1.0. Introduction to inflammation

Based on visual observation, the ancients have characterised inflammation by five cardinal signs, namely redness (rubor), swelling (tumour), heat (calor; only applicable to the body's extremities), pain (dolor) and loss of function (functio laesa). The first four of these signs were named by Celsus in ancient Rome (30-38 B.C.) and the last by Galen (A.D 130-200) [1]. However, beginning with the work of Metchnikoff and others in the 19th century, the contribution of inflammation to the body's defensive and healing process was recognised [1]. More recently, inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" [2], or "the reaction to injury of the living microcirculation and related tissues [3]. Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host.

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogen, damaged cells, or irritants [4]. Inflammation is a process by which the body deals with insult and injury. Insult may be caused mechanically (by pressure or foreign bodies), chemically (by toxins, acidity, alkalinity), physically (by temperature), by internal processes (uremia) and by microorganisms (bacteria, virus and parasites) etc. Inflammation rids the body of foreign matter and disposes of damaged cells and initiates wound healing. Furthermore, inflammation is considered as the cornerstone of pathology in that the changes observed are indicative of injury and disease.

During inflammation the sensation of heat is caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities resulting in the increased redness. The swelling is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area and in prolonged inflammatory responses deposition of connective tissue. Pain is due to the direct effects of mediators, either from initial damage or that resulting from the inflammatory response itself and the stretching of sensory nerves due to oedema. The loss of function refers
to either simple loss of mobility in a joint, due to the oedema and pain, or to the replacement of functional cells with scar tissue [5].

Inflammation is a diverse phenomenon (Figure 1), ranging from the acute inflammation associated with *S. aureus* infection of the skin (the humble boil), through to chronic inflammatory processes resulting in remodelling of the artery wall in atherosclerosis, the bronchial wall in asthma and chronic bronchitis and the debilitating destruction of the joints associated with rheumatoid arthritis.

![Figure 1](image)

**Figure 1.** Inflammation - The basic cause of many diseases.

Inflammation is controlled by mast cells that are in close proximity to autonomic nerves. Mast cells are the constituents of connective tissues containing large granules that contain heparin, serotonin, bradykinin and histamine. These substances are released from the mast cell (Figure 2) in response to injury and infection and by their degranulation, they control most of the processes of inflammation. Mast cells are responsive to other controls, for example, under the influence of progesterone they release serotonin and under the influence of estrogen they release histamine.
Another important pathway is known as the arachidonic acid cascade which is largely controlled by eicosanoids. Eicosanoids are local “hormones” made from 20-carbon essential fatty acids; they are short-lived and can affect many aspects of physiological function at the cellular level. Eicosanoids include all the prostaglandins, thromboxanes and leukotrienes. Depending on genetic as well as other factors eicosanoids transform or control prostaglandins, thromboxanes, and leukotrienes all of which are inflammatory mediators. Eicosanoids can initiate, regulate and terminate all local inflammatory responses. When inflammation affects a joint (such as in rheumatoid arthritis), the cartilage can be damaged by neutrophils and lysosomal enzymes that enter the area. This leads to a vicious cycle of repeated injury and persistent inflammation. Inflammation may also lead to mental depression which is commonly seen in chronic pain patients. Indolamine dioxygenase (IDO) is a rate-limiting enzyme in the degradation of tryptophan and is induced during inflammation by the cytokines interferon-gamma (IFN-gamma), interferon-alpha (ITN-alpha) and tumor necrosis factor-alpha (TNF-alpha) in a broad variety of cells. Elevated levels of IDO can enhance tryptophan degradation and subsequent serotonin depletion which may cause depression [6].
1.1. **Global status of inflammatory diseases**

*Figure 3.* The global map of inflammatory bowel disease: absence of colour indicates absence of data.

