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Phytochemical and In Vitro Anti Inflammatory Activity of Stem of *Pedalium murex* (L.)

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ABSTRACT

Since ancient times, herbal remedies have been a mainstay of healthcare, contributing significantly to both pharmaceutical research and international trade. A member of the Pedaliaceae family, Pedalium murex (L.) is a medicinal plant that has drawn interest due to its many therapeutic uses, which include anti-inflammatory, antibacterial, antioxidant, and anti-diabetic actions. The phytochemical profile and in vitro anti-inflammatory properties of the ethanolic extract of the stem of Pedalium murex (L.) are the main subjects of this investigation. After being gathered and verified, the plant was extracted using ethanol. Alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids, and steroids were detected by preliminary phytochemical screening. The protein denaturation method, which was used to assess the anti-inflammatory efficacy, showed dose-dependent suppression of albumin denaturation, indicating strong anti-inflammatory potential. The results demonstrate Pedalium murex's medicinal benefits.

Keywords: *Pedalium murex* (L.), Herbal medicine, Anti-inflammatory activity, Protein denaturation, Phytochemical screening, Ethanolic extract, Traditional medicine, Medicinal plants, Inflammation.

INTRODUCTION

HERBAL MEDICINES:

Herbal medicines which formed the basis of health care throughout the world since the earliest days of mankind are still widely used, and have considerable importance in international trade. Recognition of their clinical, pharmaceutical and economic value is still growing, although this varies widely between countries.

Medicinal plants are important for pharmacological research and drug development, not only when plant constitute are used directly as therapeutic agent, but also as starting material for the synthesis of drug or as models for pharmacologically active compound. Regulation of exploitation is therefore essential together with international cooperation and coordination for their conservation so as to ensure their availability for the future.

The United Nations convention on biological diversity states that the conservation and sustained use of biological diversity is of critical importance for meeting the food, health and other needs of the growing world population, for which purpose access to and hearing both genetic resources and technologies are essential.

Legislative control in respect of medicinal plants have not evolved around a structured control model. There are different ways in which countries define medicinal plants or herbs or products derived form them and countries have adopted various approaches to licensing, dispensing, manufacturing and trading to ensure their safety, quality and efficacy.

Despite the use of herbal medicines over many centuries, only a relatively small number of plant species has been studied for possible medical application. Safety and efficacy data are available for an even smaller number of plants, their extracts and active ingredients and preparation containing them.

INFLAMMATION:

Plants have the ability to synthesized a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi herbivorous mammals. At least 12000phvto compounds have been

isolated so far; a number estimated to be less than 10% of the total. The Chemical compounds in plants mediate their effect on the human boby through process identical to those already well understood for the chemical compounds in conventional drugs. Herbal medicines to have beneficial pharmacology activities, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effect. [1,2]

Inflammation is a body response to injury, pain, swelling and disturbed physiological functions. It is triggered by the release of chemical mediators from injured tissue and migrating cells[3].Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration.

Protein denaturation is a process in which protein lose their tertiary structure by application of external stress or compounds, such as strong acid or base, a concentrated inorganic salt, an oraganic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of protein is a well-documented cause of inflammation[4,5].

Inflammation can be classified as either acute or chronic. Acute inflammation is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes.

Chronic inflammation is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis. Inflammation is a common clinical conditions and rheumatoid arthritis(RA) is a chronic debilitating autoimmune disorder[6].Medicinal plants are believed to be an important source ofnew chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti- inflammatory drugs. Inflammation may be potentially harmful, causing life threatening hypersensitivity reactions and progressive organ damage[7]. NSAIDs are reported to possess prevention of the denaturation of proteins, which act as antigens and leads to auto-immune diseases[8].

Pedalium murex (L.) is a small herb distributed in tropical Africa, Ceylon, India and Mexico[9].P.murex belongs to the family Pedaliaceae, is distributed in the coastal areas of southern India[10]. P.murexd emulcent and diuretic also used for the treatment of disorders of urinary systems such as gonorrhea, dysuria incontinence of urine, etc.[11,12].Pharmacognostical study of the leaves of the plant was reported[13]. It contains alkaloids, a greenish fatty oil, small amount of resin and ash. Fruit contains a mucilaginous alkaloid, fat, resin, and gum. Caffeic acid, cumariaciddaucosterol, acid, heptatriacontonic acid[14], vanillic acid[15], ursolic acid and sitosterol were isolated from this plant. Flavonoids, triterpenoids, steroids, lipids, fatty acids, phenolic acid, amino acids and carbohydrates of Pedalium murex were reported(16). Pharmacological activities of plant possess anti-bacterial(17), anti-microbial(18), anti-oxidant(19), aphrodisiac(20),anti-hyperlipidaemic(21),nephroprotective(22),antiinflammatory(23), anti-ulcer(24) and anti-diabetic(25) studies. Hence an attempt was made to evaluate the phytochemical properties and in vitro anti-inflammatory activities ofp. murex.

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. when cells in the body are damage by microbes, physical agent or chemical agent, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal system of defense, the response of a tissue to an accidental cut is similar to the response that result from other types of tissue damage caused burns due to heat radiation bacterial or viral invasion.

When tissue cell become injured they release kinins, prostroglandins and histamine. This work collectively to causes increased vasodilation (widening of blood capillaries) and permeability of the capillaries. This least to increase the blood flow to the injured site. These substance also act as chemical messengers that attract some the bodies natural defense cells a mechanism known as chemotaxis. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues.

