Plants have long been a very important source of new drugs and many plant species have been screened to observe their therapeutic activity (Fabricant et al., 2001). Medicinal plants are promising sources for treatment of a range of diseases. Keeping these facts in mind, we are trying to build a scientific base for the traditional use of the two selected plants belonging to Pinaccae family (*Cedrus deodara* Loud. and *Pinus roxburghii* Sarg.) in neuropharmacology, wound healing and ulcer and to isolate the compound/s from most active sample by bioactivity guided parting.

1.1 NEUROPHARMACOLOGY

Neuropharmacology deals with the study of drug induced changes in the functioning of cells in the nervous system. It is concerned with the study of the neurochemical interaction of neuropeptide, neuromodulators, enzymes, secondary messenger system of the central nervous system (CNS), co-transporters, ion channels, receptor proteins and more (Meyer and Quenzer, 2004).

1.1.1 Learning and Memory

Learning is defined as acquisition of information and skills and subsequent retention of this information is called as memory. In Ayurveda, there are three aspects of mental ability eg. Dhi (process of acquisition/learning), Dhuti (process of retention) and Smriti (process of recall). Any disturbance in these aspects resulted in the loss of mental ability (Dua et al., 2009). Memory is the ability of an individual to record sensory stimuli, events, information and retain them over short or long period of time and recall the same at a later date when needed (Adnaik et al., 2008). Dementia (loss of memory) is a familiar and serious problem. It is applied to episodes during which patients forget recent events, although
they may conduct themselves properly enough and following which no memory of the period persists. Such episodes often are caused by strokes, seizures, trauma, alcoholism or intoxication. There have been a lot of possibilities to treat dementia and as per Ayurvedic science, there are various tonics to treat this ailment but clinically documentation is awaited. Since drug like galantamine, vipocetene, piracetam are being used but they have potential side effects like insomina, involuntary movements, gastric discomfort, excitation, dizziness, skin rashes and long term toxicity effects.

1.1.1.1 Types of memory

Memory can be classified on the basis of duration and nature of information it carries.

1.1.1.1.1 On the basis of duration: it is mainly of three types:

(i) Sensory memory

Sensory memory (immediate memory) corresponds approximately to the initial 200-500 milliseconds after an item is perceived. The skill to look at an item and remember what it looked like with just a second of observation or memorization is an example of sensory memory. Brain regions involved in the sensory memory probably are visual and auditory cortex.

(ii) Short term memory

It is closely related to the working memory, which is involved in temporary short term storage of declarative knowledge. Short term memory (STM) allows recall for a period of several seconds to a minute without rehearsal. Its capacity is also very limited and we know that memory capacity can be increased through a process called “chunking”. Brain regions involved in the working memory probably are perisylvian cortex and frontal lobe. STM is believed to rely mostly on an acoustic code for storing information and to a smaller extent a visual code.

(iii) Long term memory

Long term memory (LTM) can store much larger quantities of information for potentially unlimited duration (sometimes a whole life span). LTM encodes it semantically. Brain regions involved in the LTM probably are hippocampus, dorsomedial thalamus, association cortex and others (Fig. 1.1).

One of the key functions of sleep is improving consolidation of information, as it can be shown that memory depends on getting sufficient sleep between training and test and that the hippocampus replays activity from the current day while sleeping, a brief description of memory formation is given in memory model of walking brain and sleeping brain in Fig. 1.2 & 1.3.
Fig. 1.1: Taxonomy of long term memory system together with specific brain structures involved in each system.
1.1.1.1.2 On the basis of nature of information (Marialaura et al., 2004):

i. Declarative memory (Explicit)

ii. Non-declarative memory (Implicit)
(i) Declarative memory

Declarative or explicit memory is the conscious recall of knowledge about people, places, things and is particularly well developed in the vertebrate brain. This memory is more flexible than non-declarative in terms of knowledge acquired by each system and accessible to multiple response systems. Explicit memory is most eagerly studied in mammals and exclusively depends on temporal lobe and diencephalic structures (Fig. 1.1) (Bailey et al., 1996; Squire and Zola, 1996).

Declarative memory is of two type’s viz. episodic and semantic memory:

a) Episodic memory
Episodic memory provides with a crucial record of our personal experiences. It allows remembering the trip we took to any place etc. This form of memory appears to be centered in the brain’s hippocampus with considerable help from the cerebral cortex.

b) Semantic memory

It accounts for textbook learning or general knowledge. It facilitates to say, without knowing exactly when and where we learned. The semantic memory ranges from strong (recall) to weak (familiarity) but is better persistent over time.

(ii) Non-declarative memory

Non-declarative or implicit memory is the non-conscious recall of motor skills and includes simple associative forms, like classical conditioning, and non-associative forms, like sensitization and habituation (Fig. 1.1). It is not flexible as declarative memory. Non-declarative memory is more encapsulated and has less access to systems not involved in the initial learning. Implicit forms of memory can be effectively studied in both non-mammalian vertebrates and higher invertebrates. Implicit memory does not depend on temporal lobe function but rather involves the sensory, motor or associational pathways used in the expression of the learning process (Bailey et al., 1996; Squire and Zola, 1996).

It is also of two types: Procedural memory and priming.

a) Procedural memory

Procedural memory enables us to carry out commonly learned tasks without consciously thinking about them. It’s our “how to” knowledge. Riding a bike, tying a shoe, washing dishes and walking are all tasks that require procedural memory.

b) Priming

Implicit memory can also come about from priming. We are “primed” by our experiences; if we have heard something in recent times, or many more times than another thing, we are primed to recall it more quickly. In the brain, the neural pathways representing things we have experienced more often are more salient than those for things with which we have fewer experiences.

1.1.1.2. Memory processing

Memory processing has well-known into four phases:

- **Registering or encoding** an event
- **Consolidating** it in a more stable form
- **Storing** it over a certain period of time
- **Retrieving** it to guide actions.
The sensory information storage (SIS) is when brain snaps a mental picture of what we sense are taking in. The functioning of SIS observed if we close our eyes, then open and close them again as rapidly as possible. As our eyes close, notice how the visual image is maintained for a fraction of a second before fading (Heuer and Richards, 1999). This image dissolves quickly and is only meant to provide our brain with a chance to process what it has just seen. From SIS, information could move to STM. STM allows a person to retain handfuls of information. Five to nine pieces of information can be stored for an indefinite period if they always remain at the forefront of a person's mind. Once a new thought enters, something must be released to allow room for the new information. It can either transfer to LTM or can be elapsed. The limit is on quantity not time and information can be retrieved almost immediately. Information entering SIS, then moving to STM and finally to LTM can lose clarity and sharpness along the way. It is also lodged deep in the brain and may take a while to be retrieved. It takes substantial effort to convert information from STM to LTM. Repetition is a way to solidify something into LTM. The repeated exposure to a stimulus or the rehearsal of a piece of information, transfers it from STM to LTM. However, memory for a specific episode may fail due to errors during one of these four phases (Marialaura et al., 2004). A brief description of memory formation process is given in Fig. 1.4.

![Diagram of memory process](image)

**Fig. 1.4:** Schematic diagram of memory process.

### 1.1.1.3 Morris water maze in the study of learning and memory

The Morris water maze was described 20 years ago as a device to investigate spatial learning and memory in laboratory animals. In the meanwhile, it has become one of the most frequently used laboratory tools in behavioral neuroscience. The device consists of a large circular pool filled with opaque water in which a small escape platform is hidden as shown in Fig.1.5. During a number of training trials, animals learn to find the platform and escape from the pool. Surely one of the reasons for its success is its relative simplicity.
However, although the basic procedure is relatively simple, it has been used in some of the most sophisticated experiments in the study of the neurobiology and neuropharmacology of spatial learning and memory. As well, it has been used in the validation of rodent models for neurocognitive disorders. In the process, MWM testing gained a position at the very core of contemporary neuroscience research. This task has been given various names, such as Morris swimming pool, Morris maze, water maze (most notably by Morris and associates), swimming maze, spatial navigation task, etc. Now, its most common name is MWM.

![Schematic diagram of Morris water maze.](image)

**Fig. 1.5:** Schematic diagram of Morris water maze.

Spatial learning and MWM performance appears to depend upon the coordinated action of different brain regions and neurotransmitter systems constituting a functionally integrated neural network.

### 1.1.1.4 Neuropharmacology of spatial learning

The MWM task has been used in studies which involve the neurochemical systems in learning and memory and the effects of neuropharmacological manipulation on spatial functions. Morris was the first to report that intraventricular infusion of the competitive N-methyl-D-aspartate (NMDA) receptor antagonist AP5, impairs hidden-platform acquisition and probe trial performance. This report has suggested that NMDA-type glutamate receptors play a crucial role in place learning, which seems restricted to the acquisition process, but does not involve processes of storage or recall. The cholinergic system is undoubtedly the second most important neurochemical system investigated in the MWM. It was found that rats receiving intra peritoneal injection of atropine were unable to use a spatial mapping strategy to solve the hidden-platform in MWM (Sutherland *et al.*, 1982).
(i) **Excitatory amino acids**

The excitatory amino acids bind to different types of ionotropic and metabotropic receptors. Ionotropic glutamate receptors include receptors of the NMDA and α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). The intra cerebral as well as systemic application of NMDA receptor antagonists impairs acquisition of spatial information in MWM. Non-competitive NMDA receptor antagonists, like ketamine or dizocilpine, impaired MWM learning. Intraperitoneally injected ketamine dose dependently impaired hidden platform acquisition training. Subcutaneous injections of dizocilpine decreased the rate of hidden-platform acquisition when given before, but not when given after each training session.

(ii) **Inhibitory amino acids**

The gamma amino butyric acid (GABA) binds to different types of receptors of which GABA_A and GABA_B receptors have been studied most extensively. Activation of the inhibitory GABA neurotransmitter system is thought to interfere directly with spatial learning abilities, whereas the suppression of the GABA system may enhance these and other functions. The selective agonists at the benzodiazepine binding site of the GABA receptor, chlordiazepoxide and diazepam were found to impair hidden-platform acquisition training and subsequent probe trial performance.

(iii) **Acetylcholine and biogenic amines**

The importance of the cholinergic system for spatial and other kinds of learning has been well established, and acetylcholine antagonists have accordingly been shown to block these functions. Such deficits appeared to be the result of direct effects on the cholinergic system, and not of secondary effects on other neurochemical systems. Blockade of central muscarinic receptors by atropine or scopolamine impairs MWM acquisition but not recall performance, which could not be reduced to sensorimotor or motivational deficits. Under certain circumstances, stimulation of the noradrenergic system enhances MWM acquisition. The selective monoamine oxidase-B (MAO-B) inhibitor L-deprenyl thus alleviated scopolamine-induced acquisition and probe trial performance deficits. Depletion of noradrenaline seemed to affect the consolidation process rendering the memory trace less stable and/or more susceptible to interference. Since dopamine antagonists impair hidden-as well as visible-platform MWM learning, it was suggested that sensorimotor or motivational deficits might underlie the effects of these compounds. Transgenic mice lacking D1A dopamine receptors also showed severely impaired hidden and visible platform MWM acquisition. Injection of the serotonin precursor 5-hydroxytryptophane alleviated MWM deficits in aged rats. The exact role of the serotonergic system in MWM has been confounded by the heterogeneity of 5-hydroxytryptamine (5-HT) receptors and the expression of a large number of different subtypes in the central nervous system.