In the west, the incidence and prevalence of inflammatory bowel diseases (IBD) (*Figure 3*) has increased in the past 50 years i.e. 120-200/100,000 persons for ulcerative colitis (UC) and 50-200/100,000 persons for Crohn's disease (CD). Studies of migrant populations and populations of developing countries demonstrated a recent slow increase in the incidence of UC, whereas that of CD remained low, but CD incidence eventually increased to the level of UC. CD and UC are incurable; they begin in young adulthood and continue throughout life. CD begins with ulcers that develop into strictures or fistulas. Lesions usually arise in a single digestive segment in CD. Amongst the patients with CD, intestinal surgery is required for as many as 80% and a permanent stoma is required in more than 10% patients. In patients with UC, the lesions usually remain superficial and extend proximally and colectomy is required for 10-30% of patients. The mortality of patients with UC is not greater than that of the CD. It has been proposed that only aggressive therapeutic approaches based on the treatment of early recurrent lesions in patients may significantly check the progression of these chronic diseases [7]. Although progress has been made in understanding these diseases, their etiology is still unknown.
1.2. **Role of Natural products in the treatment of inflammatory diseases**

Humans have been the most privileged form of life who has dominated the rest of creatures. This most privileged class suffered from different ailments and health disorders at different stages of life. For the treatment of illness, humans started searching for the remedies in its surroundings i.e. the mother nature. The world health organization (WHO) estimates that 80% of the population living in the developing countries rely exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicines, the traditional plants play a major role and constitute the backbone of the traditional medicine. Indian Materia Medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices. Out of these drugs, 400 are of mineral and animal origin while the rest are of vegetable origin. Since less than 10% of the world’s biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity [8]. The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 B.C.) which documented oils from *Cupressus sempervirens* (Cypress) and *Commiphora* species (myrrh) which are still used today to treat coughs, colds and inflammation [8]. The Ebers Papyrus (2900 B.C.) is an Egyptian pharmaceutical record, which documents over 700 plant-based drugs ranging from gargles, pills, infusions, to ointments. The Chinese Materia Medica (1100 B.C.) (Wu Shi Er Bing Fang, contains 52 prescriptions), Shennong Herbal (~100 B.C., 365 drugs) and the Tang Herbal (659 A.D., 850 drugs) are documented records of the uses of natural products [8].

1.3. **Therapeutic potential of natural products**

The Greek physician, Dioscorides (100 A.D.) recorded the collection, storage and the uses of medicinal herbs, whilst the Greek philosopher and natural scientist, Theophrastus (~300 B.C.) dealt with medicinal herbs. During the dark and middle ages the monasteries in England, Ireland, France and Germany preserved this western knowledge whilst the Arabs preserved the Greco-Roman knowledge and expanded the uses of their own resources, together with Chinese and Indian herbs unfamiliar to the Greco-Roman world [8]. It was the Arabs who were the first to privately own pharmacies (8th century) with Avicenna, a Persian pharmacist, physician, philosopher
and poet, contributing much to the sciences of pharmacy and medicine through works such as the Canon Medicinae [8].

In India Ayurveda originated long back in the pre-vedic period. The Rigveda and Atharvaveda (5000 BC), the earliest Indian documents have references on health and diseases. During the Vedic period the Susruta samhita and the Charaka samhita were influential works on traditional medicine. The fundamental and applied principles of “Ayurveda” science of life; got organized and enunciated around 1500 B.C. Ayurveda traces its origins to the Vedas, Atharvaveda in particular, and is connected to Hindu religion. Atharvaveda (one of the four most ancient books of Indian knowledge, wisdom and culture) contains 114 hymns or formulations for the treatment of diseases. Ayurvedic system of medicine was based on nature and its products. Hundreds of medicinal plants were identified and have been traditionally used since then. Over the following centuries, Ayurvedic practitioners developed a number of medicinal preparations and surgical procedures for the treatment of various ailments and diseases. Ayurvedic medicinal preparations consist mainly of plant materials in the form of powders, semi-solid preparations, decoctions, elixirs and distillates. Many of them also contain inorganic chemical substances, minerals and animal products. Alcoholic extracts and alcoholic solutions of the ingredients, tinctures and elixirs are also frequently used in Ayurvedic medicine.

The role of natural products for the treatment of diseases has been recognized since ancient times. There has been considerable public and scientific interest in the use of natural products to combat human diseases such as inflammatory disease, cardiovascular disease, and cancer etc. In spite of major scientific and technological progress in combinatorial chemistry, drugs derived from natural products still make an enormous contribution to drug discovery today [9]. Natural products with anti-inflammatory activity have long been used as a folk remedy for inflammatory conditions such as fever, pain, migraine and arthritis.