A cascade of biochemical events propagates and matured the inflammation response involving the local vascular system, the immune system and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Several experimental protocols of inflammation are used for evaluating the potency of drugs. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants.

Inflammation process has two phases: Acute and chronic. Acute and chronic inflammation are known to be complicated processes

induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. Anti-inflammatory agents exert effect through a spectrum of different modes of action (samuelsson et al., 1978).

The acute inflammation response is characterized by an increase in vascular permeability and cellular infiltration leading to edema formation as a result of extravasations of fluid and proteins and the accumulation of leukocytes at the inflammatory site for a short time (posadas et al., 2004).

Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro- inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by mean of development of proliferative cells, which can either spread or form granuloma. Chronic inflammation may also occur due to the persistence of infection or antigen, recurring tissue injury, or a failure of endogenous anti- inflammation mechanisms.

Chronic inflammation is a multiple process medicated by activating inflammatory or immune cells (Lundberg, 2000), among which macrophages play a central role in managing many different immune pathological phenomena, including the overproduction of proinflammatory cytokines and inflammatory mediators, generated by activating and COX-2 (Walsh, 2003).

Under inflammation condition, immune cells are also stimulated by adhesion molecule activation signals in order to enhance the migration capacity to inflamed tissue and finally to form heterotypic cell clustering between the immune cells, endothelial cells and inflamed cells (Tao et al., 2009). Macrophages in the inflammatory reaction initially require of intracellular signalling events mediated by enzymes such as phosphoinositide 3-kinases (PI3K)and mitogen activated protein kinases (MAPKs)as well as transcription factors (e.g., nuclear factor [NF]-eB and activators protein [AP]-1(Sekine et al., 2006). Overall, these events lead macrophages to express pro-inflammatory genes such as inducible NO synthase (iNOS) and cyclooxygenase (COX)-2 (Burmester et al., 1997; Bresnihan, 1999). Because large amount of macrophage-derived inflammatory mediators can cause collateral or severe damage such as septic shock, rheumatoid arthritis and effective blockade of these inflammatory response is an important therapeutic target. Inflammatory diseases are a major cause of morbidity of the workforce throughout the world.

These have been called the “king of Human Miseries” (Chatterjee and pal, 1984). Pain is an objectionable sensory and emotional incident associated with actual or potential tissue inflammation. Pyrexia or fever is caused as a secondary impact of inflammation (khan et al.,2007). Inflammation, pain and fever are all associated with enhanced production of prostaglandins (Rang et al.,2003). Thus, most anti inflammation agents are expected to possess analgesics and antipyretic activity (Tripathi, et al.,2001; Dewanjee et al.,2009).

Analgesia:

Pain is an unpleasant subjective experience that is the net effect of a complex interaction of the ascending and descending nervous system involving biochemical, physiological, psychological, and neocortical processes (Chisholm-Burns et al.,2008). Pain can affect all area of a person’s life, including sleep, thought, emotion, and daily activities. There are several ways tob classify pain, but the first distinction usually made is that between acute and chronic pain. Pain is a subjective sensation which cannot be measured objectively, and its intensity is not always a direct reflection of the nociceptive inputs provoking it. Nociceptive inputs which are easily ignore by an individual in one situation may be unbearable in another (Buschmann, et al.,2002). The management and treatment of pain is probably one of the most common and yet difficult aspects of medical practice. Analgesic therapy is domain by two major classes of analgesic drugs; viz opioids and nonsteroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as gastrointestinal disturbance, renal damage (with NSAIDs drugs), etc. (Dahl and Reader, 2000; Bures et al.,2002)**.**

Inflammatory diseases:

Inflammation is a physiological response of a body to stimuli, including inflection and tissue injury. However, excessive or persistent inflammation causes a variety of pathological condition (palladino et al., 2003; kang et al., 2008). As the primary interface between the body and the external environment, the skin provides the first line of defense against traumatic injury and invasion by microbial pathogens. In addition to its properties as a physical barrier, the skin has many active defence mechanisms (kupper and fuhlbrigge, 2004) and immune activity is implicated in the pathogenesis of a large variety of inflammation skin disorders. While some of these conditions are easily remedied, treatments for chronic inflammation diseases such as psoriasis and atopic dermatitis are not 100%sucessful (chi et al.,2003). High levels of inflammatory cytokines and reactive oxygen species are proposed to contribute to the pathophysiological mechanisms associate with various inflammatory skin disorders (Trouba et al.,2002).

Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, and inflammatory bowel disease are often associated with inflammatory processes (Polya, et al., 2003; Iwalewa et al.,2007). Furthermore, oxidative and inflammatory processes are among the pathological features associated with the central nervous system in Alzheimer’s disease (AD) (Howes and Houghton, 2003).

Rheumatoid arthritis (RA) and osteoarthritis (OA)are frequent and important diseases with complex pathophysiology. There is convincing evidence that cytokines (e.g., IL-1 and TNF), prostaglandins (PG), and perpetuation of inflammation and cartilage and meniscus damage in rheumatoid arthritis and osteoarthritis. Obese individuals have high circulation levels of a range of inflammatory markers produced by adipose tissue, including TNF-a,interleukin- 1(IL-1), and IL-6(Bullo-Bonet et al., 1999; Yudkin et al., 2000). These factors, whose levels can be reduced by weight loss, are likely to contribute to vascular damage in obese individuals. Since its discovery in the early 1990s, COX-2 has emerged as a major factor in inflammatory reaction in peripheral tissues (Hinz and Brune, 2002). By extension, the COX-2 expression in the brain has been associated with pro- inflammatory activities, which are thought to be instrumental in the neurodegenerative processes occurring in acute and chronic diseases.

