures like Charaka samhita, Sushruta samhita, Bhava Prakasha etc.

**Kalmegh - Plant Profile**

**Family:** Acanthaceae

**English name:** Green chiretta, Kalmegh, Creat

**Indian name:** Sanskrit: Bhunimbah, Kiratatiktah

Kannada: Nelaberu

Hindi: Kalamegh, Kalpaath

**Distribution:** Throughout India, in the plains and also in the forests.

**Parts used:** Whole plant.

**Botanical description:** An erect branching annual shrub reaching about 0.3m to 0.9m in height with quadrangular branches; leaves simple, lanceolate, acute at both ends, glabrous, main nerves 4-6 pairs; flowers small, pale but blotched and spotted with brown and purple distant in lax spreading axillary and terminal racemes or panicles, calyx-lobes glandular pubescent, anthers bearded at the base; fruits linear capsules, acute at both ends; seeds numerous, yellowish brown, subquadrate.

**Chemical constituents:**

The major constituent of the plant is a diterpene lactone – Andrographolide. The minor constituents include various diterpene lactones, glycosides and flavonoids. Diterpene lactones are andrograpanin, deoxyoxyandrographolide, glycosides are neoandrographolide and andrographiside and flavonols include oroxylin, wogonin, andrographidins A, B, C, D, E and F.
The structure of Andrographolide is,

![Chemical structure of Andrographolide](image.png)

**Chemistry**

The chemistry of Kalmegh has been extensively studied. Whole plant of Kalmegh is considered to have a significant pharmacological property.

Andrographolide, the main crystalline bitter principle of *Andrographis paniculata* was first isolated by Gorter and has been the subject of a number of chemical investigations. The structure of Andrographolide was confirmed by Cava et al. It is reported to be a diterpenoid lactone with a hydroxyl group. The structure of Andrographolide was further confirmed by X-Ray crystallographic studies.

On subjecting the petrol extract of *Andrographis paniculata* to column chromatography, three flavonoids were isolated. Two of them where new flavonoids, namely 5-hydroxy-7,8-dimethoxyflavanone and 5-hydroxy-3,7,8,2′-tetramethoxyflavone. The other one was a known flavone - 5-hydroxy-7,8-dimethoxyflavone. UV, NMR and mass spectroscopy characterized their structures.

Pharmacological activity of Kalmegh is mainly due to the presence of diterpenes lactones and flavonoids. There are many reports where the range of andrographolide is reported. Total andrographolide were present in the range of 0.015- 8.0%. Standardization of Kalmegh and its extracts has been reported in many standard books. Andrographolide is used as marker compounds to standardize Kalmegh.
USES:

1. **Traditional and modern use:**

Kalmegh holds a prominent place in Ayurveda and Chinese medicine. The plant is mainly used for liver diseases. Leaves are used in general debility, dyspepsia, stomach ailments in infants etc. The roots are used as a tonic and stimulant. Whole plant finds its use as antispasmodic, febrifuge, stomachic, alternative, anthelmintic, anodyne, antiseptic, choleric, chologogue, diaphoretic, expectorant, immunostimulant, laxative, astringent and antipyretic. They are used in the treatment of jaundice, diabetes, malaria, cholera, dysentery, enteritis, gastritis, pneumonia, pyelonephritis, hyperdipsia, flatulence, colic, diarrhoea, haemorrhoids and oedema\(^{74-78}\).

2. **Ethnoveterinary use:**

The plant is used in treatment of datura poisoning, new castle disease, treatment of wounds, worms in the eye and abdomen, liver fluke, glossitis, constipation, tuberculosis, pneumonia, leeches in the nostrils, contagious abortion, retention of placenta, tetanus, scabies and respiratory problems of poultry\(^{78}\).

3. **Medicinal and pharmacological use:**

a. **Anti-inflammatory activity:**

**Inflammation:**

**Introduction:** Inflammation is the reaction of a tissue and its microcirculation to a pathogenic insult. It is characterized by the generation of inflammatory mediators and movement of fluid and leucocytes from the blood into extra vascular tissue. By this mechanism the host localizes and eliminates metabolically altered cells, foreign particles, microorganisms or antigens.

The clinical sign of inflammation termed phlogosis by the Greeks and inflammation in Latin were described in classical times. In the second century AD, the Roman
encyclopedist Aulus Celsus described the four cardinal signs of inflammation, namely- Rubor: redness, Calor: heat, Tumor: swelling, Dolor: pain.

**Types of inflammation:**

Depending up on the defense capacity of the host and duration of response, inflammation can be classified as acute and chronic. The components of acute and chronic inflammations are shown in Photo 1.

**Photo 1: The components of acute and chronic inflammatory responses: circulating cells and proteins, cells of blood vessels and cells and proteins of the extracellular matrix.**

A. Acute inflammation is of short duration and represents the early body reaction and is usually followed by repair.

   The main features of acute inflammations are:
   - 1. Accumulation of fluid and plasma at the affected site.
   - 2. Intravascular activation of platelets.
   - 3. Polymorphonuclear neutrophils as inflammatory cells.

B. Chronic inflammation is of longer duration and occurs either after the causative agent of acute inflammation, or the stimulus is such that it induces chronic inflammation
from the beginning. The characteristic features of chronic inflammation are presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages.

**Acute inflammation:**

The changes in acute inflammation can be conveniently described under the following two headings: -

- **Vascular events**
- **Cellular events**

**Vascular Events**: Alteration in the microvasculature (arterioles, capillaries and venules) is the earliest response to tissue injury. These alterations include haemodynamic changes and changes in vascular permeability.

**i) Haemodynamic changes:**

The earliest features of inflammatory response result from changes in the vascular flow and caliber of small blood vessels in the injury tissue. The sequence of these changes is as under: - Irrespective of the type of injury, immediate vascular response is of transient vasoconstriction of arterioles. With mild form of injury, the blood flow may be re-established in 3-5 seconds, while with more severe injury the vasoconstriction may last for about 5min.