(iv) **Neuropeptides**

Several peptides act as neurotransmitters or neuromodulators in the central nervous system, and some of these have been implicated in the processes underlying MWM
learning. Somatostatin, vasoactive intestinal peptide (VIP) and some other peptides were found to influence MWM acquisition, but their contribution to spatial learning is still unclear.

(v) Voltage-gated ion channels

Voltage-dependent calcium channels are important in the regulation of intracellular calcium levels. Memory processes involved in MWM and other kinds of learning may require the activation of calcium-dependent enzymatic processes in the neural systems that play a role in such functions. Depressed as well as excessive levels of calcium were shown to impair memory performance, indicating that the cellular mechanisms of memory depend upon optimally tuned calcium levels (Hooge and Dyan, 2001).

1.1.2 Dementia

Dementia refers to acquired global impairment of intellect, memory and personality (cognitive function) and it is not a disease but rather a group of symptoms caused by the impact of diseased brain. It is usually chronic and progressive in nature and symptoms typically include problem with memory, speech and perception with disturbances of multiple higher cortical functions including memory, thinking, orientation, calculation, learning capacity, language and judgment. Dementia can be produced by numerous pathological states that affect the brain and there is a progressive deterioration of certain parts of brain that are essential for learning and memory. Eventually the brain shrinks as the gaps develop. The disease begins with lapses in memory, swinging of mood and difficulty in finding the right words.

1.1.2.1 Epidemiology:

Worldwide, the global prevalence of dementia was estimated to be 3.9% in people aged above 60 years, with the regional prevalence being 1.6% in Africa, 4.0% in China and Western Pacific regions, 4.6% in Latin America, 5.4% in Western Europe, and 6.4% in North America (Ferri et al., 2005). More than 25 million people in the world are currently affected by dementia, most suffering from acute dementia (AD), with around 5 million new cases occurring every year (Ferri et al., 2005; Wimo et al., 2003; Brookmeyer et al., 2007). The number of people with dementia is anticipated to double every 20 years. The age-specific prevalence of AD almost doubles every 5 years after aged 65. Among developed nations, approximately 1 in 10 older people (65+ years) is affected by some degree of dementia, whereas more than one third of very old people (85+ years) may have dementia-related symptoms and signs (Von Strauss et al., 1999; Corrada et al., 2008). The study found that the incidence of AD increased with age, peaked, and then started to decline at extreme old ages for both men and women (Miech et al., 2002). Several studies from Europe observed a higher incidence rate of AD among women than men, especially among the oldest-old age groups. The incidence rates of AD were reported to be slightly lower in North America than in Europe. The incidence rate of AD among people aged 65+ years was 7.7 per 1000 person-years in Brazil (Kalaria et al., 2008). The prevalence rates of dementia in India were approximately a quarter of the rates in European countries (Scazuca et al., 2008; Rodríguez et
The incidence rate of dementia among people aged 65+ years was 3.2 per 1000 person-years in India (Kalaria et al., 2008; Chandra et al., 2001).

1.1.2.2 Symptoms of dementia:

- Progressive memory loss
- Inability to concentrate and severe confusions
- Reduce in judgment capability
- Hallucinations and delusions
- Altered sensation and perception
- Impaired recognition (agnosia): Impaired recognition of familiar objects and persons
- Altered sleep patterns: Insomnia, disturbance or change in sleep-wake cycle
- Motor system impairment
  - Impaired skilled motor function (aparaxia): Inability to reproduce geometric figures, inability to mimic hand positions and inability to dress self
  - Inappropriate movements
- Disorientation
  - Person, place, time and visual-spatial disorientation
  - Inability to interpret environmental cues
- Problem solving disorders
  - Inability to generalize and learn
  - Loss of abstract thinking and calculating ability
- Memory deficit
  - Short term memory problems (can’t remember new things)
  - Long term memory problems (can’t remember past)
- Impaired language ability (aphasia)
  - Inability to comprehend speech, read, write and repeat a phrase
  - Inability to form words and name objects
- Personality changes
  - Poor temper control, irritability, hesitant, anxiety and depression

1.1.2.3 Causes of dementia:

The causes of dementia can be classified as primary and secondary. Primary cause of dementia includes a number of neurological disorders, usually with irreversible loss and impaired through processing.

- Alzheimer, Parkinson, Pick’s and Huntington’s disease
- Vascular and Lewy-body dementia

Dementia like symptoms can also develop as a result of an underline medical condition and following are some of the more common secondary causes that can lead to dementia.
Chapter 1

Introduction

- Medications and toxins
- Alcohol induced dementia and substance abuse
- Oxidative stress
- Vitamin deficiency
- Infectious disease: Whipple’s disease, Syphilis, HIV infection
- Metabolic disorders
- Brain tumors and head injury

1.1.3 Nootropics

These are drugs, nutraceuticals, supplements and functional foods that improve mental functions like cognition, memory, intelligence, motivation, attention and concentration. The word “nootropics” develop from two Greek words ‘Noos’ (mind) and ‘Tropein’ (to turn towards). The nootropics enhanced the learning and memory acquisition as well as resistance of learned behavior that tend to impairs them. They also protect the brain against various physical or chemical injuries and facilitate the inter-hemispheric flow of information and efficient tonic cortical/sub cortical mechanism.

The basic mechanisms of nootropics are:

- Effects on energy metabolism
- Effects on cholinergic mechanism
- Effects on excitatory amino acids receptor mediatory function
- Steroid sensitivity

1.1.4 Nutrition and Memory

There is an enormous and growing amount of evidence that brain function and health are closely tied to our nutrition. Recent researches have taught us that nutrition is an important and often overlooked component of Alzheimer/dementia care. Research in this area indicated that subclinical deficiency in essential nutrients (antioxidants such as vitamins C, E and β-carotene, vitamin B12, vitamin B6, folate) and nutrition-related disorders (hypertriaacylglycerolaemia, hypercholesterolaemia, hypertension and diabetes) might be some of the nutrition-related risk factors present before cognitive impairment becomes evident. The so called "western diet" is the biggest killer in the world and more countries are adopting it. It is well-known that certain areas of the brain, such as the hippocampus and cortex, are vulnerable to a poor diet. Long-term consequence of faulty nutrition in addition of certain negative lifestyle factors, may cause Alzheimer. The contributory factors may include:

- Metabolic syndrome, insulin resistance, acetylcholine deficiency
- Unnecessary stress and the stress hormone
- Lack of antioxidant nutrients
1.1.5 Mechanistic approach of phytoconstituents for antiamnesic activity

The reactive oxygen/nitrogen species (ROS/RNS) are produced during cellular metabolism and functional activities and have important roles in cell signaling, apoptosis, gene expression and ion transportation. However, excessive ROS attack bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, and cause oxidative stress, which can damage DNA, RNA, proteins and lipids resulting in an increased risk for many diseases such as inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer’s disease, cataracts, autism and aging (Balsano & Alisi, 2009).

Nervous tissue is highly susceptible for free radical damage due to high content of lipids especially polyunsaturated fatty acids. In Alzheimer disease, biochemical and histological studies have provided evidence for increased levels of oxidative stress and membrane lipid peroxidation which may promote neuronal death in AD by multiple mechanisms that include impairment of the function of membrane ion-motive ATPases (Na\(^+\)/K\(^+\)- ATPase and Ca\(^{2+}\)- ATPase), glucose transporters and glutamate transporters. Lipid peroxidation also leads to production of the aldehyde 4-HNE that appears to play a central role in the neurotoxic actions of amyloid β -peptide (Yoshikawa et al., 2000).

Memory impairment and dementia are increasingly prevalent in the current demographic climate of an ageing population. As well as the pathological cognitive loss of neurodegenerative disease, many older persons are experiencing memory loss as part of the physiological process of ageing (Kawas, 2003). Many botanical approaches have much to offer in the improvement of cognitive function, including modulation of factors such as oxidative stress, inflammation and neurotransmission (Tripathi et al., 1996; Battacharya et al., 2000; Sairam et al., 2001; Russo et al., 2003; Russo et al., 2005; Das et al., 2002; Singh & Dhawan, 1997; Kishore & Singh, 2005).

Intracellular antioxidant enzymes and intake of dietary antioxidants may help to maintain an adequate antioxidant status in the body. Antioxidant-based drugs/formulations for prevention and treatment of such complex diseases appeared over the past three decades. There are many laboratories from India working on the antioxidant effect of compounds derived from natural sources that are capable of protecting against such damage. There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants (Osawa et al., 1994).

Polyphenols are dietary agents that can modulate many processes associated with the pathophysiology of dementia. Polyphenolic compounds can alleviate oxidative stress by acting as direct scavengers of free radicals and clearing superoxide and hydroxyl radicals and by increasing the level of antioxidant enzymes such as glutathione peroxidase. They also chelate metal ions to prevent free radical formation. Polyphenols can also combat inflammation by affecting transcription factors such as NF-κB. Some polyphenols may have the potential to inhibit excitotoxicity by regulating intracellular calcium ion concentration,
inhibiting glutamate receptors and increasing glutamate reuptake at the synapse. The cognitive
decline in dementia due to decreased availability of acetylcholine can also be countered by
polyphenols that inhibit acetyl-cholinesterase activity. Taken together, these findings suggest
that increasing the consumption of polyphenol rich food may alleviate the effects of dementia
(Abhishek D, 2016).

Flavonoids, tannins and other phenolic constituents from plant origin are potential
antioxidants and they play an essential role in the prevention of neurodegenerative diseases,
including Parkinson’s and Alzheimer’s diseases. A direct relationship between antioxidant
activity and phenolic content of plant extracts has been reported. Thus, antioxidants have been
used for their effectiveness in reducing these deleterious effects and neuronal death in many
in-vitro and in-vivo studies. The antioxidants compounds, preferentially suppressing radical
generation, and thus may be promising as effective neuropreventive agents that can plays a
pivotal role in the prevention and cure of various neurodegenerative diseases (Ameer, 2016).

1.2 WOUND

Wound is the discontinuation of lining membrane that after healing leaves a scar
for life. Different types of wounds as mentioned in Ayurveda may be endogenous in origin
due to a defect in human functional units, such as Vata (nerve impulses), Pitta (enzymes and
hormones) and Kapha (body fluids), or exogenous due to trauma such as Chinna (cut wound),
Bhinna (perforated wound), Viddha (punctured wound), Kshata (lacerated wound), Picchita
(contusion) and Ghrista (abrasion wound).