Many malignancies arise in the areas of infection and inflammation Ebert et al., 2002;Martinez-Maza and Breen, 2002). There is a growing body of evidences that chronic inflammation is strongly associated with incidence of cancer. For example, colon cancer can arise from inflammatory bowel disease such as chronic ulcerative colitis and Crohn's disease persistent more than 10 years.

Standard drugs for inflammation and side effects:

Many steroids, specifically glucocorticoids and Mineralocorticoids reduce inflammation or swelling by binding to corticoid receptors. These drugs are often referred to as corticosteroids. Long-term corticosteroids use has several severe side effectseg. Hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, anxiety effects etc. (Donihi et al.,2006).

There are over 50 different NSAIDS available (Chiroli et al.,2003) and they can be divided into different groups based on their chemical structure, pharmacokinetics and selectively towards Cox-1 or Cox-2 (FitzGerald and patrono, 2001; Bancos et al., 2009). NSAIDs can be classified (Paul et al., 2004) broadly into two types based on their chemical structure. Most NSAIDs are carboxylic acids; but a few, most noticeably phenylbutazones, are enolic acids. Carboxylic acid containing drugs includes salicylate derivatives (eg.Asprin), carbocyclic and hetrocyclic acid derivatives (eg.indomethacin)**,** fenamic acid derivatives (eg. Ibuprofen, ketoprofen, fenbufen, flurbiprofen**,** suprofen and naproxen)and phenyl acetic acid derivatives (eg. Diclofenac aceclofenac, etc) enolic acid containing drugs includes oxicam derivatives (eg. Piroxicam, tenoxicam and meloxicam) and pyrazoles (egphenylbutazone and oxyphenbutazone). Non acidic group compound include nabumenton (Derle,etal.,2006).

Most of the NSAIDS have three types of action (vane, et al.,1998):

Anti-inflammatory action for treating several conditions, including rheumatoid arthritis, osteoarthritis, musculoskeletal disorder and pericarditis.

Analgesia for treating pain of mild to moderate intensity. Their maximum therapeutic efficiency is much lower than that of the opioids, but they do not cause dependence.

Antipyretic action, which is mediated by the release of endogenous pyrogen from monocytes and macrophages in the presence of infection or inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDS) typically relieve inflammation and associated pain by inhibiting cyclooxygenase enzymes involved in the production of prostaglandins. These enzymes exist in two isoforms (COX-1 and COX-2) coded by distinct genes on different chromosomes (polya, et al., 2003).NSAIDS can cause liver damage (Purcell et al., 1991). Renal failure forced et al., 2001), aseptic meningitis (Nguyen and Juurlink, 2004) and can interfere with bone fracture healing (wheeler and batt, 2005). NSAIDS use is associated with a high risk of upper gastrointestinal symptoms and lesions such as oesophagitis, gastritis, peptic ulcers, and their severe complications including bleeding and perforation (cryer and kimmey,1998) and results mostly from inhibition of cox-1 in the gastric mucosa.

Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandin synthesis and/or production (Todd and Sorkin, 1988; skoutakis et al., 1988). The drug also affects polymorphonuclear leukocyte function in vitro, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation, and neutral protease production (Mahgoub,et al., 2002). Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (Al- Tuwaijri and Mustafa, 1992). However, it may cause side effects, including gastrointestinal disorders when administered by the oral route and cutaneous lesions by intramuscular injection (Lopes et al., 2006; suwalsky et al., 2009). There are several published reports of cases of diclofenac associated hepatotoxicity (Purcell et al., 1991; Aydin et al., 2003).

Indomethacin is used in the treatment of disorder such as rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. Indomethacin produces its therapeutic and toxic effect by inhibiting prostaglandin synthesis in various tissues (Lione and scialli, 1995). However, long time use of indomethacin causes gastrointestinal complications, Including intestinal perforation (cassadyet al., 1989); bronchopulmonary dysplasia and respiratory distress syndrome (Eronen et al., 1994).

Aspirin is the most widely used drugs in the world today, because of its ability to act as anti-inflammatory medicine (Serhan et al., 2004; Schwab et al., 2007). However, patient with a history of peptic ulcer or other gastrointestinal disorders, are prone to

gastroduodenal lesions on prolonged use of aspirin. The toxicity of aspirin is both dose- and disease-dependent.

Ibuprofen is also a commonly and successful used NSAIDs. However, long term use of ibuprofen, sulindac, phenylbutazone, and piroxicam has been associated with hepatotoxicity (Manoukian and carson, 1996). The coxibs likerofecoxib, lumiracoxib and etoricoxib were reported to be associated with reduced gastrointestinal toxicity from the upper gastrointestinal tract when compared to non- selective NSAIDs; however, there are also reports that coxibsare associated with serious cardiovascular events (pilotto et al., 2009) and hepatotoxicity (Alegria et al., 2002). Based on all these findings, the US Food and Drug Administration (FDA) in 2005 mandated that all NSAIDs should cardiovascular events, along with warming about potential severe life- threatening gastrointestinal event. The same has been delivered by the European Medicines Agency (EMEA)as well as by a large numberof national drug agencies all over the world. Thus, a careful evaluation of the risk profiles for adverse events before prescribing non-selective NSAIDs and coxibs is strongly recommended(Layton et al., 2008).