- Next follows persistent progressive vasodilatation which involves mainly the arterioles, but to a lesser extent, affects other components of the microcirculation like venules and capillaries. The change is obvious within half an hour of injury. Vasodilatation results in increased blood volume in microvascular region, which is responsible for redness and warmth at the site of acute inflammation.

- Progressive vasodilatation, in turn may elevate the local hydrostatic pressure resulting in transudation of fluid into the extravascular space. This is responsible for swelling of the local site of acute inflammation.
- Slowing or stasis of microcirculation occurs next. Slowing is attributed to increase permeability of micro-vasculature that result in increased concentration of red cells and thus raised blood viscosity.

- Stasis or slowing is followed by leucocytic margination or peripheral orientation of leucocyte (mainly neutrophils) along the vascular endothelium. The leucocytes stick to the vascular endothelium briefly and then move and migrate through the gaps between the endothelial cells in to the extra-vascular space. This process is known as emigration.

The features of hemodynamic changes in inflammation are best demonstrated by the lewis experiment. Lewis induced the changes in the skin of inner aspect of forearm by firm stroking with a blunt point. The reaction so elicited is known as triple response or red line response and consists of the following: -

• Red line appears within a few second following stroking and results from local vasodilatation of capillaries and venules.

• Flare is the bright reddish appearance or flush surrounding the red line and results from vasodilatation of the adjacent arterioles.

• Wheal is the swelling or edema of the surrounding skin occurring due to transudation of fluid in the extra-vascular space.

ii) Altered vascular permeability:

Pathogenesis: In and around the inflamed tissue, there is an accumulation of edema fluid in the interstitial compartment which comes from blood plasma by its escape through the endothelial walls of peripheral vascular bed. In the initial stage, the escape of fluid is due to vasodilatation and consequent elevation in hydrostatic pressure. This is transudate in nature. But subsequently the characteristic inflammatory edema exudates, appears by increased vascular permeability of
microcirculation. The appearance of inflammatory edema due to increased vascular permeability of microvascular bed is explained on the basis of starling’s hypothesis. In normal circumstances, the fluid balance is maintained by two opposing sets of forces:

- Forces that cause outward movement of fluid from microcirculation.
- Forces that cause inward movement of interstitial fluid into circulation.

Whatever little fluid is left in the interstitial compartment is drained away by lymphatic and, thus no edema results normally. However in inflamed tissues, the endothelial lining of microvasculature becomes more leaky. Consequently, intravascular osmotic pressure decrease and osmotic pressure of the interstitial fluid increases resulting in excessive outward flow of fluid into the interstitial compartment which is exudative inflammatory edema.

Anti-inflammatory activity of natural agents.

Natural products (eg. plants) or natural product derived compounds represent great structural diversity which is not commonly seen in synthetic compounds and natural products are generally believed to be safe. Thus, natural products (and traditional medicine) offer great hope in the identification of bioactive compounds and their development into drugs for the treatment of inflammatory diseases. Since ancient times, many people suffering from inflammation were treated with phytochemicals. This is evident from the discovery of the first anti-inflammatory, analgesic drug aspirin which was based on the known analgesic, and anti-pyretic property of the bark of willow-tree since 400 BC by the Greeks and Romans.

Flavonoids (Quercetin, luteolin) a major class of polyphenolic compounds widely distributed through out the plant kingdom are found to possess interesting anti-inflammatory activity. They are known as nature’s tender drug. They possess various
biological and pharmacological activities including anticancer, anti-viral, anti-inflammatory, immunomodulatory and antithrombotic activity. Of these pharmacological activities, the anti-inflammatory capacity of flavonoids has long been utilized. Flavonoids possess antioxidative and free radical scavenging activities. They could regulate cellular activities of the inflammation related cells, mast cells, macrophages, lymphocytes and neutrophils. Some flavonoids inhibit histamine release from mast cells and others inhibit T-cells proliferation. Certain flavonoids modulate the enzyme activities of arachidonic acid (AA), metabolizing enzymes such as phospholipase A\textsubscript{2} (PLA\textsubscript{2}), Cyclooxygenase (COX) and Lipooxygenase (LOX) and NO producing enzyme, Nitric Oxide Synthase (NOS). An inhibition of these enzymes by flavonoids reduces the production of AA, PG (end product of COX and LOX pathway), LT and NO, crucial mediators of inflammation.

Proposed possibility of anti-inflammatory property of flavonoids is their ability to inhibit neutrophil degradation. This is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells. As a form of plant extract, flavonoids could improve the symptoms of acute inflammatory as well as chronic inflammatory disorders including rheumatoid arthritis (RA), and some allergic disorders.

i. Tajuddin et al\textsuperscript{79}, has studied anti-inflammatory activity of aqueous extract of \textit{Andrographis paniculata} by carrageenan induced edema. The extract showed significant anti-inflammatory activity in the doses of 20 mg/100 gm body weight orally.

ii. \textit{Andrographis paniculata} is reported to be a potent anti inflammatory agent by Lin et al\textsuperscript{80}. In his study, extracts and their active constituent andrographolide were evaluated for antioxidant, antioedema and analgesic activities. It was observed that at a dosage
of 100 mg/kg, aqueous extract and andrographolide showed antioedema and analgesic activities.

**iii.** Medicinal Plant Research Institute, Ministry of Public Health, Thailand\(^81\) found anti-inflammatory activity of *Andrographis paniculata* in rats using method for testing carrageenan-induced hind paw edema. Dried powder, ethanolic and water extract of *Andrographis paniculata* given orally to rats showed anti-inflammatory property when tested by carrageenan-induced hind paw edema and inhibition of white blood cells, infiltration and granuloma development were reported by actions of these extracts. Mechanism of anti-inflammatory actions is also reported. Anti-inflammatory action of *Andrographis paniculata* is related to i) inhibition of nitric oxide (NO) production from inflammatory macrophages by neoandrographolide ii) inhibition of NO production by decreasing expression of inducible nitric oxide synthase (iNOS) in macrophages by andrographolide\(^82,83\) and iii) inhibition of neutrophil adhesion and transmigration and by prevention of reactive oxygen species\(^84\).