*Boswellia serrata* is native to India and has been used in traditional Ayurvedic medicine for the treatment of inflammatory diseases in India [10]. The gum resin of *Boswellia serrata* called ‘salai guggul’ or ‘Indian olibanum’ and is obtained from the bark of *Boswellia serrata* after injury. Many commercial formulations of salai guggul in the form of ointments, creams and capsules are available in the market [11]. As a
result of its alleged safety, boswellia was considered superior over mesalazine in terms of a benefit-risk evaluation [12]. Research has shown that it is perhaps the triterpenoid boswellic acids in the *Boswellia serrata* gum resin which exert the anti-inflammatory action [13]. Boswellic acids inhibit the 5-LOX (Lipoxygenase) enzyme, thereby reducing the production of the potent inflammatory mediators, the leukotrienes [14].

Bromelain is a crude aqueous extract which is obtained from both the stem and fruit of the pineapple plant and contains a number of proteolytic enzymes [15]. A large body of scientific research shows that bromelain is a potential product for treatment of osteoarthritis [16]. Bromelain was first reported to be used as an anti-inflammatory for use in both rheumatoid arthritis and osteoarthritic patients in 1964 [16]. The mechanism of anti-inflammatory action of bromelain is known [16]. They suggest that bromelain’s anti-inflammatory action is mediated by increasing serum fibrinolytic activity, reducing plasma fibrinogen levels and decreasing bradykinin levels (which results in reduced vascular permeability) and hence reducing oedema and pain; by decreasing levels of PGE$_2$ and thromboxane A$_2$ (TXA$_2$); and by modulation of certain immune cell surface adhesion molecules.

Lyprinol is a stabilized lipid extract obtained from the New Zealand green-lipped mussel (NZGLM) and is used to relieve the symptoms of arthritis. The oil of the NZGLM contains a complex mixture of triglycerides, sterol esters, polar lipids and free fatty acids [17].

Ternatin, a tetramethoxy flavone (Figure 4) isolated from *Egletes viscosa*, was shown to have anti-inflammatory activity in rat carrageenan-induced pleurisy test [18].

![Figure 4. Ternatin](image-url)
Quercitrin and rutin display beneficial effects in experimental inflammation which is induced by trinitrobenzene sulfonic acid in the rat model [19]. The mechanism by which flavonoids exert their anti-inflammatory effects involves the inhibition of COX and LOX activities, eicosanoid biosynthesis and neutrophil degranulation.Selective flavonoids such as quercetin inhibit both COX and LOX activities [20]. Pelzer et al., (1998) investigated the anti-inflammatory activity of 30 flavonoids isolated from several plants of the compositae family and found that all the flavonoids tested have anti-inflammatory activity depending on both their structure and the method used for the assay [21].

Just et al., (1998) isolated three saponins (Fruticesaponin A, Fruticesaponin B, ruticesaponin C) from Bupleurum fruticescens and investigated their anti-inflammatory effects. All of them exerted anti-inflammatory activity in the mouse oedema assay; however Fruticesaponin B has the highest anti-inflammatory activity [22]. Silva et al., (2002) isolated a new steroidal saponin from the leaves of Agave attenuata and investigated its anti-inflammatory activity using the capillary permeability assay. It inhibits the increase in vascular permeability caused by acetic acid [23]. Aescin, the main active constituent of Aesculus hippocastanum, is a complex mixture of triterpenoid saponin glycosides. It has been shown to have anti-oedematous, anti-inflammatory and venotonic properties in different animal models [24]. Two triterpenoid saponins (Figure 5), kalopanaxsaponin A and pictoside A, have been isolated from the stem bark of Kalopanax pictus and were found to exhibit significant anti-inflammatory activity at the oral dose of 50 mg/ml [25].

![Figure 5. Kalopanaxsaponin A and Pictoside A](image-url)
Chapter V

Probably the most famous and well-known example to date would be the synthesis of the anti-inflammatory agent, acetylsalicyclic acid (1) (aspirin) [26] derived from the natural product, salicin (2) isolated from the bark of the willow tree Salix alba L. Investigation of Papaver somniferum L. (opium poppy) resulted in the isolation of several alkaloids including morphine (3) a commercially important drug, first reported in 1803.

![Chemical Structures](image1)

It was in the 1870s that crude morphine derived from the P. somniferum was boiled in acetic anhydride to yield diacetylmorphine (4) (heroin) which was readily converted to codeine (5), a painkiller.

![Chemical Structures](image2)

Pilocarpine (6) found in Pilocarpus jaborandi (Rutaceae) is a L-histidine-derived alkaloid, which has been used as a clinical drug for the treatment of chronic open-angle glaucoma and acute angle-closure glaucoma for over 100 years. In 1998, its oral preparation was approved for the treatment of Sjogren's syndrome, an autoimmune disease that damages the salivary and lacrimal glands [27].