Inflammatory mediators:

The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger. Macrophages play major roles in the immune and inflammatory response involved in host defence. Activated macrophages secrete a number of different inflammatory mediators, including tumour necrosis factor-a (TNF-a), interleukin-1a(IL-1a), interleukin-6 (IL-6), reactive oxygen species (ROS), prostaglandin E2 (PGE2), NITRIC OXIDE (NO), etc, (Kaplan ski et al., 2003; Bosca, et al., 2005).

Cyclooxygenase (COX):

COX is the key enzyme that catalyses the first two steps in the biosynthesis of the prostaglandins (PGs). The COX pathway leads to the generation of the prostaglandins and thromboxane, which mediate the pain and edema associated with inflammation. There are two isoforms of COX; COX-1 and COX-2, COX-1 is detectable, but COX-2 is not detectable in most normal tissues, however, COX-2 can be induced by many factors such as pro-inflammatory cytokines, phlogistic factors, etc. studies indicate that COX-2 plays an important role in inflammation (Oshima et al., 1996; shu et al., 2006). Thus, those agents that could suppress the activity or protein expression of COX-2are likely to be a valuable medicine for anti-inflammation and pain ease. Thus, decreasing of synthesis and activity of COX-2 can result in anti- inflammatory action both in localized and systemic condition (salveminietal., 1993).

Prostaglandins:

Prostaglandins (PGs) are generated by a variety of cell types, including activated macrophages (Harris et al., 2002). The rate-limiting enzyme in PG synthesis is cyclooxygenase (COX). Prostaglandins are the end products of the metabolism of arachidonic acid by cyclooxygenase (COX) and prostaglandin synthesis (PGS), and comprise a series of classical pro-inflammatory mediators like PGD2, PGE2, PGF2a, and PGI2.

Arachidonic acid:

The lipoxygenase pathway utilizes arachidonic acid by 5 lipoxygenases to produce the lipoxygenase product e.g. leukotrienes (LTs) which are also involve in inflammatory reactions as pro inflammatory mediators. Leukotrienes, i.e.LTC4 and LTD4 cause edema together with increased microvascular permeability.

Thromboxane:

Thromboxane A2 (TXA2) is an arachidonic acid metabolite produced during the catalysis of arachidonic acid by the sequential action of COX and thromboxane synthase (TXS), and is well established as a potent vasoconstrictor. This metabolite participate in various physiological and pathological process ranging from synaptic transmission to inflammation (Turini and DuBois, 2002). Platelets represent the best know cell type to produce TXA2 in response to various stimuli. However, many other cells and tissues are also able to synthesize TXA2(Nakahata, et al., 2008).

Leukotrienes:

Leukotrienes (LT) are end products of the metabolism of arachidonic acid by 5-lipoxygenase. Leukotrienes have physiological roles in innate immune response and pathological roles in a variety of inflammatory and allergic diseases, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, allergic rhinitis, but most prominently in bronchial asthma (Werz and steinhilber, 2005).

Polyunsaturated fatty acid (PUFA):

Linoleic acid (LA) and a-linolenic acid (ALA) belong to them. 6 and n.3 series of polyunsaturated fatty acid (PUFA). LA and ALA are precursors for the synthesis of higher unsaturated species: arachidonic acid deriving from LA, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) deriving from ALA. One possible mechanistic explanation for these anti- inflammatory and antitumorigenic effects may be that an increased consumption of EPA and DHA result in an increased incorporation of these fatty acid into phospholipids at the expense of arachidonic acid. Consequently, they also replace arachidonic acid as a substrate for COX and LT that result in a reduced formation of PGE2,TXA2, LTB4 and LTE4 (Huwiler and pfeilschifter, 2009).

Histamine:

Histamine (HA) is a biogenic amine that affects a variety of functions in the human body. It has been known to play a role in inflammation, gastric caid secretion, and neurotransmission (Passani et al., 2007; Huang and Thurmond, 2008). Multiple receptors exist for histamine in mammalian tissues and these have been classified into 4 distinct receptor types (H1R, H2R, H3R, and H4R), all of which are G-protein coupled receptors (GPCRs) (schneider et al., 2002). Histamine appears to play a complex role in pain modulation. Histamine released from mast cells is an established mediator of inflammation, increase vascular permeability (such as seen with acetic acid)causes an immediate, sustained reaction that is prolonged over 24h (Okoli et al., 2007).

Nitric oxide (NO):

It is widely known that nitric oxide (NO), synthesized from L arginine by nitric oxide synthase (NOS), is involved in diverse physiological processes. An excess in NO production is largely thought of as causing a variety of inflammatory diseases, such as sepsis, psoriasis, arthritis, multiple sclerosis, and systemic lupus erythromatosus (Clancy et al., 1998).