**iv.** Major active component of the plant *Andrographis paniculata* is andrographolide and it has been shown to possess many pharmacological effects including antioxidant and anti-inflammatory activities by Sheeja et al\(^85,86\).

**b. Antidiaarrhoeal activity:** Alcoholic extract of the plant showed significant activity against diarrhoea induced by *Escherichia coli* enterotoxins\(^87\).

**c. Antimalarial activity:** Alcoholic extract when studied against *Plasmodium berghei* NK 65 in *Mastomys natalensis* suppressed the level of parasites in dose dependent manner\(^88\).

**d. Antiviral activity:** Dry extract of the plant showed efficacy in initial treatment of common cold and sinusitis by decreasing the symptoms\(^89,90\).
e. **Antipyretic activity:** Oral administration of juice of the plant normalized induced pyrexia condition in rats\(^9\).  

f. **Antiatherosclerotic activity:** The plant alleviated atherosclerotic artery stenosis induced by both de-endothelialisation and a high cholesterol diet. It also lowered the restenosis rate after experimental angioplasty\(^9\).  

g. **Cardiovascular activity:** n-butanol extracts of the plant produced a significant fall in the mean arterial blood pressure of anaesthetized rats\(^9\).  

h. **Hypotensive activity:** Aqueous extract of the plant exhibited dose-dependent hypotensive effect on systolic blood pressure of rats\(^9\).  

i. **Immunomodulator activity:** Ethanolic extract of the plant-induced stimulation of antibody and delayed-type hypersensitivity response to sheep RBC in mice\(^9\).  

j. **Antifertility activity:** Dry leaf powder of the plant when fed orally to male albino rats produced antispermatogenic and antiandrogenic effect.  

k. **Hepatoprotective activity:** Administration of alcoholic extract of the plant showed significant antihepatotoxic action in *P.berghi* K173 induced hepatic damage in *M.natalensis*\(^9\). Extracts of *Andrographis paniculata* showed significant hepatoprotective activity when studied on hepatotoxicity-induced rats\(^9\).
3.2 Microbial contamination of medicinal plants

- Crude drugs are often contaminated to a greater or lesser extent by microorganisms of various kinds. If untreated, the herbs will result in rapid spoilage. To assess the quality of a crude drug, not only must its identity and amount of actives be checked, but also the degree of microbial contamination.

Post harvest and post processing herbs are always contaminated with microorganisms from the plant themselves, from soil, air, water and dust. Wide spectrum of microorganisms and microbial loads are reported previously in medicinal plants. Probably the most commonly documented reason for toxic or adverse reactions to herbal products is the presence of microbial load.

- Raw materials must be properly processed right from the time of sowing up to storage, but this does not happen always. Such was the case for an herb and spice exporter in Guatemala, who was puzzled that certain herbs repeatedly failed the buyer’s quality standards, despite thorough washing. Research revealed that when herbs were harvested, the cut stems were placed on the ground, where they continued to absorb moisture and resulted in formation of microorganisms that contaminated the final product.

- Herbs are biological substances and hence will naturally contain large numbers of a variety of microorganisms. Acceptable levels for the microbial contamination of herbs and herbal products have been designated in various authorities such as the British Pharmacopoeia (BP). Not surprisingly, surveys of crude medicinal herbs have found high levels of total aerobic organisms, Enterobacteriaceae, coliform organisms, yeasts and molds. A survey of 138 medicinal herbs found that while none of the samples contained enterohemorrhagic E. coli, Salmonellae, Pseudomonas aeruginosa, Listeriae, Staphylococcus aureus or Candida albicans, four samples were E. coli
positive, two samples were presumptively _Campylobacter jejuni_ positive and nine herbs contained a potentially aflatoxigenic mold\textsuperscript{101}.

- It is expected that the manufacturing processes will target a substantial reduction in the bioburden (for example by extracting with ethanol-water mixtures) and so what is more relevant are the levels in finished products. A survey of 425 licensed herbal products by the UK Medicines Control Agency found that the majority of products complied with European Pharmacopoeia (EP) standards\textsuperscript{15}.

- However, some products, especially the solid oral dosage forms, were found to contain undesirable organisms such as _Enterobacter_ spp. (31\% of all samples), _Enterococcus faecalis_ or _faecium_ (23\%) and _Clostridium perfringens_ (6\%). Also 22\% exceeded the EP standard for total aerobic bacterial count. Most surveys of medicinal herbs for aflatoxins have not found excessive levels\textsuperscript{102}. However, researchers in India and Egypt have found significant levels of contamination in some herb samples\textsuperscript{103}. This might be expected since the higher heat and humidity in tropical regions could favor the development of fungal growth if inadequate drying procedures are used. A follow-up study by the Indian group detected aflatoxins in seed samples of two different Indian herbs\textsuperscript{104}.

- In another study, some herbal concoctions were investigated for bacterial and fungal quality by serially diluting and plating on nutrient agar and potato dextrose agar plates in triplicate and incubating at room temperature for 24 and 96 hours respectively, to allow bacterial and fungal growth. It was observed that the level of microbial contamination exceeded the tolerable limits\textsuperscript{105} and there was presence of a large number of pathogenic organisms.
Favet J\textsuperscript{106} has analysed twenty drugs to determine the microbial contamination. Total viable aerobic count and total fungal count showed variations in the sample. 

\textit{Salmonella} and \textit{Aspergillus flavus} was never found.

Herbal drugs stored in the markets are hazardous for human health as reported by Efuntaye MO\textsuperscript{107}.