1.2.1 Burn wound

Wounds are breaks in skin and can be caused due to various reasons, but the most
common ones are burn, scrapes, lacerations, punctures and cuts (Nguyen et al., 2009). Burns
are defined as coagulative destruction of tissue by thermal, chemical or electrical injury. Burns
remain a huge public health problem in terms of morbidity and long term disability, especially
in the developing countries (Heimbach, 1999). Infection is a main complication of burn injury
and is responsible for 50-75% of hospital deaths (Mokaddas et al., 1998). Many of the
synthetic drugs pose problems, forcing researcher to search for alternative drugs (Purna and
Babu, 1998). In India, medicines based on herbal origin have been the basis of treatment and
cure for various diseases and physiological abnormalities under practice such as Ayurveda,
Siddha and Unani. Moreover, Indian folk medicine comprises numerous prescriptions for
therapeutic purposes such as inflammation, skin infections, wounds healing, leprosy,
diarrhoea, scabies, venereal disease, ulcers, snake bite and so on. More than 80% of the
world’s population still depends upon traditional medicines for various skin diseases. An
herbal drug in wound management causes disinfection, debridement (removal of unhealthy
tissue from a wound) and providing a moist environment to encourage the establishment of
the suitable environment for natural healing process (Purna and Babu, 2000).
1.2.1.1 Epidemiology

In India about 7,00000 patients are admitted to hospitals per year, while very few are looked after in specialist burn units (Potokar et al., 2007). About 90% of burns occur in the developing world and 70% of these are in children. Survival of injuries greater than 40% TBSA is rare in the developing world (Jay et al., 1977).

1.2.1.2 Etiology

The etiology of burns varies according to age, activity and socio-economic circumstances (Fig. 1.6). Children are more likely to be burned in conditions of social disruption, whether this takes the form of poor socio-economic infrastructure, poor adult supervision or blatant child abuse. Electrical injuries are usually low voltage but can produce life threatening cardio respiratory complications and unique injuries. High voltage injuries are far less common and are usually associated with significant underlying muscle damage.

(i) Thermal injury

Contact with flame, hot surface or hot liquid can causes a degree of cellular damage to the skin that varies with the temperature and duration of exposure. As the temperature rises, increasing molecular collisions resulting in altered molecular conformation and the disruption of intermolecular bonds. This process leads to disruption of ion channels, resulting in sodium and water intake. As the temperature raises more, protein denaturation occurs, oxygen radicals are liberated and eventually cells die with the formation of the burn scar.

(ii) Chemical injury

Chemical contact may also damage protein structures (Mortiz and Heuriquex, 1947).

(iii) Microbial Etiology

Bacteria rapidly colonize open skin wounds after burn. Microbes colonizing the burn wound originate from the patient’s skin, respiratory and gastrointestinal flora. Microorganisms may also be transferred to a patient’s skin surface via contact with contaminated external environmental (Barret and Herndon, 2004).

1.2.1.3 Pathology

The epidermis is the exterior thinner layer responsible for protective evaporation of water from the body and is constantly replenished by cell division in the basal layer of the epidermis. The dermis is the deeper thicker layer providing strength and durability, it contains
the accessory structures which provide an epithelial reservoir from which partial thickness wounds can heal by a process known as re-epithelialisation (Roth and Hughes, 2004).

**Fig. 1.6**: Etiopathogenesis of wound.

A burn wound is tri-dimensional in nature and pathologically consists of three concentric zones in surface and depth. The zone of coagulation represents irreversible tissue necrosis, which comprises the dead tissues that form the burn scar that is located at the center of the wound nearest to the heat. The zone of stasis is an area of impaired circulation secondary to the release of vasoactive mediators and platelet aggregation, which given correct management, can be salvaged and needs to be protected, and optimized at all stages of recovery. The zone of hyperemia is caused by the release of inflammatory mediators and results in vasodilatation. It is this zone which causes much of the systemic fluid perturbation. The evolution of the injury in a burn wound is a dynamic process and it may take up to 3-4 days for the size and eventual depth of cell death to become evident. This will depend on the effectiveness of treatment received and the potential salvage of the zone of stasis. Burn
wounds from flames tend to be deeper and of a greater severity than scald burns. No objective clinical methods are available to determine the depth of thermal injury and no standardized method has been adopted. Most burns are a combination of superficial and deeper burns and the best assessment can be made 3-4 days after the injury when wound evolution has been completed.

Burns are divided into various thicknesses, each of which relate to an anatomical level within the skin microstructure (Sheridan and Tompkins, 2005).

(i) Superficial partial thickness:

Destruction of only superficial layers of the skin. There is enough preservation of dermal elements to ensure re-epithelisation. These wounds will epithelialise spontaneously within 3 weeks. These wounds characteristically have an erythematous, moist, homogenous surface with blister formation, are painful and hypersensitive to touch, blanch readily and have a normal to firm texture on palpation.

(ii) Indeterminate depth (deep dermal burn):

Destruction of epidermis and varying amounts of dermis. The dermis is affected and depending on the recovery of the zone of stasis may have enough remaining dermal elements to heal spontaneously but usually in a delayed manner. These wounds are difficult to assess during the first 3 days after injury due to the ongoing evolution within the burn wound which can be modulated by infection and dehydration. The wounds present with a mottled pink and white dry surface and may blister. Pain is perceived as discomfort and the wound is often less sensitive to pinprick than the surrounding normal skin. Hypertrophic scar formation is commonly encountered in the long term.

(iii) Unequivocally full thickness:

Total irreversible destruction of all elements of the skin with or without extension into the deeper tissues and structures. These wounds will not heal spontaneously within 3 weeks and have unsatisfactory functional and cosmetic results (Robert et al., 2004).

1.2.1.4 Pathophysiology

The initial burn wound may only appear to affect the integumentary system but the insult is widespread with a multi organ cascade of effects.

(i) Hemodynamic changes:

Thermal injury alters endothelial integrity and function. The subsequent systemic inflammatory response syndrome (SIRS) effects are mediated by the widespread release of inflammatory mediators, primarily from the zone of hyperemia. This results in obligatory
isotonic fluid loss from the intravascular spaces into the burn area. The capillary changes occur very rapidly and are maximal within the first 3-12 h following small burns and up to 24-48 h after injury for larger burns. The fluid loss is not confined to the burnt area, but involves the whole body when the total body surface area (TBSA) > 30%. This obligatory sequestration of fluid will lead to the formation of edema with eventual loss of effective circulating fluid volume. If this loss is extensive, hypovolaemia and shock will develop. It remains a common cause of death.

(ii) **Hyper metabolic response:**

Although a thermal offense occurs immediately, the systemic response to inflammatory mediators reaches a peak 5-6 days after the injury. Increased metabolic rates are often encountered in burns and may be as high as 50-100% above normal in major burns. This response is characterized by gluconeogenesis, severe protein catabolism, lipolysis and fat redistribution. Afferent stimuli from the burn wound i.e. pain cause the release of cytokines, tumour necrosis factor and thromboxane, which stimulate the hypothalamus to reset the core temperature. In addition, catabolic agents, i.e. glucagon and cortisol are increased and anabolic hormones (insulin and growth hormone) are uniformly decreased during the 1st period of the injury.

(iii) **Cardiovascular changes:**

Apart from hypovolaemia, the following changes are observed:
- Increased haemotocrit due to isotonic fluid loss from the intravascular space, erythrocyte heat damage, stasis and impaired microcirculation both in burned and unburned tissue, with ultimate compromise of substrate delivery and resultant propagation of the inflammatory cascade.
- Myocardial depression mediated primarily by tumor necrosis factor (TNF), this is predominantly seen at burns exceeding >50% TBSA.
- Increased systemic vascular resistance, catecholamine, both noradrenaline and adrenaline release with an increased sensitivity of peripheral vessels to adrenaline and acute phase reactants. Respiratory complications: Although the respiratory tree is well protected, there are several manifestations of respiratory burns.
- Renal failure: Numerous aspects may alter renal function, that is by a low perfusion state due to under or delayed resuscitation e.g. burn shock or alternatively due to electrical or extensive burns which may cause haemolysis and renal failure.

(iv) **Susceptibility to infection:**

The burnt patient is prone to microorganism invasion because of impaired local defense mechanisms (loss of outer skin barrier, presence of dead tissue and impaired local blood flow) and impaired systemic immune defenses due to a decrease in phagocyte and lymphocyte function, compounded by impaired humoral and cell-mediated immunity. These factors together with an exposure to a high-risk environment for nosocomial infection and
recurrent invasive procedures result in sepsis being the leading cause for delayed mortality in burns. Gram-positive organism infection predominates during the first week. Seventy percent of the wounds harbour organisms from an exogenous source (mostly Gram-positive) and 30% of the organisms are from an endogenous source predominantly the gastrointestinal tract, mostly gram negative organisms.

(v) The gastrointestinal tract:

The bowel is very susceptible to injury during the periods of hypovolaemia and lack of enteral feeding. Release of stress hormones induces mesentric vasoconstriction and lead to decreased gut immune function and gut mucosal-integrity, predisposing the patient to bacterial and endotoxin translocation of endogenous flora into the systemic circulation (Mast, 1992).

1.2 Wound healing

Wound healing is a complex process and it runs through a number of phases, which either run parallel or are closely interlinked through some chemical, biochemical and cellular pathways. The epidermis and dermis forming a protective barrier against the external environment. Once the defensive barrier is broken, the common physiologic process of wound healing is instantly set in action (Stadelmann et al., 1998). A treatment could influence the healing of wound by intervening in any one or many phases of healing. The current allopathic therapy of wound healing like antibiotics has many side effects (Srikanth et al., 2008).

The classic model of wound healing is divided into three or four sequential, yet overlapping, phases: (1) haemostasis, (2) inflammatory, (3) proliferative and (4) remodeling (Fig. 1.7).

Upon injury to the skin, a set of complex biochemical actions takes place in a closely orchestrated cascade to restore injures. In minutes post-injury, platelets aggregate at the injury site to form a fibrin clot. The fibrin clot acts to control active bleeding to obtain haemostasis (Midwood et al., 2004).

1.2.2.1 Inflammatory phase

During the inflammatory phase clotting takes place in order to prevent blood loss and different factors are released to magnetize cells that phagocytise debris, damaged tissue and bacteria, and release factors that begin the proliferative phase of wound healing.
Fig. 1.7: The process of normal wound healing.

1.2.2.1 Clotting cascade

When tissue is initially injured, blood makes contact with collagen, trigger blood platelets to begin secreting inflammatory factors. Platelets also express glycoprotein’s on their membranes that allow them to stick to one another. Fibrin and fibronectin cross-link together and form a plug that prevents further blood loss. The fibrin-fibronectin plug is the structural support for the wound until collagen is deposited. The clot is ultimately lysed and replaced with granulation tissue and then later on with collagen.

1.2.2.1.2 Platelets

Platelets present in the highest numbers soon after a wound and release a number of mediator into the blood, including extracellular matrix (ECM) proteins, cytokines and growth factors. They also release other pro-inflammatory mediators like 5-HT, histamine, bradykinin, prostaglandin (PG), prostacyclins and thromboxane. These mediators increase cell proliferation, migration and to cause blood vessels to become dilated and porous (Theoret, 2004).