Indian system of medicine:

Ayurveda, meaning the "science of life," is said to be oldest and most complete medical system in the world and dates back to 5000B.C. There is no denying the benefits of Ayurvedic treatments that several Indians and others across the globe have experienced. The diagnostic and treatment procedures used are unique and are still valid today as are its foundational principles of panchamahabhutha (five basic elements of nature), tridosha (three humours) and prakrithi (individual constitution)(Venkatasubramanian et al.,2007).

Ayurveda has vast literature in Sanskrit and various Indian languages, covering various aspects of diseases, therapeutics, and pharmacy (Dev et al.,1999). The original source of Ayurveda is the vedas and the texts known as the samhitas, which are treaties on health care and describe medical procedures, including surgery and a form of massage of vital energy points (Ebadi et al., 2007). The earliest references to such plants are found in the Rig vedaand the Atharva veda, dating back to the second millennium B.C.

The first recorded treatise fully devoted to the concepts and practice of Ayurveda; its primary focus was therapeutics (charaka Samhita et al., 1949sharma et at., 1981).This text sets out all the fundamental principles of Ayurveda but concentrates most of its attention on digestion (described as internal fire, or Agni). Another early classic, the susruta Samhita, focuses on surgical techniques (Majumdar et at., 1971,Krishnamurthy et at., 1991).

The Astanga Hridayam written in about 500 A.D. said about most of the detailed principles of ayurveda, including the dosha and subdosha(grade et al.,1954 sharma et al., 1979). The Madhava Nidana (800-900A.D) was the next important milestone and it is most famous ayurvedic work on the diagnosis of diseases. As per Ayurveda, every material(Dravya) is a manifestation of five elements (earth, water, fire, air and space) in different proportion. The material could be living as well as nonliving things. Depending on the predominant combination of the elements, nature can be categorized into three doshas, namely vata, pitta,and kapha.

Vata: Linked to the wind the force that controls movements and the functioning of the nervous system in the body.

Pitta: The force of heat and energy, linked with the sun, that controls digestion and all biochemical processes in the body.

Kapha: The force of water influenced by the moon, the stabilizing influence that controls fluid metabolism in the body.

When balanced these three forces ensure that the body is healthy, but when they are "abnormal" or unbalanced, disease follows(Thomas et al., 1997). India has a rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing systems of treatment i.e. Ayurveda and unani systems since ancient times(suranta et al ., 2008).

Role of WHO in herbal medicine :

To traditional healthy system (including herbal medicine) as holistic that of viewing man in his totality within a wide ecological spectrum and emphasizing the view that ill health or diseases is brought about by an imbalance or disequilibrium of man in his total ecological system and not only by the causative agent and pathogenic evolution system WHO probably implying that the indigenous system drugs (including herbal medicine) restore the imbalance leading to the cure ill health or diseases. However it helped the inclusion of proven traditional remedies in national drug policies and regulatory approval by developing countries. In 1991 WHO developed guidelines for the assessment of herbal medicine, and the 6th international conference of Drug Regulatory Authorities held in Ottawa in the same year ratified the same.

The salient features of WHO guidelines are:

- **Quality assessment:** crude plant materials or extract plant preparation and finalized product.
- **Stability:** self life.
- **Safety assessment:** Documentation of safety based on experience and toxicological studies.
- **Assessment of efficacy:** Documented evidence of traditional use and activity determination (Animal and Human).

Advantages:

- Herbal medicine has a long history of use and better patient tolerance as well as acceptance.
- Medicine plants have a renewable source, which is only hope foe sustainable supplies of cheaper medicines for the growing world population.
- The cultivation and processing of medicinal herbs and herbal products is environment friendly.
- Availability of medicine plants is not a problem, especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity.
- Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
- Sometimes herbal medicines prove more effective than the traditional prescribed medicines.
- The herbal medicine is costly. Herbs cost is much less when compared to the prescription medications.
- The herbal medicine is the non existence of side effects. Also, they tend to offer long lasting benefits in terms of overall wellness.

Disadvantages:

- The quality of herbal products may vary between batches, brands or manufacture. This is much more difficult to prescribe the proper dose of an herbal medicine.
- Herbal medicines have the potential to cure many ailments the curing period is usually longer duration when comparing to the conventional medication.
- Herbal medicines can cause allergic reactions in some cases. Before resorting to herbal medication you need to ensure that you are not allergic to the particular herb that going to consume.
- There are many herbal remedies and medications that cause high blood pressure in the vessels of the lungs.

The main objects to carry out the research in Indian medicinal plants:

Plants have provided a large variety of potent drugs to alleviate suffering from diseases to mankind. In spite of spectacular advances in synthetic drugs in recent years, some of the drugs of plant origin have still retained their importance. The use of plant based drugs of the western world is increasing and thus is because of the belief that many medicines are known to be free from side effect.

The recent development of natural drugs :

A total of 122 biologically active compounds has been identified, derived only form 94 species of plants. A conservative estimate of the number of flowering plants occurring on the planet is 2,50,000. Of these, only about 6% have been screened for biologically activity and a reported 15% have been evaluated only phytochemically. Consistent findings should be carried out to discover a probable abundance of medicinal extracts in these plants (Turker and Usta 2008).

The use of traditional medicine plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO 1996). Furthermore an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutic from these plants as well as form traditionally used rural herbal remedies(UNESCO1998).