![Chemical Structures](image3)

_Tripterygium wilfordii_ (Lei Gong Teng) is a Chinese herb that has been shown to be quite effective in treating inflammation. It is a toxic herb that should be used...
with caution as it can cause internal bleeding, kidney damage, decrease in blood cell counts, decreased bone mineral density in females, hair loss, immune system dysfunction and even death. PG490-88, a diterpene-diepoxide (7), is a semisynthetic analogue of triptolide which is isolated from *Tripterygium wilfordii*. It is used for the treatment of autoimmune and inflammatory diseases in the People’s Republic of China [28, 29].

Grandisines A (8) and B (9) are two indole alkaloids which were isolated from the leaves of the Australian rainforest tree, *Elaeocarpus grandis*. Grandisine A (8) contains a unique tetracyclic skeleton, while Grandisine B (9) possesses an unusual combination of isoquinuclidinone and indolizidine ring systems. Both 8 and 9 exhibit binding affinity for the human δ-opioid receptor and are potential leads for analgesic agents [30].

The first notable discovery of biologically active compounds from marine sources can be traced back to the reports of Bergmann on the isolation and identification of C-nucleosides, spongouridine (10) and spongothymidine (11) from the Caribbean sponge, *Cryptotheca crypta* in the early 1950s [31]. These compounds were found to possess antiviral activity and the synthesis of its structural analogues led to the development of cytosine arabinoside (Ara-C) as a clinical anticancer agent, together with (Ara-A) as an antiviral agent 15 years later [31].
There are many anti-inflammatory natural products derived from marine sponges. Eighty four anti-inflammatory compounds dominated by isoprenoid derived metabolites, especially sesterterpenes (means 2.5 terpenes) have been isolated from marine sponges [32]. Manoalide (12) is probably the most well-known of all the anti-inflammatory products from sponge and was originally isolated by de Silva and Scheuer in 1980 from the sponge *Luffariella variabilis* [33].

The global marine pharmaceutical pipeline consists of three Food and Drug Administration (FDA) approved drugs, one EU registered drug, 13 natural products (or there derivatives) in different phases of the clinical trial and a large number of marine chemicals in the pre-clinical pipeline [34]. Some examples include, Ziconotide (Prialt®, Elan Corporation) a peptide first discovered in a tropical cone snail, which was approved in December 2004 for the treatment of pain. Plitidepsin (13) (Aplidin®, PharmaMa), a depsipeptide was isolated from the Mediterranean tunicate *Aplidium albicans* [35,36]. Plitidepsin (13) is effective in treating various cancers, including melanoma, small cell and non-small cell lung, bladder as well as non-hodgkin lymphoma and acute lymphoblastic leukemia and is currently in Phase II clinical trials [34,37].
Sativex, a mixture of dronabinol 14 and cannabidol 15 obtained from the cannabis plant, is the world's first pharmaceutical prescription medicine that was launched in Canada (April 2005) and was later approved by Health Canada (August 2007) as adjunctive analgesic for severe pain in advanced cancer patients [38]. Sativex has been recommended by FDA to enter directly in Phase III trials and as of November 2009, GW Pharmaceuticals have completed the recruitment for Phase II/III trial against cancer pain.
Natural product-derived drugs launched during 2005-2010; lead compounds, and therapeutic area.