A study conducted by Adriana et al\textsuperscript{108} showed that the increasing consumption of natural drugs has made their use a Public Health issue, due to the possibility of accessing products without quality. The concern about quality was mainly due to the potential microbiological contamination of the products, by their natural origin. Ninety-one samples of sixty-five herbal species were evaluated in relation to microbial contamination. About 10 generas are contaminated with mycotoxins mainly \textit{Aspergillus} and \textit{Pencillium} genera, which has the capability of producing Ochratoxins, aflatoxin and citrinine. Presence of toxigenic moulds represents potential risk of mycotoxin contamination Results indicated that 92.3\% of the herbal drugs failed to comply with the pharmacopoeial parameters of acceptance and therefore, it suggests that regulatory and educational measures are needed in order to guarantee the quality of these products to reduce the risk of consumer’s health.

McKee\textsuperscript{98} has quoted in a review that herbs can often be a major source of microbial contamination and this suggests a need for better control in production, processing and usage of these products.

Herbs and spices are exposed to a wide range of microbial contamination during their cultivation, harvest, processing, storage, distribution and sale. Sources of microbial contamination include the macro environment (i.e., soil or plant in which the products are grown), dust, insects, faecal material and contaminated water\textsuperscript{109}. In addition, microbial contamination can arise from poor handling and poor hygiene practices by
Most herbs and spices are known to be contaminated at the point of origin by varying degrees with aerobic and anaerobic bacteria. The microbial content of herbs and spices has been thoroughly investigated since 1930’s through to present day. Members of anaerobic spore forming species are found but usually in low numbers. Thermophillic organisms are also indigenous to spices but their presence is generally at low levels. Many of researchers investigated different commodities and levels of contamination by different microorganisms. They found that the contamination levels range from plate counts of 0 to $10^8$ microorganisms CFU/gm (Colony Forming unit/gm) for standard plate count, 0 to $10^6$ for coliforms and 0 to $10^7$ for yeasts and moulds, depending on the product. Some products are more susceptible to mould contamination, including pepper (white and black), chilies and chili powder, coriander, caraway and other herbs.

- Martins et al. evaluated the nature and content of microbiological contamination of 62 samples of seven medicinal plants (Chamomile, leaves of orange tree, flowers of linden, corn silk, marine alga, pennyroyal mint and garden sage) using conventional microbiological methods. Practically all samples (96.8%) were contaminated with Bacillus cereus; 19.2% of them with levels higher than $10^3$ CFU/gm. The highest levels were found in corn silk samples ($>10^7$ CFU/gm). The fungal contamination of seven medicinal plants was reported and the mean level of fungal population was $10^5$ CFU/g.

- Most of herbs and spices are known to be contaminated to varying degrees with fungi species of Aspergillus, Fusarium and Alternaria which are common types of fungi in air, soil and many agricultural commodities studied by Aziz et al.

The use of a suitable decontamination processes is therefore an important step towards the consumer safety and therapeutical efficiency.
3.3 Sterilization methods used for microbial decontamination

- There are a number of conventional methods been applied for microbial decontamination of medicinal plants. Both chemical and physical methods have been used with varying degrees of success. Fumigation with ethylene oxide (ETO), methyl bromide (MB) or propylene oxide (PO) is the main chemical method used commercially in several countries. Physical methods include steam, heat sterilization and irradiation\textsuperscript{114}.

a. **Chemical treatment with Ethylene oxide:** is commonly used to decontaminate herbs and spices, with varying degree of success. But use of ethylene oxide is prohibited in many countries because it reacts with organic components to leave the harmful residues - ethylene chlorohydrins and ethylene bromohydrin on herbs, which are carcinogenic. Ethylene oxide (ETO) is considered by the International Agency for Research on Cancer (IARC) to be a human carcinogen\textsuperscript{115}. As a fumigant, ETO gas is currently used as a way to disinfect spices potentially contaminated with pathogenic bacteria, such as *Salmonella*, in New Zealand, the United States and Canada. However, in Europe, ETO is banned due to concerns of the potential toxicological risks to workers and consumers. Many countries permit irradiation as an alternative treatment to ETO fumigation\textsuperscript{116}. Fumigation may result in reduction of volatile oil content\textsuperscript{116} and can dull the colour.

b. **Sterilization by heat:** Processes used for the decontamination of dried products, such as high pressure steam, often include organoleptic degradation or achieve only a low destruction rate. Indeed, most decontamination processes involving heating induce severe damage to the products.

c. **Ultra violet (uv), Infrared and Microwave:** The uv irradiation as a method of sterilization is not effective because of its low penetrating power\textsuperscript{117}. Infrared and
microwave irradiation have proved to be of limited value because these methods are basically forms of heating and consequently have the same disadvantages of the use of heat.

d. Ionizing radiation: The use of ionizing radiation as a physical method of microbiological decontamination of food, including spices and medicinal plants, was approved by the Codex Alimentarius Commission in 1983. The experts agreed that decontamination by ionizing radiation is a safe, efficient, environmental clean and energy efficient process. Irradiation is used to inactivate food-borne microorganisms, to reduce quality losses during storage and to guarantee the hygienic quality of several foodstuffs such as poultry, meat, spices and herbs\textsuperscript{118}.

3.4 Types of radiation: Expert committee of WHO/IAEA/FAO has approved three types of ionizing radiation to be used in food and other medicinal material irradiation (WHO, 1998). They are:

1. Gamma rays from radioisotopes Cobalt-60 or Cesium-137. They are most often used in irradiation application because of their high penetrating power and low cost.