The pharmaceutical Research and Development Committee, Ministry of Chemicals, Government of India also underscores the importance of traditional knowledge (Mashelkar et al.,1999). The increasing use of traditional therapies demands more scientifically sound evidence for the principle behind such therapies and for effectiveness of medicines. Recent advances in the analytical and biological sciences, along with innovations in genomics and proteomics can play an importance role in validation of these therapies. Western scientific community views traditional medicines cautiously and stresses the concerns related to research, development and quality (Patwardhan et al., 2003: fabricant and Farnsworth et al., 2001).

A large proportion of such medicinal compound have been discovered with the aid of ethnobotanical knowledge of their traditional uses. The rich knowledge base of countries like Indian and china in medicinal plantsand healthcare has led to the keen interest by pharmaceutical companies to use this knowledge as a resource of research and development programs in the pursuit of discovering novel drugs (krishnaraju et al., 2005).

The rapid pace of research and development in herbal medicine has made it an interdisciplinary science. If any scientific monography of a medicinal plant is seen, it can be concluded that knowledge of alternative and complementary systems of medicines like Ayurveda, botany, pharmacognosy and phytochemistry, biochemistry, ethnopharmacology and toxicology is an integral part of herbal medicine. There has been an explosive growth of the herbal drug industry recently.

Data analysis has shown that more and more people are consulting the herbal medicine practitioners. The WHO has also identified the importance of herbal medicines. According to a study from the U.S.,60-70% patients living in rural areas are dependent on herbal medicine for their day to day diseases. Several authors have reported favorable result with herbal drugs(mostly in the form of extracts) either in animal or in human studies (padma et al., 2005.

LITERATURE REVIEW

R Manikandan, et el., (2025) was conducted study about the morphological analysis cobalt oxide nanoparticle (Co3O4NPs) mediated by leaves extract of Pedalium murex L and its Antibacterial activity, Antifungal, Antioxidant and Anticancer (MCF-7 cell line) study. The study investigates the eco-friendly synthesis, characterization, and biomedical applications of cobalt oxide nanoparticles (Co_3O_4NPs) synthesized using Pedalium murex L. leaf extract. The nanoparticles were characterized using various spectrometric techniques, such as UV, IR, and fluorescence spectroscopy, as well as X-ray diffraction, DLS, SEM with EDS, and TEM for morphological analysis. The synthesized Co_3O_4NPs demonstrated significant antibacterial and antifungal activity against pathogens like Staphylococcus aureus, E. coli, P. aeruginosa, B. cereus, Candida albicans, and Trichoderma viride. Additionally, their antioxidant and anticancer activities were evaluated using DPPH and MCF-7 cell line assays.

Rao, A. S., et al., (2024) was conducted a study about review on ethnomedicine, phytochemistry, pharmacology, and toxicology of Pedalium murex L. Journal of Herbal Medicine. Pedalium murex L. (Gokhru) is a medicinal succulent plant known for its diverse therapeutic properties across various traditional systems. This review highlights key research on its ethnomedicinal uses, phytochemical composition, pharmacological benefits, and toxicological safety. It emphasizes the plant's potential in treating conditions like kidney stones, inflammation, and diabetes, while also noting the need for further studies on its safety and efficacy.

Rathore,s., et el.,(2024) was conducted study about evaluation of medicinal values of Indian plants Pedalium murex. Pedalium

murex, a member of the Pedaliaceae family, is traditionally used for various medicinal purposes such as enhancing appetite, purifying blood, and treating conditions like cough, asthma, skin diseases, anti-inflammatory and heart problems. While once abundant, its population is declining. Phytochemical analysis of its flowers revealed the presence of tannins and flavonoids, highlighting their potential for pharmacological applications. This study encourages further exploration of the plant's flowers for future drug formulations.

A Arumugama, et el., (2024) was conducted study about the formulation and evaluation of liposomal encapsulated pedalitin cream cookies from Pedalium murex. The study focuses on the eco-friendly synthesis of cobalt oxide nanoparticles (Co3O4NPs) using Pedalium murex L. leaf extract as a stabilizing and reducing agent. The synthesized nanoparticles were characterized using UV, FTIR, and fluorescence spectrometry, along with X-ray diffraction, dynamic light scattering, SEM, EDS, and TEM for their size and morphology. The Co3O4NPs exhibited notable antibacterial and antifungal activities against various microorganisms, including Staphylococcus aureus and Candida albicans. Additionally, antioxidant and anticancer activities were evaluated, showing promise for biomedical applications. This greener synthesis method reduces hazardous chemicals and solvents.

Subbulakshmi Madasamy, et el., (2023) was conducted study about the bio fabricated of nickel oxide nanoparticle form Pedalium murex leaf extract: A promising approach for biomedical and environment application. This study focuses on the green biosynthesis of nickel oxide nanoparticles (NiO NPs) using methanolic leaf extract of Pedalium murex (MEPM) at room temperature. The synthesized NiO NPs were characterized by UV-Vis, XRD, FT-IR, and SEM. The antioxidant and antibacterial properties of MEPM and NiO NPs were evaluated, with the NiO NPs showing superior antioxidant activity (68%) compared to MEPM. NiO NPs also exhibited stronger antimicrobial effects against various pathogens, including E. coli and Klebsiella pneumoniae. Additionally, the NPs demonstrated high catalytic efficiency in dye degradation.

Overall, the study suggests that MEPM-mediated NiO NPs are a promising, eco-friendly, and cost-effective approach for various biomedical applications.