<table>
<thead>
<tr>
<th>Year</th>
<th>Trade name</th>
<th>Lead Compound</th>
<th>Disease area</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Dronabinol / Cannabidiol (Sativex®)</td>
<td>Dronabinol / cannabidiol</td>
<td>Pain</td>
</tr>
<tr>
<td>2005</td>
<td>Fumagillin (Flisint®)</td>
<td>Fumagillin</td>
<td>Antiparasitic</td>
</tr>
<tr>
<td>2005</td>
<td>Doripenem (Finibax® / DoribaxTM)</td>
<td>Thienamycin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>2005</td>
<td>Tigecycline (Tygacil®)</td>
<td>Tetracycline</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>2005</td>
<td>Ziconotide (Prialt®)</td>
<td>Ziconotide</td>
<td>Pain</td>
</tr>
<tr>
<td>2005</td>
<td>Zotarolimus (EndeavorTM stent)</td>
<td>Sirolimus</td>
<td>Cardiovascular surgery</td>
</tr>
<tr>
<td>2006</td>
<td>Anidulafungin (EraxisTM / EcaltaTM)</td>
<td>Echinocandin B</td>
<td>Antifungal</td>
</tr>
<tr>
<td>2006</td>
<td>Exenatide (Byetta®)</td>
<td>Exenatide-4</td>
<td>Diabetes</td>
</tr>
<tr>
<td>2007</td>
<td>Lisdexamfetamine (VyvanseTM)</td>
<td>Amphetamine</td>
<td>Attention deficit-hyperactivity disorder (ADHD)</td>
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<tr>
<td>2007</td>
<td>Retapamulin (AltabaxTM/AltargoTM)</td>
<td>Pleuromutilin</td>
<td>Antibacterial</td>
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<tr>
<td>2007</td>
<td>Temsirolimus (ToriselTM)</td>
<td>Sirolimus</td>
<td>Oncology</td>
</tr>
<tr>
<td>2007</td>
<td>Trabectedin (YondelisTM)</td>
<td>Trabectedin</td>
<td>Oncology</td>
</tr>
<tr>
<td>2007</td>
<td>Ixabepilone (IxempraTM)</td>
<td>Epothilone B</td>
<td>Oncology</td>
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<td>2008</td>
<td>Methylnaltrexone (Relistor®)</td>
<td>Naltrexone</td>
<td>Pain</td>
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<tr>
<td>2009</td>
<td>Everolimus (Afinitor®)</td>
<td>Sirolimus</td>
<td>Oncology</td>
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<tr>
<td>2009</td>
<td>Telavancin (VibativTM)</td>
<td>Vancomycin</td>
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<tr>
<td>2009</td>
<td>Romidepsin (Istodax®)</td>
<td>Romidepsin</td>
<td>Oncology</td>
</tr>
<tr>
<td>2009</td>
<td>Capsaicin (Qutenza®)</td>
<td>Capsaicin</td>
<td>Pain</td>
</tr>
<tr>
<td>2010</td>
<td>Monobactam aztreonam (CaystonTM)</td>
<td>Aztreonam</td>
<td>Antibacterial</td>
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</table>
Natural products as anti-inflammatory agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Structure</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>Curcuma longa</td>
<td><img src="image" alt="Curcumin structure" /></td>
<td>[39]</td>
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<tr>
<td>Parthenolide</td>
<td>Tanacetum parthenium</td>
<td><img src="image" alt="Parthenolide structure" /></td>
<td>[40]</td>
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<tr>
<td>Cucurbitacins</td>
<td>Wilbrandia ebracteata</td>
<td><img src="image" alt="Cucurbitacins structure" /></td>
<td>[41]</td>
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<tr>
<td>1,8-Cineole</td>
<td>Eucalyptus obliqua</td>
<td><img src="image" alt="1,8-Cineole structure" /></td>
<td>[42]</td>
</tr>
<tr>
<td>Pseudopterosins</td>
<td>Pseudopterogorgia elisabethae</td>
<td><img src="image" alt="Pseudopterosins structure" /></td>
<td>[43]</td>
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<tr>
<td>Amrubicin hydrochloride</td>
<td>Streptomyces peucetius</td>
<td><img src="image" alt="Amrubicin structure" /></td>
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</tbody>
</table>
The natural products discovered so far have played a vital role in improving the human health and have been the drugs of choice despite facing a tough competition from their synthetic counterparts, due to their safe and long lasting effects. Current interest leads to the search for new natural products with anti-inflammatory activity. Extensive scientific research deals with the finding, extracting, pharmacological effects and mechanism by which natural products exert their activity. In summary, we propose that a combination of metabolomics technologies with natural product discovery processes will be highly beneficial. By increasing the number of identifications in our metabolomics data we may provide novel structures to be tested for bioactivity for a disease under investigation.

In view of the importance of natural products in the human health system, the main objectives of this work are as follows:

1. Ethanolic extraction of the dried plant material followed by the fractionation with different solvents of increasing polarity.
2. Isolation of the active principles from the biologically active fraction(s) of the plant.
3. Structure elucidation of isolated compounds and biological evaluation of the ethanolic extract and its fractions along with the isolated natural compounds for their anti-inflammatory and analgesic activities with gastric ulceration studies.
References