2. Electron beam generated from machine sources (accelerators) operated at or below an energy level of 10 MeV.

3. X-ray generating from machine sources operated at or below an energy level of 5 MeV.

- A large number of facilities in the world use gamma irradiation for sterilization of medicinal plants. Gamma radiation from Cobalt – 60 source is a well established industrial process in India. It is very efficient and convenient technique for achieving a high level sterility. There are several advantages with the use of gamma radiations compared to Electron Beam and X – ray machines. Cobalt - 60 is a monoenergetic
radiation source with half life of 5.27 years. It is readily available from simple nuclear reactions in nuclear reactors. The major difference in gamma radiation and EB lies in their penetration powers, where gamma radiations can penetrate deep inside the products, the electron beams do not have good penetration power. Though X – rays in the energy ranges of 8 – 10 Mev have penetrations comparable to those of gamma rays, they are not yet very popular. Probably the fact that at these ranges of energy, X – rays may also cause nuclear transformations and prohibit their use in commercial scale. The maximum energy of gamma radiations from Cobalt – 60 is only 1.33 Mev, which is not sufficient to cause nuclear transformations of any type in the products being processed. Gamma radiations from Cobalt – 60 or Cesium – 137 are therefore considered more useful for sterilization. Radiation Sterilization is a cold process, with a temperature rise of not more than a few degrees centigrade. The process is particularly suitable for industrial scale sterilization of heat sensitive products, enclosed in air and moisture proof packs in shipping cartons. The radiation dose required for the destruction of fungi and majority of bacteria varies from 1.5 to 15kGy. Usually bacterial spores are more resistant than the vegetative forms. A radiation dose of 25kGy (2.5Mrad) is the officially accepted dose for medical product sterilization in many countries. The FDA (USA) and UK panel have accepted the concept of dosimetric release for radiation sterilized medical devices manufactured under GMP. No post sterility microbiological testing is required with radiation if dosimetric release procedures are followed. Radiation sterilization at a dose of 25kGy provides such a high safety factor that, tests for sterility are generally considered redundant. However, as mentioned elsewhere, one must strictly adhere to good manufacturing practices (GMP) during the manufacturing stage of the healthcare products so as to attain high sterility assurance levels.
Taking into consideration, gamma irradiation is considered to be the most reliable method for microbial decontamination of medicinal plants. Gamma irradiation results in a much lower level of microbial contamination and is often the only treatment effective to meet standards set by processors operating under Hazard Analysis Critical Control Points (HACCP) or International Organization for Standardization (ISO) (WHO, 1994).

**Gamma Irradiation:**

A gamma radiation plant essentially consists of,

- Radiation source housed in a concrete cell
- Automatic conveyor system
- Control and Interlock system
- Service laboratories
- Storage area for Processed/ Non-processed products
PHOTO 2 - ISOMETRIC VIEW OF GAMMA IRRADIATION PLANT

PHOTO 3 - PRODUCT FLOW IN IRRADIATION CELL

Radiation Source Frame housing Cobalt-60 pencils (550 kCi)

Traverse the path along the direction of arrows

Complete one cycle, 5 cycles required in five different shelves

The product Boxes enter here for irradiation
3.5 **Units of Radiation:** When an ionizing radiation is emitted from a radioactive source, it penetrates into the medium (e.g. medicinal plants and their phytopreparations). All or part of the radiation energy is absorbed by that medium. The quantity of energy absorbed by the medium is called the absorbed dose which is measured in “rad”. It is defined as a unit equivalent to the absorption of 100 ergs/g of matter. The new unit used now according to the international system is the gray “Gy”. It is equal to the absorption of 1 J/Kg.

\[ 1 \text{ Gy} = 100 \text{ rad} \]
\[ 1 \text{ kGy} = 100 \text{ Krad} \]
\[ 10 \text{kGy} = 10^6 \text{ rad} = 1 \text{ Mrad} \]

The amount of ionizing radiation energy absorbed in a unit of time is called the “dose rate”. Radiation exposure was measured in Röentgen (R) and now in Coulomb (WHO, 1998).

3.6 **How does the process of radiation work?**

Radiation processing of medicinal plant is carried out inside an irradiation chamber shielded by 1.5 to 1.8 meter thick concrete walls. Products, either pre-packed or in-bulk, placed in suitable containers is sent into the irradiation chamber with the help of an automatic conveyor. The conveyor goes through a concrete wall labyrinth, which prevents radiation from reaching the work area and operator room. When the facility is not in use, the radiation source is stored under 6 meter deep water. The water shield does not allow radiation to escape into the irradiation chamber, thus permitting free access to personnel to carry out plant maintenance. For treating herbs, the source is brought to the irradiation position above the water level after activation of all safety devices. The goods in aluminium carriers or tote boxes are mechanically positioned...
around the source rack and are turned round their own axis, so that contents are irradiated on both the sides. Absorbed dose is checked by placing dosimeters at various positions in a tote box or carrier. The quantity of dose is measured in terms of unit, called Gray, abbreviated as Gy. It is the unit of absorbed radiation energy. One gray is equivalent to 1 Joule per kilogram. The old unit of dose is ‘rad’. 1 Gray is equivalent to 100 rad. Irradiation is a direct, simple, and efficient one-time process. It works by disrupting the biological processes that lead to destruction of microorganisms.

**Cost of Irradiated Food**

When we realize about the risk benefit ratio of the product, the cost will not be a major concern. Processing with gamma irradiation brings benefits to consumers in terms of availability, storage, life, distribution and improved hygiene of medicinal plant products. Irradiation costs may range from Rs.1 to Rs.3 per kilogram for high dose applications such as treatment of spices for microbial decontamination. The costs could be brought down in a multipurpose facility treating a variety of products around the year.