Vennila Devi Kannan, et el., (2023) was conducted study about the ecofriendly bio-synthesis and spectral characterization of copper nanoparticles using fruit extract of Pedalium murex L in vitro evaluation of antimicrobial, antioxidant and anticancer activities on human lung cancer A549 cell line. This study investigates the synthesis of copper nanoparticles (CuNPs) using Pedalium murex L. fruit juice and characterizes them via SEM, FTIR, XRD, and UV-Vis methods. The CuNPs were spherical, with sizes ranging from 20 to 50 nm. FTIR analysis revealed that biomolecules in the fruit juice acted as capping and reducing agents. The study also evaluated the fruit extract's antioxidant activity and found that CuNPs exhibited dose-dependent cytotoxicity and promoted apoptosis in A549 lung cancer cells. These results suggest that CuNPs synthesized from Pedalium murex L. fruit juice possess anti-cancer properties, making them potential candidates for lung cancer treatment.

Suresh velayudham, et el., (2023) was conducted study about the Neuroprotective effect of ethanolic extract of Pedalium murex linn leaf in 3- nitropropionic acid induced neurodegeneration. This study explores the effects of ethanolic extract of Pedalium murex Linn leaf (EEPML) on neurodegeneration induced by 3-Nitropropionic acid (3-NPA) in a Sprague Dawley rat model. Rats were treated with EEPML (200 and 400 mg/kg) for 14 days, and 3-NPA (10 mg/kg) was administered on day 14. The study assessed anticholinesterase activity, DPPH radical scavenging, behavioral tests (rotarod, open field), acetylcholine content, acetylcholinesterase (AChE) activity, and superoxide dismutase (SOD) levels. EEPML reduced AChE activity, improved memory, learning, and motor coordination, and restored acetylcholine levels in neurodegenerated rats, suggesting its potential antioxidant and cholinesterase inhibitory effects.

Palanisamy jeyakumar,et el., (2023) was conducted study about the diet induced animal model anti-obesity, phytochemical profiling, and in silico analysis of culinary plant gokhru (Pedalium murexL.) mucilage . This study explores the anti-obesity effects of Pedalium murex L. mucilage (PMM) in high-fat diet-induced obese rats. The research includes HR-LCMS phytochemical profiling and in silico evaluations, identifying compounds like 7(14)-Bisabolene-2, 3, 10,11tetrol, Moschamine, and N-Feruloyltyramine with potential anti-obesity activity. In vivo, rats fed PMM showed significantly reduced serum levels of total cholesterol, LDL, and triglycerides, along with increased HDL levels.

Anju verma (2022) was conducted study about [3:46 PM, 2/12/2025] Rubanraj: A Comprehensive Review: Development on Phyto-pharmacological Studies of Pedalium murex Linn. This review summarizes the research on Pedalium murex Linn., focusing on its cytomorphology, traditional uses, pharmacognostic and phytochemical studies, and biological activities. As the demand for herbal medicines increases, Pedalium murex offers a wealth of bioactive compounds like terpenoids, flavonoids, and saponins, with a wide range of therapeutic effects, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic properties. This plant, found in tropical regions such as Africa, Sri Lanka, India, and Mexico, shows promise as an effective herbal medicine with fewer side effects.

M Narayanan.,et el.,(2022) was conducted study about the pharmaceutical potential of crude ethanol extract of Pedalium murex(L.) This study assessed the antibacterial, anti-inflammatory, antioxidant, and cytotoxic properties of various solvent leaf extracts of

Pedalium murex. The ethanol extract exhibited the highest phytochemical content, including saponins, flavonoids, and alkaloids, and showed significant antibacterial activity against skin infection-causing bacteria like Staphylococcus aureus, Streptococcus pyogenes, and Corynebacterium sp. The ethanol extract also demonstrated excellent antioxidant activity and supported the proliferation of HEK 293 cells without significant cytotoxicity. These results suggest that ethanol extract components could be used in drug formulations for skin infections with minimal side effects.

Tyagi, Petal.,(2021)was conducted a study about review Comparative study of the pharmacological, phytochemical and biotechnological aspects of Tribulus terrestris Linn. and Pedalium murex Linn.Tribulus terrestris L. and Pedalium murex L. are key medicinal herbs used in Ayurvedic treatment, known for their bioactive compounds like steroidal saponins, flavonoids, alkaloids, and carbohydrates. These compounds, isolated using techniques such as HPLC, UPLC, and GC–MS, contribute to the herbs' therapeutic properties, including diuretic, antibacterial, antifungal, anti-inflammatory, and anticancer activities. While both herbs have been studied for their pharmacological benefits, research on Plant Growth Promoting Rhizobacteria (PGPR), molecular work, and biotechnological applications, especially for P. murex, remains limited. This review highlights the need for further exploration of these herbs in clinical, pharmacological, and biotechnological fields.

Choudhary, S et al., (2021)was conducted study about Medicinal Importance of Chota Gokhru and Bada Gokhru in Ayurveda and Modern Science. Tribulus terrestris (laghugokshuru) and Pedalium murex (brihatgokshuru) are two well-known medicinal plants in Ayurveda, both recognized for treating urinary disorders and kidney-related issues. While the fruits of T. terrestris are slightly astringent and primarily used for urinogenital and kidney disorders, P. murex has a sweeter taste and is commonly used as an aphrodisiac, anti-inflammatory.