1.2.2.1.3 Vasoconstriction and vasodilation

Instantly after a blood vessel is rupture, ruptured cell membranes release inflammatory mediators like thromboxane and PG. These mediators constrict the blood vessel
ensuing avoid blood loss and to collect inflammatory cells in the area. The vasoconstriction lasts five to ten minutes and is followed by vasodilatation with peaks at about 20 minutes post-wounding. The main mediator responsible for vasodilatation is histamine. They also cause blood vessels to become porous, proteins from the bloodstream leak into the extra vascular space, causing tissue to become edematous. They also facilitate the entry of inflammatory cells like leukocytes into the wound spot from the bloodstream (Muller et al., 2003).

1.2.2.1.4 Polymorphonuclear neutrophils

Within an hour of wounding, polymorphonuclear neutrophils (PMNs) attracted to the site by fibronectin, growth factors and kinins and become the predominant cells in the wound for the first two days. Neutrophils phagocytise debris and bacteria. They also rinse the wound by secreting proteases that break down damaged tissue. Once they have completed their tasks are engulfed and degraded by macrophages (Santoro and Gaudino, 2005). The helper T cells also enter the area and secrete cytokines which cause more T cells to divide and to increase inflammation and enhance vasodilation and vessel permeability.

1.2.2.1.5 Macrophages

Macrophages are vital for wound healing. The monocytes from the bloodstream enter the wound area through blood vessel walls by growth factors released by platelets and other cells. Once they are in the wound site monocytes mature into macrophages. The main role of macrophages is to phagocytise bacteria and damaged tissue. They also secrete a number of factors such as growth factors and other cytokines, particularly during the 3rd and 4th post-wounding days. These mediators attract cells involved in the proliferation stage of healing and may control the contraction stage. Macrophages are inspired by the low oxygen content of surroundings to produce factors that bring and speed angiogenesis and also stimulate cells that reepithelialize the wound, create granulation tissue and build a new ECM.

1.2.2.1.6 Decline of inflammatory phase

As inflammation fall down, less inflammatory factors are released and the numbers of neutrophils and macrophages are reduced at the wound site. These modifications indicate the finishing of inflammatory phase and establishment of proliferative phase. The presence of macrophages in fact delays wound contraction and thus the desertion of macrophages from the wound may be vital for successive phases to occur. Since inflammation plays roles in combating infection, clearing debris and inducing the proliferation phase, it is a crucial part of healing. Yet, inflammation can lead to tissue damage if it lasts too long. Therefore, the reduction of inflammation is often a goal in therapy. The presence of dirt or other stuff can expand the inflammatory phase leading to a chronic wound (Lansdown et al., 2001).

1.2.2.2 Proliferative phase
After two or three days of wound, fibroblasts commence to penetrate the wound site, represent the onset of the proliferative phase even before the inflammatory phase has ended. Like in other phases, steps in the proliferative phase do not happen in a series but rather partially overlap. The proliferative phase is also called the reconstruction phase (Ruszczak, 2003).

### 1.2.2.1 Angiogenesis

The process of angiogenesis occurs along with fibroblast proliferation when endothelial cells migrate to the area of the wound. Since the activity of fibroblasts and epithelial cells requires oxygen and nutrients, angiogenesis is vital for other stages in wound healing. The tissue in which angiogenesis has occurred usually looks red due to the presence of capillaries. Stem cells of endothelial cells develop pseudopodia and drive through the ECM into the wound site to begin new blood vessels. The endothelial cells need collagenases and plasminogen activator to degrade the clot and part of the ECM for migration. The zinc-dependent metalloproteinases digest basement membrane and ECM to allow cell migration, proliferation and angiogenesis.

### 1.2.2.2 Fibroplasia and granulation tissue formation

Concurrently with angiogenesis, fibroblasts begin accumulating in the wound site two to five days after wounding with peak numbers at one to two weeks and ends two to four weeks after wounding. In the first two or three days, fibroblasts mostly proliferate and migrate, after that they are the main cells that put down the collagen matrix in the wound site. At the end of the first week, fibroblasts are the chief cells in the wound. After migration and adherence to the fibronectin, fibroblasts deposit ground substance into the wound bed and later on collagen.

Granulation tissue functions as elementary tissue and begins to materialize in the wound previously during the inflammatory phase and continues growing until the wound bed is covered. Its consist of inflammatory cells, endothelial cells, fibroblasts, myofibroblast, new blood vessels and the components of a new provisional ECM. The composition of provisional ECM is unlike the ECM in normal tissue and its components derive from fibroblasts including fibronectin, collagen, elastin, glycoprotein, proteoglycans and glycosaminoglycans. The fibronectin build a hydrated matrix and facilitate cell migration. Later on provisional matrix is replaced with an ECM that more closely resembles that found in non-injured tissue. Transforming growth factor beta (TGF-β) and fibronectin encourage proliferation, migration to the wound bed and fabrication of ECM by fibroblasts. Hypoxia still contributes to fibroblast proliferation and secretion of growth factors (Mulvaney and Harrington, 1994).

### 1.2.2.3 Collagen deposition

Fibroblasts begin secreting collagen by the second or third post wounding day with peak deposition at one to three weeks. Collagen deposition is vital because it increases the strength of the wound, the only thing holding the wound closed is the fibrin-fibronectin
clot. Collagen production continues quickly for two to four weeks, after that its destruction matches its production so there is no net collagen gain. This homeostasis signals the beginning of the maturation phase. Granulation slowly ceases and fibroblasts decrease in number in the wound once their work is done.

1.2.2.2.4 Epithelialization

The formation of granulation tissue in an open wound allows the reepithelialization phase to take place. Basal keratinocytes from the wound boundaries and dermal appendages such as hair follicles, sebaceous glands and sweat glands are the major cells responsible for this phase. They proceed in a sheet across the wound site and proliferate at its edges, ceasing progress when they gather in the middle. Migration of keratinocytes begins as early as a few hours after wounding without proliferation. Though, epithelial cells need viable tissue to migrate, so deep wound must be initially packed with granulation tissue. Therefore the onset time of migration is uneven and may happen about one day after wounding. The proliferation of cells on the wound margins start on the second and third day post-wounding in order to give more cells for migration (Son et al., 2005). Before they start to migrate, cells dissolve their desmosomes and hemidesmosomes, which anchor the cells by intermediate filaments in their cytoskeleton to other cells and to the ECM. The keratinocytes detach from the basement membrane and are able to enter the wound bed. Epithelial cells mount over one another in order to migrate and making a growing sheet called as epithelial tongue. The first cells attach to the basement membrane form the stratum basale. These basal cells continue to migrate across the wound bed and the more quickly this migration occurs, the less of a scar there will be (Bayram et al., 2005). While keratinocytes migrate, they move over granulation tissue but under the scab (if one was formed), separating it from the underlying tissue. Epithelial cells have the ability to phagocytize debris that would otherwise delay their path. The migration of keratinocyte is enhanced by a moist environment, while a dry one leads to formation of a bigger, tougher scab. The keratinocytes must dissolve the clot, debris, and parts of the ECM in order to get through. They secrete plasminogen activator, activates plasminogen and turning it into plasmin to dissolve the scab. The migration of cell is occur over living tissue, so they must excrete collagenases and proteases like matrix metalloproteinases (MMPs) to dissolve damaged parts of the ECM in their way. As keratinocytes continue migrating, fresh epithelial cells should be formed at the wound boundaries to replace them and to provide more cells for the advancing sheet. Proliferation after migration normally initiates a few days after wounding and occurs at a higher rate than in normal tissues (Hinz, 2006). Growth factors stimulated by MMPs and integrins cause cells to proliferate at the injury edges. Keratinocytes also secrete growth factors and basement membrane proteins, which help in epithelialization and in other phases of healing.

Growth factors are also essential for the natural immune defense of skin wounds by stimulation of the production of antimicrobial peptides in keratinocytes. They carry on migrating across the wound bed until cells from either side meet in the center, at which point contact inhibition causes them to discontinue migration. At the end of migration, the keratinocytes secrete the proteins that form the new basement membrane. Basal cells start to
divide and differentiate in the same manner as they do in normal skin to reestablish the strata found in reepithelialized skin (Eichler and Carlson, 2005).

1.2.2.5 Contraction

Contraction is a key phase of wound healing, however if continues for too long, it may lead to disfigurement and loss of function. This phase commences around a week following wounding, once fibroblasts have differentiated into myofibroblasts, with peaks at 5 to 15 days and continues even after the wound is completely reepithelialized. A large wound can become 40 to 80% smaller after contraction. During contraction most wounds have an axis of contraction, which allows for greater organization and alignment of cells with collagen. Initially, contraction occurs without myofibroblast involvement but later on fibroblasts are stimulated by growth factors and differentiate into myofibroblasts. Myofibroblasts are similar to smooth muscle cells and are responsible for contraction. They are attracted by fibronectin and growth factors and move along fibronectin linked to fibrin in the provisional ECM in order to reach the wound edges. They form connections to the ECM at the wound edges, attach to each other and to the wound edges by desmosomes. Myofibroblasts have several adhesions which pull the ECM during contraction. Once actin in myofibroblasts contracts, the wound edges are pulled together and fibroblasts give up collagen to strengthen the wound. The contraction phase ends while myofibroblasts stop contracting and commit apoptosis. Finally breakdown of the provisional matrix leads to a decrease in hyaluronic acid and an increase in chondroitin sulfate, which progressively triggers fibroblasts to stop migration and proliferation. These actions indicate the onset of the maturation stage (O’Leary et al., 2002).

1.2.2.3 Maturation and remodeling phase

The maturation phase begins when the levels of collagen production and degradation match. This phase can take place for a year or even longer, depending on the size of the wound. During maturation, type III collagen is gradually degraded and the stronger type I collagen is laid down in its place. Firstly unsystematic collagen fibers are rearranged and cross-linked. The tensile strength of the wound increases with the strength approaching 50% that of normal tissue by three months and finally becoming as much as 80%. When activity at the wound site is reduced, the scar loses its red appearance because blood vessels that are no longer desired are removed by apoptosis (O’Leary et al., 2002).

1.3 PEPTIC ULCER

Peptic ulcer is the most common gastrointestinal disorder in clinical practice with increasing incidence and prevalence attributed to an imbalance between the protective (mucus, bicarbonate, and prostaglandins) and the aggressive (Reactive oxygen species and acidity) factors (Dharmani et al., 2004). The common causes are stress, continuous use of tobacco, alcohol misuse, non-steroidal antiinflammatory drugs (NSAIDs) and infection by Helicobacter pylori (H. pylori). The current medical treatment for peptic ulcer is based upon
the inhibition of gastric acid secretion by inhibiting proton pump in the parietal cells of gastric mucosa. Histamine H$_2$ receptor blockers are being used to control acid secretion. Considering the several side effects like impotence, gynaecomastia, arrhythmias and hematopoietic changes of modern prescription, indigenous drugs possessing fewer side effects should be looked for as a better alternative to the treatment of peptic ulcer (Akhtar et al., 1992; Saravanan et al., 2011). This has been the rationale behind the development of new antiulcer drugs and search for novel molecule. Drugs of plant’s origin are gaining popularity and investigating for the various disorders including peptic ulcer. Since decades, many indigenous drugs have been known to possess antiulcer activity (Sharma and Mishra, 2014).