### 3.7 Skepticism about Irradiated herbs

There are misconceptions in the minds of practitioners, health care providers and consumers about irradiated products. The main fear is about the toxicity and loss of pharmacological activity of irradiated products. However, scientific research has proved that consumption of irradiated herb is absolutely harmless. The safety of food processed by radiation has been examined carefully, both at the national and international levels. On the basis of extensive studies with laboratory animals carried out in different countries including India, FAO/IAEA/WHO Joint Expert Committee
has recommended that the items irradiated up to an average dose of 10 kilo Gray be accepted as safe from the health angle and do not present any toxicological hazards. In fact, the doses of irradiation required for the treatment of commodities are far below this stipulated limit. The committee has further recognized radiation as a physical process like thermal processing and not as a food additive. The irradiation process involves passing of products through a radiation field allowing the product to absorb desired radiation energy. The product itself never comes in contact with the radioactive material. Gamma rays, X-rays and electrons prescribed for radiation processing do not induce any radioactivity in medicinal plant products. In comparison to other processing and preservation methods the nutritional value is least affected by irradiation.

Extensive scientific studies have shown that irradiation has very little effect on the main nutrients such as proteins, carbohydrates, fats, and minerals. Vitamins show varied sensitivity to food processing methods including irradiation. For example, vitamin C and B₁ (thiamine) are equally sensitive to irradiation as well as to heat processing. Vitamin A, E, C, K and B₁ in foods are relatively sensitive to radiation, while riboflavin, niacin and vitamin D are much more stable. The change induced by irradiation on nutrients depends on a number of factors such as the dose of radiation, type and packaging conditions. The Joint Expert Committee of the Food and Agriculture Organization (FAO), World Health Organization (WHO), and International Atomic Energy Agency (IAEA), in 1980 concluded that irradiation does not induce special nutritional problems. The committee also rejected the possibility of development of chromosomal abnormalities by the consumption of irradiated products.
3.8 Efficacy of gamma irradiation on medicinal plants:

Radiation treatment is an emerging technology in an increasing number of countries and more and more clearances on radiation decontaminated foods are issued or expected to be granted in the near future\textsuperscript{119}. Ionizing radiation has the ability to ionize compounds, thereby creating highly reactive free radicals. The killing effect of radiation can be attributed to breaking of chemical bonds of essential macromolecules such as DNA or to the ionization of water which results in forming highly reactive radicals such as H, OH, etc. These free radicals split carbon bonds of macromolecules in living organisms, thereby killing the organisms. Since no heat is generated in this form of destruction of microorganisms, radiation sterilization is commonly known as “cold sterilization”. Herbs and spices are suitable for treating with ionizing radiation, as the process does not affect the chemical/physical properties of the material, yet at a dose of 10kGy the microbial population is reduced by at least $10^5$ CFU/g\textsuperscript{120}. The effect of different radiation doses on the microbial content of spices has been studied in detail and it has been confirmed that doses between 10 and 20kGy lead to complete sterilization of spices where the original level of contamination was of the order $10^7$ CFU/g (IAEA\textsuperscript{121}).

- In general, it was found that moulds, fungi and coli forms are eliminated by doses lower than those required for bacteria. Studies indicated that minimum doses as low as 4-5kGy will destroy these organisms while some bacteria and yeasts require minimum doses of 10kGy to reach non-detectable levels\textsuperscript{122,123}.

- Fang and Wu\textsuperscript{123} showed that the sterilization dose recommended for most herbs was 10kGy for dry herbs, 7kGy for herbal medicines and 5kGy for more sensitive herbal medicines.
Pathogenic bacteria that may be present in herbs, spices or other vegetable seasonings can be inactivated by a relatively low absorbed dose. Microorganisms belonging to the Enterobacteriaceae family are generally susceptible and can be killed at a dose of 4-6kGy. The number of mesophilic aerobic microbes usually decreases by 2-3 orders of magnitude after being treated with 5kGy. Of the spore forming bacteria, the most frequent genus in spices is Bacillus. The number of anaerobic bacteria spores is normally low and an absorbed dose of 5kGy kills them.

Kneifel and Berger have reported that a dose of 2 to 3kGy are sufficient to control or reduce many food borne pathogens, such as Salmonella, Escherichia coli and Vibrio, which may be found on herbs and spices.

Ramamurthy et al reported that a dose of 2kGy was sufficient to eliminate Coliforms, Listeria and Yersinia in Capsicum. Trampuz et al studied the effect of gamma irradiation on Staphylococcus epidermidis and Escherichia coli and found that doses of 2.8 and 3.6kGy, respectively were enough to eliminate them.

Aziz noticed that irradiation at 5kGy was lethal dose for all fungi and yeast contaminating the dried foods. He has analyzed the effect of gamma radiation on the viability of fungi-contaminated medicinal plants. They found that the viable counts of these flora decrease approximately with the level of radiation dose, the effective dose for the elimination of these microorganisms being about 5kGy, for all the medicinal plants studied e.g. fennel, ginger, black cumin, saffron, cinnamon and chamomile. Swailam and Abdulla found that irradiation dose of 5 and 10kGy reduced total fungal counts by about 57.4% and 97.5% respectively in Caraway, anise and qarad (medicinal plants).

Several authors have reported the treatment of medicinal herbs with gamma irradiation to prevent the microbial load. Various physiochemical and sensory
characters of the plants were observed after irradiation and the method was found to be suitable.

- Seventeen species of Thai medicinal herbs were microbially decontaminated by gamma irradiation at doses of 7.7 and 8.8 kGy. The samples were tested qualitatively and quantitatively for total aerobic bacteria, *Staphylococcus* species, *Salmonella* species, coliform bacteria, fungi and gamma irradiation was found suitable to microbially decontaminate the samples\(^{131}\).

- Department of AYUSH has carried out work on gamma irradiation of some 20 herbs and based on the study, a dose of 5 – 10kGy was recommended for microbial decontamination of Ayurveda plant based drugs and formulations.

- Authors\(^{132}\) have reported certain generalized factors about microbial contamination levels and corresponding resistivities to irradiation. Total aerobic plate count is the most informative measure of contamination and the treatment dose needed to reduce initial contamination to tolerable level is between 4 and 30kGy in a typical scenario and between 8 – 40kGy under the worst case scenario for raw materials and botanical products.

- In another study, effects of gamma irradiation (5, 10 and 20kGy) on the physiological activity of Korean soybean fermented foods were investigated. Further physiological activity was evaluated and was observed that up to 10kGy there was no significant change\(^{133}\).

- Maity JP et al\(^{134}\), has reported the effects of gamma irradiation on some common storage edible seeds in India. 6kGy dose of gamma irradiation was applied for the exposed seeds. It was observed that there was depletion in fungal population.
Black cumin samples were irradiated under 2.5, 6, 8 and 10kGy doses. It was observed that increase in doses increased the peroxide and free fatty acid values. But oil content, iodine value, rancimat value, refractive index and percentage of unsaturated fatty acids decreased. Another important parameter was undetectable reduction in total bacterial count and total count of yeast and moulds\textsuperscript{135}.

Prasad et al\textsuperscript{136} has reported the effect of $\gamma$-irradiation on the volatile oil constituents of some Indian spices. The volatile oils of commercial samples of clove, cardamom and nutmeg were gamma-irradiated at 10kGy and were isolated and subjected to gas liquid chromatography. There were no qualitative and major quantitative changes in irradiated clove and cardamom. But irradiated nutmeg showed a 6-fold increase in content of myristicin and decrease in elimicin.

Effect of $\gamma$-irradiation was studied on the volatile oil constituents of turmeric by Suchandra chatterjee et al\textsuperscript{137} at a dose of 10kGy on subjecting to GLC and GC/MS. No detectable difference was observed in irradiated samples.

Hye-Young S et al\textsuperscript{138} has carried out studies to determine the effect of gamma irradiation on volatile flavor components of Angelica gigas Nakai. A total of 116 compounds were identified and quantified by GC/MS. It was found that yields of active substances were increased after irradiation.

Chemical, sensory and microbiological changes of gamma irradiated coconut cream powder was studied by Norimah Y et al\textsuperscript{139}. Samples were gamma irradiated between 0-15kGy. Iodine value, periodic value and thiobarbituric acid values were analyzed. Along with this, sensory characters like taste, odour and overall acceptance were studied. Microbiological studies were carried out and no colonies were detected after irradiation. Based on the results, gamma irradiation at a dose of 5kGy was found to be optimum dose to decontaminate the sample.
Seeds of *Pimpinella anisum* were gamma-irradiated from 0-20kGy by Al-Bachir\textsuperscript{140}. Microbial population, total and inorganic soluble solids in water extract and sensory properties were evaluated after 0, 6 and 12 months of storage. Results showed that gamma irradiation reduced aerobic plate counts of aniseed and also there were improved sensory characteristics. There were no significant differences in other parameters.

Al Bachir\textsuperscript{141} has studied the effects of gamma irradiation from 0-2.5kGy on fungal load, chemical and sensory characteristics of *Juglans regia* \textit{L}. Fungal load, proximate composition, chemical changes and sensory properties were evaluated immediately after irradiation and after 12 months of storage. Results indicated that gamma irradiation reduced fungal load and there was no significant difference in chemical and sensory characters. But after 12 months of storage, decreased total acidity and peroxide values and increased iodine value and volatile basic nitrogen were observed and also there was a negative effect on sensory characteristics.

Microbiological, chemical and sensory characteristics of licorice root powder after subjecting it to gamma-irradiation from 0-20kGy were studied by Al Bachir et al\textsuperscript{142} at 0 and 12 months of storage. Results indicated that gamma-irradiation reduced the counts of micro-organisms on licorice root powder. However, mineral ions from irradiated products were lower and glycyrrhizinic acid and maltose concentration were higher and there was no significant differences in sensory characters.

Piggott and Othman\textsuperscript{143} have studied the effects of irradiation on volatile oils of black pepper. Samples were irradiated at 10, 20 and 30kGy and stored for 1, 30 and 90 days at 24° c. Composition of volatile oil was analyzed by Capillary Gas Chromatography and no systematic changes were observed.
Lycium fruit was exposed to several doses of gamma-irradiation (0 - 14kGy) by Hsiao-Wei et al\textsuperscript{144} to evaluate decontamination efficiency, changes in chemical composition and in sensory characteristics. After 10kGy of irradiation Bacillus cereus was the only survivor. It was found that 14kGy is the optimal decontamination dose for Lycium fruit for retention of its sensory quality and extension of shelf-life.

Branka K et al\textsuperscript{145} has studied the microbiological decontamination of botanical raw material and corresponding pharmaceutical products by irradiation and has suggested that 4-30kGy is sufficient to treat typical contamination or 10-40kGy for severe cases.

According to Farkas\textsuperscript{119}, several decontamination methods exist but the most versatile treatment is processing with ionizing radiation. It is a safe, efficient, environmentally clean and energy efficient process and is considered as an end product decontamination procedure. It is also said that radiation decontamination of dry ingredients, herbs and enzyme preparation with doses of 3-10kGy is proved to be a viable alternative to fumigation with microbicidal gases. It is an emerging technology in an increasing number of countries and more and more clearances on radiation decontaminated products are issued or expected to be granted in the near future.

Effect of gamma radiation was studied on Cascara bark upto four months after the irradiation process by Ciranni et al\textsuperscript{146}. HPLC and ESR studies were carried out and results showed that there was no difference in un-irradiated and gamma irradiated samples.

Effects of gamma irradiation on anti-microbial activity of Anacardium occidentale was investigated by Gustavo et al\textsuperscript{147}. Exposure to radiation caused changes in physical and chemical constituents of phenolic extracts of leaves of cashew, increasing levels of tannins. Gamma radiation caused an increase in antimicrobial activity of extracts against Staphylococcus aureus.
Kumari et al\textsuperscript{32} have reported that gamma-radiation dose up to 5kGy could be safely used to hygienize Triphala, which is a mixture of \emph{Emblica officinalis}, \emph{Terminalia chebula}, and \emph{Terminalia bellirica}. A high level of microbial contamination was observed in Triphala samples obtained from different sources. On gamma radiation processing, there was a sharp decline in log CFU with increasing radiation dose and a complete decontamination at 5kGy. Water extracts of irradiated samples showed linearly increasing concentration of gallic acid (3.3 to 4.5 times), total phenolic contents (2.16 to 2.87 times), and antioxidant properties with increasing radiation dose up to 25kGy. Aflatoxin B1 and ochratoxin could not be detected in the samples.