The term peptic ulcers are usually used to include duodenal ulcers and gastric ulcers. Acute peptic ulcers often are multiple, and range in size from a few milliliters to 3 cm in diameter. The lesions are shallow, extend through the mucosa, and have well-defined margins. Sub acute peptic ulcers consist of lesions that are in transition between being acute and chronic. Such lesions are deeper than the acute ones, and are capable of penetrating through the mucosa, sub mucosa and occasionally the muscular layer. Chronic ulcers are almost always single, although they may be surrounded by scars of previously healed acute or sub acute ulcers. The floor of the ulcer is usually clear and covered with fibrous tissue. Peptic ulcers heal from the floor upward. In superficial ulcers, healing is completed and gastric glands may regenerate. In chronic ulcers, healing is slow. The mucosa is replaced by smooth, scarred tissue that is devoid of glands.

1.3.1 Epidemiology

The prevalence of ulcer in the general population varies widely from 40-50% in developed countries to as high as 90% in some part of developing world. The point prevalence of peptic ulcer was 4.72% and the lifetime prevalence was 11-22%. The duodenal to gastric ulcer ratio was 17.1:1. Duodenal and gastric ulcer were common in men. The prevalence of peptic ulcer increased with age, with a peak prevalence of 28.8% in the 5th decade of life. Peptic ulcer was not related to socio-economic status (Khuroo et al., 1989). The reported frequency of ulcer in India has ranged from 31-85%, most centre reported a figure of around 80% (Singh et al., 2002).

1.3.2 Gastric anatomy

The gastric epithelial lining consists of rugae that contain microscopic gastric pits, each branching into gastric glands made up of highly specialized epithelial cells. Glands within the gastric cardiac cover <5% of the gastric gland area and contain mucous and endocrine cells. The 75% of gastric glands are found within the oxyntic mucosa and include mucous neck cells, parietal cells, chief cells and endocrine cells. Pyloric glands contain mucous cells and endocrine cells (Fauci et al., 2008).
1.3.3 Classification of peptic ulcer:

(i) Duodenal ulcers

Duodenal ulcers (DU) are chronic in nature and recur if treated improperly. The lesions are usually deep, and sharp at the edges, and are capable of penetrating through the mucosa, submucosa, and into the muscularis. The lesion is usually clear, but at times it may contain blood or exudates along with inflammatory cells. In over 95% of patients with DU, the ulceration occurs in the first portion of the duodenum. The lesion is about 1 cm in diameter.

(ii) Gastric ulcers

Gastric ulcers (GU) occur approximately 10 years later in life than DU. GU is usually deep, and the lesion is surrounded by inflammation. They usually occur in the fundus of the stomach and are accompanied by antral gastritis due to the colonization by \( H. \) pylori. GU affects the lesser curvature of the stomach and appears as a single lesion. It is not uncommon for patients to develop both DU and GU simultaneously.

(iii) Oesophageal ulcer

This type of ulcer occurs in the lower end of oesophagus. Esophageal ulcers are often associated with a bad case of acid reflux, or gastro esophageal reflux disease (GERD).

1.3.4 Symptom

Symptoms of peptic ulcers depend on the location of the ulceration, and the patient’s age. Some patients may experience minimal symptoms (i.e. the elderly), while others may complain of symptoms from moderate to severe. Epigastric pain located in an area 3 to 4 cm between the xiphoid process and the umbilicus is the most common symptom. The pain may be accompanied by burning, cramp like sensation, tightness, nausea and vomiting. The pain is steady, mild or moderately severe and may radiate to the back. Pain may occur at night, usually within a few hours after retiring. DU pain is usually absent upon awakening in the morning, but develops in mid-morning, is relieved by food, but reappears within 2 to 3 h (Sultalley, 2002).

1.3.5 Etiology

Most peptic ulcers occur in the presence of acid and pepsin once \( H. \) pylori, NSAIDs or other factors disrupt normal mucosal defense and healing mechanisms. Potential causes of peptic ulcer are:

- \( H. \) pylori infection (90%)
- Drugs e.g. NSAIDs and corticosteroids
- Hyperacidity e.g. Zollinger Ellison syndrome
Chapter 1

Introduction

- Cigarette smoking and alcohol
- Stress and oxidative stress

1.3.6 Pathophysiology

Gastric and duodenal ulcers occur because of an imbalance between aggressive factors (gastric acid and pepsin) and mechanisms that maintain mucosal integrity (mucosal defense and repair) as shown in Fig. 1.8. Ulcer formation is the net result of a lack of homeostasis between factors within the gastrointestinal tract responsible for the breakdown of food (e.g. gastric acid and pepsin) and factors that promote epithelial defense and repair (e.g. mucus, PG and bicarbonate).

(i) Gastric acid and pepsin

Hydrochloric acid and pepsin are the primary substances that cause gastric mucosal damage in peptic ulcer diseases (PUD). Hydrochloric acid is secreted by the parietal cells, which also contain receptors for histamine, acetylcholine and gastrin hormone. Acid (as well as H. pylori infection and NSAIDs used) is an independent factor that contributes to the disruption of mucosa. Augmented acid secretion has been observed in patients with duodenal ulcers and may be a consequence of H. pylori infection (Del et al., 2003, Sachs et al., 2000). Patients with gastric ulcer usually have normal or reduced rates of acid secretion (hypochlorhydria). The basal acid output follows a circadian rhythm, with the peak acid secretion occurring at night and the lowest in the morning (Del et al., 2003). Pepsinogen released during food digestion is converted to pepsin in the presence of an acidic environment and plays a key role in the initiation of protein digestion, proteolysis of collagen and as a signal for the release of other digestive enzymes such as gastrin and cholecystokinin. Pepsinogen is secreted by the chief cells located in the gastric fundus. Pepsin is activated by acid pH (1.8 to 3.5), inactivated reversibly at pH 4 and irreversibly destroyed at pH 7. Pepsin appears to play a role in the proteolytic activity concerned in ulcer formation.

(ii) Mucosal defense and repair

Mucosal defense and repair mechanisms guard the gastro duodenal mucosa from harmful endogenous and exogenous substances. Mucosal defense mechanisms include mucus and bicarbonate secretion, native epithelial cell resistance and mucosal blood flow (Del et al., 2003). The viscous nature and near-neutral pH of the mucus-bicarbonate barrier protect the stomach from the acidic contents in lumen. Mucosal restore after injury is linked to epithelial cell restitution, growth, and regeneration. The maintenance of mucosal integrity and repair is mediated by the production of endogenous PG. The PG stimulates bicarbonate and mucus secretion and increase mucosal blood flow. Bicarbonate ions are secreted in to the unstirred mucus layer, neutralizing hydrogen ions. Fast cell turnover and a rich mucosal blood flow are important protective elements. This phenomenon enables the stomach to initially withstand the damaging effects of irritating agents. Changes in mucosal defense that are induced by H.
*pylori* or NSAIDs are the most important cofactors in the formation of peptic ulcers (Elta et al., 2003, Suerbaum and Michetti, 2002).

(iii) *Helicobacter pylori*

*H. pylori* are a spiral shaped, pH sensitive and gram negative bacterium that resides between the mucus layer and surface epithelial cells. The combination of its spiral shape and flagellum permits it to move from the lumen of the stomach to the mucus layer. The acute infection is followed by transient hypochlorhydria, which allow the bacteria to survive in the acidic media. The exact process by which *H. pylori* initially induces hypochlorhydria is uncertain. *H. pylori* may produce large amounts of urease, which hydrolyzes urea in the gastric juice and converts it to ammonia and carbon dioxide. The local buffering effect of

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**Fig. 1.8:** The development of gastric and duodenal ulcers.
ammonia creates a neutral microenvironment within and surrounding the bacterium, which protects it from the lethal effect of acid. *H. pylori* also produce acid inhibitory proteins, which allow it to adapt to the low pH environment of the stomach (Go, 2002; Suerbaum and Michetti, 2002). A number of bacterial and host factors contribute to the ability of *H. pylori* to cause gastro duodenal mucosal injury. Pathogenic mechanisms include: (a) direct mucosal damage (b) alterations in the host immune/inflammatory response and (c) hypergastrinemia leading to increased acid secretion. Direct mucosal damage is produced by virulence factors (cytotoxin and growth inhibitory factor), bacterial enzymes (lipases, proteases, and urease), and adherence. Lipases and proteases degrade gastric mucus, whereas ammonia is toxic to gastric epithelial cells. The bacterial adherence enhances the uptake of toxins into gastric epithelial cells. *H. pylori* infection alters the host inflammatory response and damages epithelial cells either directly by cell-mediated immune system or indirectly by activated neutrophils or macrophages attempting to phagocytose bacteria or bacterial products (Del et al., 2003; Suerbaum and Michetti, 2002).

(iv) **Nonsteroidal antiinflammatory drugs**

Nonselective NSAIDs cause gastric mucosal damage by two important mechanisms: (a) direct or topical irritation of the gastric epithelium and (b) systemic inhibition of endogenous mucosal prostaglandin synthesis. Direct irritation of the mucosal lining by NSAIDs occurs because NSAIDs are weak acids. Topical irritation is therefore most pronounced with more acidic NSAIDs such as aspirin. Although the initial injury is initiated topically by the acidic properties of many of the NSAIDs, systemic inhibition of the protective prostaglandins plays the predominant role in the development of gastric ulcer. Cyclooxygenase (COX-1 and COX-2) is the rate limiting enzyme in the conversion of arachidonic acid to PG and is inhibited by NSAIDs (Wolfe et al., 1999). COX-1 produces protective PG that regulates physiological processes such as gastro intestinal (GI) mucosal integrity, platelet homeostasis and renal function. COX-2 is induced (up regulated) by inflammatory stimuli such as cytokines and produces PG involved with inflammation, fever, and pain. Adverse effects (e.g., GI toxicity or renal toxicity) of NSAIDs are associated with the inhibition of COX-1, whereas antiinflammatory actions result from inhibition of COX-2.

Nonselective NSAIDs including aspirin irreversibly inhibits platelet COX-1 resulting in decreased platelet aggregation and prolonged bleeding times, which may potentiate upper and lower GI bleeding. Neutrophil adherence may damage the vascular endothelium and may lead to a reduction in mucosal blood flow, or may liberate oxygen-derived free radicals and proteases. Leukotrienes are inflammatory substances that may contribute to mucosal injury through stimulatory effects on neutrophil adherence (Silverstein et al., 2000).

**1.3.7 Diagnosis**

Peptic ulcers can be diagnosed by direct visualization using an endoscope or by using contrast radiography to view the ulcer crater. If PUD is suspected in a young patient
with no alarm symptoms, the primary aim should be confirmation of \textit{H. pylori} infection and subsequent eradication. \textit{H. pylori} infection can be diagnosed using a number of techniques, including urease detection. Several different urease tests are commercially available, which use a pH indicator to detect ammonia generated by the urease produced by \textit{H. pylori}. These tests require endoscopic biopsy from the gastric antrum. The urease breath test is a simple test used commonly to diagnose \textit{H. pylori} infection and to confirm its eradication. Stool tests, which detect the specific \textit{H. pylori} antigen, are less expensive than invasive tests and easy to perform.

\subsection*{1.3.8 Treatment of peptic ulcer disease}

The treatment of chronic PUD varies depending on the etiology of the ulcer (\textit{H. pylori} or NSAID), whether the ulcer is initial or recurrent and on associated complications. Overall treatment is aimed at relieving ulcer pain, healing the ulcer, preventing ulcer recurrence and reducing ulcer-related complications.

\subsubsection*{1.3.8.1 Non pharmacological therapy}

Patients with PUD should avoid exposure to factors known to worsen the disease, exacerbate symptoms, or lead to ulcer stress and avoid cigarette smoking, alcohol consumption, foods or beverages that exacerbate ulcer symptoms and NSAID or aspirin use. The high success rates of medical therapies have reduced the number of surgical procedures performed and relegated surgery primarily to elective situations. For this reason, surgical interventions are generally reserved for complicated or refractory PUD. Some surgical procedures include: (i) vagotomy and pyloroplasty; (ii) highly selective vagotomy or (iii) vagotomy combined with antrectomy. Complications of vagotomy are rare but can include dumping syndrome, bile reflux, diarrhea, malabsorption and gastric atony (Wells \textit{et al.}, 2006).

\subsubsection*{1.3.8.2 Pharmacological therapy}

Earlier therapy was based on “no acid, no ulcer.” But after the discovery of \textit{H. pylori}, eradication of \textit{H. pylori} and prevention of NSAID induced disease is the basis of treatment, although acid secretion is still important in the pathogenesis of PUD.

\textbf{(i) Acid neutralizing drugs: Antacids}

Before unspoken the important role of histamine in stimulating parietal cell activity, neutralization of acid with antacids constituted the main form of therapy for peptic ulcers. But they are now rarely used as the primary therapeutic agent, instead are often used by patients for symptomatic relief of dyspepsia. The frequently used antacids are mixtures of aluminum hydroxide and magnesium hydroxide. Aluminum hydroxide may cause constipation and phosphate depletion, while magnesium hydroxides cause loose stools. Calcium carbonate and sodium bicarbonate are potent antacids with varying levels of potential
problems. The long term use of calcium carbonate can lead to milk-alkali syndrome (hypercalcemia and hyperphosphatemia).

(ii) H$_2$ Receptor antagonists

The commonly used agents are ranitidine, cimetidine, Famotidine and nizatidine, each has different potency but all significantly inhibit basal and stimulated acid secretion to comparable levels. Currently, this class of drug is frequently used for treatment of active ulcers (4-6 weeks) in combination with antibiotics directed at eradicating *H. pylori*. Patients may develop tolerance to H$_2$ blockers. Reversible systemic toxicities were also reported with H$_2$ receptor antagonists including pancytopenia, neutropenia, anemia and thrombocytopenia, with a occurrence rate varying from 0.01 to 0.2% (Fauci et al., 2008).

iii) Proton pump inhibitors

The commonly used agents are omeprazole, pantoprazole, esomeprazole, lansoprazole, and rabeprazole inhibit proton pump and potently inhibit all phases of gastric acid secretion. The onset of action is rapid with a maximum effect between 2-6 h and duration of inhibition lasting up to 72-96 h. The half-life is about 18 h therefore; it can take between 2-5 days for gastric acid secretion to return to normal levels once these drugs have been discontinued. Their efficacy is maximized if they are administered before a meal.

(iv) Cytoprotective agents

**Sucralfate**

Sucralfate is a complex sucrose salt and it is insoluble in water. It forms a viscous paste within the stomach and duodenum and binding primarily to sites of active ulceration. It may act as a physicochemical barrier, stimulating mucous and bicarbonate secretion, enhancing prostaglandin synthesis, mucosal defense and repair. Toxicity is rare, with constipation being most common (2-3%).

**Bismuth-containing preparations**

Colloidal bismuth subcitrate (CBS) and bismuth subsalicylate (BSS, Pepto-Bismol) are the most commonly used preparations. The possible mechanisms include ulcer coating, prevention of more pepsin/acid-induced damage and it also stimulate prostaglandins, bicarbonate and mucous secretion. Undesirable effects with short-term usage may include constipation, black stools and darkening of the tongue. While long term usage with high doses, especially with the eagerly absorbed CBS, may lead to neurotoxicity (Fauci et al., 2008).

(v) Prostaglandin analogues
Misoprostol is the most commonly used drug and it act through augmentation of mucosal defense and repair. It also enhances mucous bicarbonate secretion, mucosal blood flow and decrease mucosal cell turnover (Hardman et al., 2001).

**(vi) Treatment of H. pylori**

Amoxicillin, tetracycline, clarithromycin, metronidazole and bismuth compounds are the most commonly used therapeutic agents for therapy of *H. pylori*. No particular agent is effective in eradicating the organism, therefore combination therapy like triple therapy (bismuth subsalicylate/ ranitidine/ omeprazole + metronidazole/ clarithromycin + tetracycline/ amoxicillin) and quadruple therapy (omeprazole + bismuth subsalicylate + metronidazole + tetracycline) provides the maximum efficacy.

**(vii) Herbal treatment**

There are many plants which posses the antiulcer activity like *Byrsonima crassa*, which contain catechins and flavonoids as secondary metabolites. In this direction especial interest are given for those compounds that have a catechol nucleus that has been related with the antioxidant activity involved in the scavenging of the reactive oxygen species on the surface of gastric mucosa, hence protecting cells from injury (Sannomiya et al., 2005). Flavonoids, terpenoids and tannins are one of most important compounds with antiulcer and gastro protective activity (Wahida et al., 2007). In *Cordia verbenacea* flavonoids, saponins, glycosylated triterpenoids, alkaloids, xanthones, phenols and steroidal aglycones are present. Amongst these secondary compounds, xanthones, saponins, triterpenoids and flavonoids are referred as antioxidant compounds with antiulcer and gastroprotective properties (Roldao et al., 2008). The plant *Scoparia dulcis* inhibits gastric acid secretion by inhibition of the proton pump (Vela et al., 2007). A triterpenoid isolated from aqueous extract and decoction of *Passiflora suberosa* barks has been found to be effective as an anti-ulcer agent because it protects the mucosa from acid effects by selectively inhibiting PGF$_2$ (Pasquale et al., 1995). The methanolic extract of *Ficus arnottiana* have shown antiulcer activity, probably due to the antioxidant activity of flavonoids present in the extract (Gregory et al., 2009). High content of triterpenoid and saponins obtained from *Polyscias Balfouriana* is established for its anti stress property (Sandhya et al., 2010). Therefore, the search for an ideal antiulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse and side effects which comes under the allopathic treatment (Sairam and Goel, 2002).

**1.3.9 Mechanistic approach of phytoconstituents for antiulcer activity**

In India, like other parts of the world, market available medicines are widely used for the prevention and treatment of ulcer, but long term use of such synthetic medicines ultimately causes gastro-duodenal risks. Plants are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer. Nearly 240 medicinal plants and 21 plants based compounds were identified as antiulcer
agents so far (Sandhya et al., 2013). The healing potential of the plants is due to their ability to synthesize the aromatic substances such as phenols and flavonoids, which serves as defense mechanism against such diseases (Carlo et al., 1999).

Phenolic compounds have attracted special attention due to their health promoting characteristics. Many studies have been carried out that strongly support the contribution of polyphenols to the prevention of peptic ulcer. Polyphenols display a number of pharmacological properties in the GIT area, acting as antisecretory, cytoprotective, and antioxidant agents (Sumbul et al., 2011). The polyphenolic compounds have also been reported to stimulate PGE formation (Alanko et al., 1999). Polyphenols clearly improves the status of different oxidative stress biomarkers (Williamson et al., 2005; Cook et al., 1996).

Flavonoids have been reported to act in the gastrointestinal tract with antispasmodic (Lima et al., 2005), antisecretory, antidiarrheal (Di et al., 1993), antiulcer, and antioxidant properties (La Casa et al., 2000; Martin et al., 1998). Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed (Borrelli and Izzo, 2000; Di Carlo et al., 1999; Galati et al., 2001). They protect the gastric mucosa against a variety of ulcerogenic agents via several mechanisms of action, mainly free radical scavenging and antioxidant properties, increased mucus production, antisecretory action, and inhibition of the Helicobacter pylori growth (Di Carlo et al., 1999).

Tannins prevent ulcer development due to their protein precipitating and vasoconstricting effects. Their astringent action can help to precipitate microproteins on the ulcer site, thereby, forming an impervious layer over the lining, which hinders induced gastric ulcer in rats, as evidenced by the gut secretions, and protects the underlying mucosa from reduction in the ulcer scores (Nwafor et al., 2000; Nwafor et al., 1996; Rehaily et al., 2002). Triterpenes confers on it various health benefits, such as lipid-lowering and antioxidant effects.

1.4 INFECTIONS

Infections are caused by various deadly pathogens which are being rendered drug resistant due to the random practice of self medication. Poverty and ignorance have definitely added to the global threat of drug resistance. Several synthetic antimicrobial are rendered useless by these drug resistant microorganisms. So screening of herbal recourses is going on throughout the globe to isolate components with antibacterial efficacy which will be effective in controlling various pathogens.

1.4.1 Mechanistic approach of phytoconstituents for antimicrobials activity

The components with phenolic structures were highly active against the microorganisms and are known to be either bactericidal or bacteriostatic agents depending upon the concentration used. (Pelczar et al., 1988; Suresh et al., 1992; Belaiche et al., 1995; Charai et al., 1996; Hili et al., 1997; Dorman & Deans, 2000).
Alcohols are known to possess bactericidal rather than bacteriostatic activity against vegetative cells potentially acting as either protein denaturing agents, solvents or dehydrating agents (Pelczar et al., 1988).

Aldehydes, notably formaldehyde and glutaraldehyde, are known to possess powerful antimicrobial activity. It has been proposed that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement, which may explain their activity, suggesting an increase in electronegativity increases the antibacterial activity (Moleyar & Narasimham, 1986). Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms (Kurita et al., 1979 & 1981).

The presence of an oxygen function in the framework increases the antimicrobial properties of terpenoids. The bacteriostatic and fungistatic action of terpenoids was increased when carbonylated (Naigre et al., 1996).

An increase in antibacterial activity dependent upon the type of alkyl substituent incorporated into a nonphenolic ring structure. An alkenyl substituent (1-methylethenyl) resulted in increased antibacterial activity. In addition, the susceptible organisms were principally Gram-negative, which suggests alkylation influences Gram reaction sensitivity of the bacteria (Pelczar et al., 1988).