3.9 Effect of gamma irradiation on chemical constituents:

- Rajendra et al\textsuperscript{148} has reported that application of high dose irradiation if required for microbial decontamination of dried Welsh onion was feasible as it enhanced the total concentration of volatile compounds by 31.60\% and 24.85\% at 10 and 20kGy respectively.

- Gupta \textit{et al}\textsuperscript{149} studied the effect of gamma irradiation (7.5-10kGy) on major fatty acids (Oleic, linoleic, linolenic, palmitic, stearic), saponins including Ginsenosides in ginseng-red powder. No effect of irradiation on major fatty acid composition and no significant changes in saponins concentration were observed.

- Swailam and Abdullah\textsuperscript{128} reported that gamma irradiation doses (5, 10 and 15kGy) used, resulted in fluctuation changes in the relative percentage of the major fatty acids identified in irradiated samples.

- Extensive research has shown that proteins, essential amino acids, minerals, trace elements and most vitamins do not represent significant losses during irradiation even at doses over 10kGy\textsuperscript{150}.
A study conducted by X. Fan et al\textsuperscript{151} from U.S. Department of Agriculture showed that fresh cilantro irradiated at 2kGy retained its sensorial quality and shelf-life. Irradiation did not have a consistent effect on antioxidant power or phenolic content during the 14-day storage. The total aerobic plate count of irradiated cilantro was significantly lower than that of non-irradiated controls immediately after irradiation and during the entire storage period.

Nine spice and aromatic herb samples were gamma irradiated by Lucia et al\textsuperscript{152} at a dose of 10kGy. Irradiation resulted in a general increase of quinone radical content in all of the investigated samples, as revealed by electron paramagnetic resonance spectroscopy. The fate of these radicals after storage for 3 months was also investigated.

Prakash et al\textsuperscript{153} has studied the effects of gamma irradiation on microbial load, total aflatoxins and phytoconstituents content of \textit{Trigonella foenum-graecum}. Gamma irradiation at a dose of 2.5kGy resulted in 2 log reduction of the total aerobic microbial count. A complete sterilization was, however, observed at 10kGy. The total aflatoxin level decreased gradually with increase in gamma irradiation dose as compared to its un-irradiated counterparts, whereas the high performance liquid chromatography (HPLC) profile showed no change in the levels of phytochemicals upto the gamma irradiation dose of 10kGy. HPLC profiles, however, differed in peak areas, and retention times of the components. These results suggest that gamma irradiation at a dose of 5.0kGy was very effective for microbial decontamination because it did not adversely affect the active components of \textit{Trigonella foenum-graecum}.

Effects of gamma irradiation on hygienic quality and extraction yields in twenty-one kinds of Korean medicinal herbs were investigated by Mi-Jung Kim et al\textsuperscript{154}. Gamma
irradiation at 5–10kGy inactivated contaminating microorganisms. The total extraction yield in fifteen kinds of the investigated medicinal herbs increased by 5–25% at a dose of 10kGy.

- Fungal burden, toxigenic molds and mycotoxin contamination were evaluated in gamma irradiated medicinal plants stored before and after 30 days of irradiation treatment by Aquino et al. The samples were treated using a $^{60}$Co gamma ray source with doses of 5 and 10kGy. Non-irradiated samples were used as controls of fungal isolates. Gamma radiation treatment can be used as an effective method for preventing fungal deterioration of medicinal plants subject to long-term storage.

### 3.10 Effect of gamma irradiation on bioactive substances:

- Lots of researches is done to find out the efficacy of gamma irradiation on biologically active substances like flavonoids, anthocyanins, essential oils, glycosides, triterpene saponins, oleanosides and plants mucus. These bioactive compounds did not change significantly after irradiation. Pharmacological activity of medicinal herbs has been found satisfactory after microbiological decontamination by irradiation.

- Koseiki et al studied the effect of radiation doses (0, 10, 20 and 30kGy) on the flavonoids, essential oils and phenolic compounds of Brazil medicinal herbs. From the described pharmacological tests, it was concluded that irradiated samples showed identical therapeutical action as non-irradiated preparations after exposure to a dose of 10, 20 and 30kGy of ionizing radiation.

- The irradiation of traditional medicines and herbal products does not result in any negative chemical changes or important losses of active components. After irradiation upto 17.8kGy, the content of the main biologically active substances of two medicinal herbs (ginko and guarana) was not modified.
Lai *et al*\textsuperscript{158} noticed that the total volatile compounds were decreased by more than 50% in irradiated (5 and 10kGy) dry shiitake (*Lentinus edodes* Sing). Irradiation increased the concentration of some minor volatile compounds, such as 3-methyl-2-butanol and 1-hexanol. However, the major flavour compounds including eight-carbon and sulphur-containing compounds were significantly reduced. The ratio of the eight-carbon compounds, such as 3-acetone, 3-octanol and 1-octen-3-ol, to total volatiles decreased from 72% in the control to 21% in the 10kGy irradiated samples.

Uchman *et al*\textsuperscript{159} (1983) reported that the treatment of herbs with higher doses (> 6 kGy) had been suggested to have a dose-dependent reduction effect on the volatile oil content of black pepper. Mishra *et al*\textsuperscript{160} reported that the dose of 5kGy led to a decrease in 6-gingerol, the compound responsible for the pungency of ginger.

Lee *et al*\textsuperscript{161} showed that the pungency and red colour caused by capsanoids and capsanthin, were not altered by irradiated (3, 7 and 10kGy) red pepper powder.