Furthermore, the stereochemistry had an influence on bioactivity. It was observed that α-isomers are inactive relative to β-isomers, e.g. α-pinene; cis-isomers are inactive contrary to trans-isomers, e.g. geraniol; compounds with methyl-isopropyl cyclohexane rings are the most active; or unsaturation of the cyclohexane ring further increases the antibacterial activity, e.g. terpinolene, terpineol and terpineolene (Hinou et al., 1989). However β-sitosterol present in Lagerstroemia parviflora is reported to possess significant antibacterial action as reported by Mazumder et al in 2003. The same compound is also present in our plant which has contributed to the antimicrobial activity of the plant.

Investigations into the effects of terpenoids upon isolated bacterial membranes suggest that their activity is a function of the lipophilic properties of the constituent terpenes (Knobloch et al., 1986), the potency of their functional groups and their aqueous solubility (Knobloch et al., 1988). The froth forming saponins reduces the surface tension surrounding the cell wall of the microbes which helps the death of bacteria by bursting of the cell wall.

1.5 RATIONALITY OF HERBAL MEDICATION INSTEAD OF SYNTHETIC

Herbal drug is a chief constituent in traditional medicine and a common constituent in Ayurvedic, Homeopathic, Naturopathic and other medicine systems. It is estimated that about 80% of people in developing countries still relays on traditional medicine for their primary health care (Mukherjee, 2002; Bodeker et al., 2005; Bandaranayake, 2006).
According to Grünwald, the phytomedicine market has grown at an expressive rate worldwide since 1985. The world health organization promotes drug development from traditional medicines partly due to the saving in time and cost that makes the products affordable and accessible, leading to cheaper and cost-effective primary healthcare. Conventional drug development is slow and expensive and often the finished products are unavailable and unaffordable to resource-limited countries.

Modern medicines have their own limitations against diseases with a complex pathology like dementia and ulcer, demonstrating a need of substitute medication from an alternative system of medicine. Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods (Sairam et al., 2001). In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in disease prevention or management. Drugs of herbal origin reduce the offensive factors and have proved to be safe, clinically effective, relatively less expensive, globally competitive, and with better patient tolerance (Goel et al., 2002). These drugs may be slow in action, but mostly augment the defensive factors and are reliable and safe. Therefore, there has been the rationale for the development of new memory enhancing and antiulcer drugs that offer better protection and decreased relapse (Balsano & Alisi 2009; Sumbul et al., 2011).

The problem with modern medicine is also that it is only pathology oriented. The use of herbal remedies is deeply embedded in the history of human kind. Herbal medicines have a wide range of therapeutic use and are suitable for chronic treatments. They usually cost less than synthetic drugs. One of the distinctions between pharmaceutical medicines and herbs is that pharmaceuticals typically contain just one physiologically-active chemical, frequently a synthetic version of an original plant constituent. Herbal medicines, in contrast, have enormous intrinsic biological and pharmacological complexity (Griffin & O'Grady, 2006).

1.6 MECHANISTIC APPROACH OF HERBS AT CELLULAR LEVELS

The reactive oxygen/nitrogen species (ROS/RNS) are produced during cellular metabolism and functional activities and have important roles in cell signaling, apoptosis, gene expression and ion transportation. However, excessive ROS attack bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, and cause oxidative stress, which can damage DNA, RNA, proteins and lipids resulting in an increased risk for many diseases such as inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer’s disease, cataracts, autism and aging (Balsano & Alisi, 2009). Intracellular antioxidant enzymes and intake of dietary antioxidants may help to maintain an adequate antioxidant status in the body. Antioxidant-based drugs/formulations for prevention and treatment of such complex diseases appeared over the past three decades. There are many laboratories from India working on the antioxidant effect of compounds derived from natural sources that are capable of protecting against such damage. There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants (Osawa et al., 1994).
Nervous tissue is highly susceptible for free radical damage due to high content of lipids especially polyunsaturated fatty acids. In Alzheimer disease, biochemical and histological studies have provided evidence for increased levels of oxidative stress and membrane lipid peroxidation which may promote neuronal death in AD by multiple mechanisms that include impairment of the function of membrane ion-motive ATPases (Na+/K+-ATPase and Ca2+-ATPase), glucose transporters and glutamate transporters. Lipid peroxidation also leads to production of the aldehyde 4-HNE that appears to play a central role in the neurotoxic actions of amyloid β-peptide (Yoshikawa et al., 2000). Both AD brain pathology and epidemiological studies have implicated oxidative stress and inflammation as causal factors in AD. Diets enriched in antioxidants, such as vitamin C, and E and alphalipoic acid improve learning and memory reduce plaque load in aged rats, where as anti-inflammatory agents reduce plaque load in transgenic mice (Devasagayam et al., 2004).

Memory impairment and dementia are increasingly prevalent in the current demographic climate of an ageing population (Australian Institute of Health and Welfare 2010). As well as the pathological cognitive loss of neurodegenerative disease, many older persons are experiencing memory loss as part of the physiological process of ageing (Kawas, 2003). Many botanical approaches have much to offer in the improvement of cognitive function, including modulation of factors such as oxidative stress, inflammation and neurotransmission (Tripathi et al., 1996; Bhattacharya et al., 2000; Sairam et al., 2001; Russo et al., 2003; Russo et al., 2005; Das et al., 2002; Singh & Dhawan, 1997; Kishore & Singh, 2005). Flavonoids, tannins and other phenolic constituents from plant origin are potential antioxidants and they play an essential role in the prevention of neurodegenerative diseases, including Parkinson’s and Alzheimer’s diseases. A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported. In recent years, there has been increasing interest in investigating polyphenols from botanical source for possible neuroprotective effects against neurodegenerative diseases as reported from the Department of Biochemistry, University of Missouri, USA.

In India, like other parts of the world, market available medicines are widely used for the prevention and treatment of ulcer, but long term use of such synthetic medicines ultimately causes gastro-duodenal risks. The healing potential of the plants is due to their ability to synthesize the aromatic substances such as polyphones, which serves as defense mechanism against such diseases (Carlo et al., 1999). The mechanisms of action involved in gastric protection are increase of gastric blood flow, the mucosal PG content (Alcaraz & Hoult, 1985; Moroney et al., 1988), the amount of neutral glycoproteins in the gastric mucosa (Carlo et al., 1999), and inhibition of histamine secretion from mast cells (Bronner & Landry, 1985), the gastric proton pump (Carlo et al., 1985), the lipoxygenase pathway (Moroney et al., 1988), platelet activating factor synthesis (Izzo et al., 1994), lipid peroxidation (Alarcón de la Lastra et al., 1994) and _H. pylori_ growth (Beil et al., 1995). They are excellent free radical scavengers (Baumann et al., 1980; Cavallini et al., 1978; Salvayre et al., 1982) and enhancing glutathione peroxidase activity significantly (Martin et al., 1998). Addition of nutraceuticals in daily habit is the alternate therapy to protect from different diseases by preventing oxidative
stress and improving the stores of critical elements such as antioxidants, vitamins, and so forth (Chatterjee & Bandyopadhyay, 2014).

1.7 PLANTS PROFILE

1.7.1 *Cedrus deodara* Loud.

It is generally called as deodar and is a species of cedar native to the western Himalayas. It is growing to a large height and wide girth and also living to a larger age. It is having soft grayish green or blue needles, drooping branches and growing rapidly to 40-50 feet tall and 20-30 feet wide (Bhattacharyya *et al.*, 1988; Yadav and Bhattacharyya, 1992).

1.7.1.1 Classification


1.7.1.2 Morphological characteristics

* Cedrus deodara *Loud. is a large evergreen tree often reaching 60 meter in height (Fig. 1.9A). Branches and branchlets are horizontal, and leaves are 2.5 to 5 cm. long, needle like as shown in Fig. 1.9B (Farjon, 1990). Bark is grayish or radish brown with vertical and diagonal fissures (Fig. 1.9 C). It is monocious plant, although male and female cones appear on different branches. Female cones are barrel shaped, cylindrical, 2.5 to 4.5 cm. in length and borne singly at the tip of the dwarf shoots (Fig. 1.9 B). Flowers are bisexual and appear in the month of the autumn. Fruit shape is oval, brown in color and covering is dry or hard with 3 to 6 inches in length (Shah, 2006).

1.7.1.3 Phytochemical constituents

The main chemicals in woods of *Cedrus deodara* Loud. include wikstromal (7-9%), matairesinol (9-13%), dibenzylbutyrolactol (7-11%) (Rao *et al.*, 2003a; Singh *et al.*, 2007), 1, 4 diaryl butane, benzofuranoid neo lingam (Agrawal *et al.*, 1982), cedrin (6-methyldihydromyricetin), taxifolin, cedeodarin (6-methyldihyridromyricetin), dihydromyricetin, cedrinoside (Agrawal *et al.*, 1980), deodardione, diosphenol, limonene carboxylic acid (Krishnappa *et al.*, 1978), (−)-matairesinol, (−)-nortrachelogenin and dibenzylbutyrolactolignan (4, 4', 9-trihydroxy-3, 3'-dimethoxy-9, 9'-epoxylignan) (Tiwari *et al.*, 2001). A new dihydroflavonol named deodarin (3, 4, 5, 6-tetrahydroxy-8-methyl dihydroflavonol) has been isolated from the stem bark (Adinarayana & Seshadri 1965). The compounds obtained from ethanolic extract of needles were characterized as β-sitosterol, ethyl stearate, ethyl laurate, ferulic acid, shikimic acid, protocatechuic acid, 10-nonacosanol, methylconiferin, dibutyl phthalate, (E)-1-O-p-coumaroyl-beta-D-glucopyranoside, 5-p-trans-coumaroylguinic acid, 9-hydroxy-dodecanoic acid, 3-beta-hydroxy-oleanolic acid
methyl ester, phthalic acid bis-(2-ethylhexyl) ester, beta-glucoside and 1-[3-(4-hydroxyphenyl)-2-propenoate]-α-D-glucopyranosidea (Zhang et al., 2010a; Zhang et al., 2010b) taxifolin, quercetin, myricetin, 2R,3R-dihydromyricetin and cedrusone A (Zhang et al., 2011; Liu et al., 2011a). A diterpene acid, centdaroic acid has been reported from roots (Srivastava et al., 2001). Five flavonoids cedrusone A, myricetin, 2R,3R-dihydromyricetin, quercetin, and 2R,3R-dihydroquercetin were isolated from pine needles (Liu et al., 2011b).

The essential oils from woods were reported to contain a sesquiterpenes-L II isohemacholone, sesquiterpenes L III deodarone and atlantone (Shankaranaryan et al., 1977a), α-himacholone (12.5%), β-himacholone (43%), α-pinene, β-pinene, myrcene (Yan-qiu et al., 2008), himachalene, cis-atlantone and α-atlantone (Makhaik et al., 2005). Singh and Agarwal, 1986 also reported the presence of himacholol (3%) and β-himachalene (31%) in the essential oil. A new compound, 3-p-trans-coumaroyl-2-hydroxyquinic acid, and seven known compounds viz. protocatechuic acid, 2R,3R-dihydromyricetin, massonianoside B, tran-sp-coumaric acid-4-O-β-D-glucopyranoside, (6S,9R)-Roseoside, massonianoside B and myricetin-3-O-β-D-glucopyranoside were isolated from the methanolic extract of pine needle (Yan Ping et al., 2015). The compounds isolated from the pollen grains were dehydroabiatic acid, 15-hydroxydehydroabiatic acid, 7α,18-dihydroxydehydroabietaanol, naringenin, β-sitosteryl β-D-glucoside, 7β,15-dihydroxydehydroabiatic acid, hexadecane-1,16-diol 7-caffeoyl ester (Ohamoto et al.,1987a), 7β,18-dihydroxydehydroabietaanol, 7β, hroxydehydroabiatic acid, 15-methoxyabiatic acid, 15-Hroxydehydroabiatic acid and 9-caffeoyloxyhexadecanol (Ohamoto et al.,1987b).

Fig. 1.9: The plant Cedrus deodara Loud.: (A) Tree, (B) Needles & cone, and (C) Stem wood.
1.7.1.4 Pharmacological activity

A number of workers have reported a range of activities of Cedrus deodara Loud. in various in-vivo and in-vitro investigation models. Different parts of this plant have been establish to showed analgesic, antiinflammatory, antibacterial, antispasmodic, antihyperglycemic, immunomodulatory, insecticidal, anticancer, antiapoptotic, molluscidal, antisarcoptic, anxiolytic and anticonvulsant activities.

1.7.1.5 Toxicological activity

Organ specific effects of Cedrus deodara showed that it has anti-inflammatory effects, not irritating to skin and no significant changes were observed in the body weight, organ weight or organ/body weight rations of the treated animals, serum oxaloacetic transaminase and pyruvic transaminase levels remained unaltered, as did blood glucose and blood urea nitrogen values. Neither spontaneous nor treatment related histopathological changes were observed. These data suggest that oil was devoid of any adverse effects on skin or on liver and kidney functions of rabbits (Tisser & Young, 2014; Shinde et al., 1999a, 1999b, 1999c; Dhar et al., 1968; Tandan et al., 1998; Saab et al., 2012).

1.7.1.6 Ethnopharmacological uses

Cedrus deodara Loud. oils and gum have medicinal values and are used in treatment of inflammations, fever, dyspepsia, insomnia, urinary discharge, hiccough, bronchitis, ozoena, itching, tuberculous glands, leucoderma, piles, ophthalmia, disorder of mind, disease of skin and blood. Leaves are used in treatment of inflammation and tuberculous glands. Wood is bitter and is used as diuretic, diaphoretic, carminative. It finds wild application in treatment of rheumstism, skin disease, fever, palsy, piles, prolapus recti, epilepsy, pulmonary and urinary disorder. The whole parts of Cedrus deodara Loud. are useful in Ayurveda system of medicine for the treatment of insomnia, disorder of mind, disease of skin and blood (Kirtikar and Basu, 1991). Oils is used as antidote, analgesic, diaphoretic and is used in treatment of ulcers, boils, bruises, injuries to joint, skin diseases and tuberculous glands. Bark is astringent and is useful for diarrhea, dysentery and fever. Turpentine oils are used for treatment of ulcer, skin diseases and leprosy (Shah, 2006). Cedrus deodara Loud. used in preparation of V-gel, which is commonly used as antiseptic (Pandey, 2000).

1.7.2 Pinus roxburghii Sarg.

It is generally known as chir and saral (Hindi), chir pine (English), bhadradaru and pitadru (Sanskrit). Chir has long been known for its medicinal value (Shah et al., 2006).
1.7.2.1 Classification

Kingdom - Plantae, Sub kingdom - Trachiobionta, Division - Coniferophyta, Class - Pinopsida, Order - Pinales, Family - Pinaceae, Genous - Pinus, Species - roxburghii.

1.7.2.2 Morphological characteristics

It is a large tree reaching 30-50 meter with a trunk diameter of up to 2-3 meter (Fig. 1.10A). The leaves are yellowish green needle-like, 20-35 cm long and are present in cluster of three. The cones are green or brown, ovoid, 12-24 cm long and 5-8 cm broad (Fig. 1.10B). They open slowly over the next year, or after being heated by a forest fire to release the seeds. The seeds are 8-9 mm long with a 40 mm wing and are wind-dispersed. The bark is thick, reddish brown and deeply fissured at the base of the trunk, thinner and flaky in the upper crown (Fig. 1.10C).

1.7.2.3 Distribution

It is originate at the height of 500 to 2500 meter above sea level. In India it is found in Kashmir, Himachal Pradesh, and Uttaranchal. The range extends from northern Pakistan, across northern India (Jammu and Kashmir, Punjab, Himachal Pradesh, Uttarakhand, Sikkim) and Nepal to Bhutan.

1.7.2.4 Phytochemical constituent

The major constituents of wood volatile oils are caryophyllen (16.75%), thunbergol (16.29%), 3-carene (14.95%), cembrene (12.08), α-thujene (10.81%), terpinolen (7.17%), α-pinene (4.8%), α-caryophyllene (3.7%), sabinene (3.59%), verticilol (1.84%), 4-terpineol (1.79%), and myrcene (1.28%) (Salem et al., 2014). Hasan & Amjid (2009) reported that major components in the essential oil of stem were α-pinene (41.9%), camphene (0.9%), 3-carene (16.3%), β-phellandrene (0.7%), γ-terpinene (0.2%), limonene (1.7%), p-cymene (1.9), 1-terpineol-4-ol (0.2%), α-terpineol (1.8%), borneol acetate (1.1%), terpinyl acetate (0.8%), farnesene (0.6%), butanoic acid, 3-methyl- 2-phenylethylester (0.3%), β-caryophyllene oxide (1.0%), camphorphyline (12.3%), α-terpinyl acetate (0.8%) and farnesy lactate (0.2%). Makaik et al., (2005) reported that the major constituents in the volatile oil of resin were α-pinene (18.1%), careen (51.8%), and longifolene (13.8%).

The main components in the essential oils from bark are α-pinene (31.29%), 3-carene (28.05%), cembrene (4.86%), longifolene (4.42%), thunbergol (4.11%), β-pinene (2.99%), sylvestrene (2.4%), terpineol (2.05%), terpinyl acetate (1.56%), elemol (1.46%), methyl dehydroabietae 91.37%), myrcene (1.36%), bornyl acetate (1.1%), α-cadinol (1.08%), and phenethyl isovalerate (1%) (Salem et al., 2014). Satyal et al., (2013) reported that the essential oil from bark was dominated by sesquiterpenes, particularly (E)-caryophyllene and α-humulene as well as monoterpene alcohols including terpinen-4-ol and α-terpineol. The bark also contains 7-10% of tannins (Anonymous, 2003). Sugars were extracted from the bark.
and the amounts of sugars were glucose (1.25-2.49%), fructose (1.2-2.9%) and arabinose (1.17-1.87 %) (Ahmad et al., 1990a).

The major chemical constituents found in the essential oil from needle are α-pinene (39%), 3-carene (33.37%), β-pinene (4.6%), longifolene (2.76%), sylvestrene (1.18%), caryophyllene (1.07%), and cembrene (1.05%) (Salem et al., 2014). Zafar et al., (2010) reported that the major components in the essential oils of needles was α-pinene (29.3%), β-myrcene (1.1%), 3-carene (14.2%), terpinyl acetate (1.0%), α-terpineol (4.5%), borneol acetate (2.2%), α-longipinene (1.2%), caryophyllene (21.9%) and caryophyllene oxide (3.1%). Qadir and Shah (2014) reported that the principle constituents of the fruit oil from India were α-pinene (60.8%) and β-pinene (30.2%).

The seed oil was analyzed for fatty acid components and was rich in oil with high linoleic acid oil and polyunsaturated fatty acid contents (Ahmad et al., 1990b). Oleoresin was reported to contains terpenoids, α-pinene, camphene, β-pinene, α-terpinene, limonene, terpinene, terpinolene, α-terpineol, car-3-ene (Swales & Dev, 1979; Mishra et al., 1988). The turpentine contained significant amounts of α-pinene, β-pinene, and Δ-3-carene. Abietic and isopimaric acid were the major resin acids (Coppen et al., 1988). Coppen et al., 1988 also reported the presence of α-pinene (22.8%), camphene (0.4%), β-pinene (14.1%), Δ3-carene (50.6%), α-phellandrene (0.1%), α-terpinene (0.4%), limonene (0.9%), β-phellandrene (0.7%), γ-terpinene (0.5%), terpinolene (3.8%), longipinene (0.2%), longicyclene (0.2%), saturene (0.1%), longifolene (3.4%), β-caryophyllene (0.2%), and α-terpenyl acetate (0.3%) in the resin. Longifolene, an important member of the sesquiterpene class of the major mevalonoid group of natural products, was first isolated from Indian turpentine oil from Chir pine (Jadhav & Nayak, 1980). Phytochemical investigation indicate that the methanolic extract of the stem bark contains 1,5-dihydroxy-3,6,7-trimethoxy-8-dimethylallyloxy-xanthone and 1-hydroxy-3,6-dimethoxy-2-β-D-glucopyranoxanthone (Rawat et al., 2006). Friedelin, ceryl alcohol and β-sitosterol were isolated from the unsaponifiable matter of the petroleum ether extract of the bark (Beri, 1970). Chatterjee et al., (1977) also reported the presence of hexacosylferulate in the stem bark.

1.7.2.5 Pharmacological activity

The plant Pinus roxburghii Sarg. has been reported to have antidiyslipidemic, antioxidant, antiinflammatory, analgesic, hepatoprotective and antimicrobial activities.

1.7.2.6 Toxicological activity

Oral administration of pine extract for 6 months and 12 months did not showed any organ specific toxic effects in dogs and rats. They have no potential for causing mutations or birth defects, and have no adverse effect on fertility, pregnancy or nursing (www.mdidea.com). Single and 14 days repeated dosing in rats and dogs also had no influence on body weight, feed consumption, blood chemistry, and haematology at any dose level. There were no treatment related findings on gross and detailed necroscopy, organ weights, organ weight ratios and histology. Moreover, short and long term human studies
showed no adverse influence on liver and kidney function, hematology, and did not cause any adverse events (Frevel et al., 2012).

Fig. 1.10: The plant *Pinus roxburghii* Sarg.: (A) Tree, (B) Needles & cone, and (C) Stem wood.

### 1.7.2.7 Traditional and Ayurvedic uses

Plant is bitter, pungent, heating, antiseptic and is used in disease of eye, ear, throat and skin, ulcer, bronchitis, diaphoresis, giddiness, inflammation and itching. Gum is bitter, acrid, heating, oleaginous, purgative, carminative, expectorant, aphrodisiac, fattening, diuretic, anthelmintic, analgesic and is used in disease of eye, liver, spleen, vagina and uterus, dyspepsia, ulcer, diaphoresis, scabies, asthma, chronic bronchitis, inflammation, piles, ear discharge, toothache, lumbago and tuberculous glands. Resin is used as a remedy for gonorrhea and its plaster is applied to buboos and abscesses for suppuration. Wood is stimulant, diaphoretic and is used in cough, fainting and ulceration. Wood and oleoresin are used in snakebite and scorpion sting (Shah, 2006; Kunwar *et al.*, 2009).