Renuka Mahajan,et el., (2021) was conducted study about the phytochemical analysis and standardization of Pedalium murex linn. extract through HPLC methods. This study aimed to screen phytochemicals and standardize the extract of Pedalium murex fruits using HPLC. The aqueous ethanolic extract was obtained via maceration and analyzed for total phenolic and flavonoid content. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, and other compounds. The total phenolic content was 27.1±0.72 mg/g, and flavonoid content was 17.6±0.79 mg/g. HPLC analysis confirmed the presence of quercetin in the extract and fractions. The study established the phytochemical profile and validated quercetin's presence, contributing to standardization methods for Pedalium murex extract.

Leeba balan, et el., (2021) was conducted study about the synthesis of silver nanoparticle form pedalium murex L. and its Antiproliferative activity against breast cancer (MCF-7) cells. This study reports the low-cost synthesis of silver nanoparticles (AgNPs) using the leaf extract of Pedalium murex, which offers an eco-friendly alternative to conventional methods. Characterization via UV-Visible spectroscopy, scanning, and transmission electron microscopy confirmed the formation of AgNPs with a surface resonance peak at 425 nm and an average size of 11 nm. The synthesized nanoparticles demonstrated strong antimicrobial activity at 12 µg/ml and showed dose-dependent cytotoxic effects against MCF-7 breast cancer cells, with an IC50 of 65.60 µg/mL. These findings suggest that Pedalium murex-derived AgNPs have promising biomedical applications, particularly for cancer treatment.

AIM AND SCOPE OF THE WORK

The Pedalium murex(L.) with wide range of pharmacological, biological activities and having interesting phytochemical constituents. The plant has been used for a wide range of disease as reflected from collected literature.

The selection of the plant Pedalium murex(L.) was in view of its

- Easy availability
- Therapeutic value and
- Degree of research work that can be done

In this context the present study has been undertaken with the following objectives

- Collection and authentication of plant and the plant parts
- Extraction of dried plant materials

- Preliminary phytochemical screening
- In-vitro anti-inflammatory activity.

PLANT PROFILE

FIGURE: 1

FIGURE: 2

FIGURE:3

FIGURE:4

Botanical information:

Botanical name : Bada gokhru Family : Pedaliaceae **Vernacular Names:**

English : large caltrops

Tamil : yanainerunji

Hindi : Bara gokhuru, kadavagokhru Malayalam: Aananjerinjil, kakkamullu, kattunjerinjil. Odia : badogo, ekuro, gokara, gokshura.

Sanskrit : sthulagokshurah

Kannada : Aane-neggilu

Telugu : yenuga palleru, pedda palleru

Taxonomic classification:

Kingdom : plantae Sub kingdom : plantae

Super divison : spermatophyta. Divison : magnoliophyta

Class : magnoliopsida Superorder : asteridae Order : lamiales

Family : Pedaliaceae

Genus : Pedalium

Species : Pedalium microcarpum, murex burmanni.

Distribution:

It is distributed in tropical Africa, the Indian subcontinent and southeast Asia.

Description:

It is a creeper that is about 2 to 3 feet long having branches spread all over, leaves are in pairs of 5 to 8 is of irregular shape. Flowers are small and yellow coloured. Fruits are round and possess 5 to 12 compartments and each compartment contains a seed. The seed contain aromatic oil. Roots are 4 to 5 inch long, brown in colour and bear a sweet aroma . the plant flowers in early winters followed by fruiting.p.murex L is a succulent herb found near sea coast of south India and some tropical areas of India. It appears during the month of July – September. It grows luxuriously in fertile soils and crop ;land as a weed at temperature of 25-30 degrees.

Chemical constituents:

- Alkaloids: found primarily in the fruits, stem alkaloids contribute to the plants medicinal properties.
- Flavonoids: These compounds are abundant in the leaves, stem and have antioxidant and anti-inflammatory properties.
- Saponins: present in the roots, stem and leaves, saponins are know for their potential health benefits, including anti-cancer and anti-diabetic effects.
- Tannins: Found in the stem and roots, tannins possess astringent properties and can help in wound healing.
- Steroids: These compounds are present in various parts of the plant and are believed to contribute to its aphrodisiac and fertility-enhancing properties.
- Carbohydrates: These provide energy and structural support to the plant.
- Resins: These compounds have various medicinal properties, including anti- inflammatory and antimicrobial effects.

Traditional uses:

- Urinary disorder: used to treat urinary discharge, urinary retention, and vesical calculi.
- Digestive issues: used to treat ulcers, intestinal colic and digestive tonics.
- Reproductive health: used to treat infertility, impotency, and as an aphrodisiac.

- Blood purification: used as a blood purifier.
- Anti-inflammatory: used to treat asthma, heart problems and other inflammatory conditions.
- Anti-microbial: used to treat fevers, wounds, and other infections
- Health tonic: used as a health tonic and appetizer.

MATERIAL AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL :

The aerial parts of Pedalium murex(L) were collected from Anthiyur in the month of December , Erode district , Tamilnadu. India. The plant Material was authenticated by **Dr .P .RADHA ; Reasearch officer (botany) Sci &Vc ; siddha Medicinal Plants Garden / Mettur Dam, Tamilnadu–636401.** And a voucher specimen { P240125254M } was deposited at the, SSM College of pharmacy , Erode (638212) Tamilnadu, India.

EXTRACTION OF PLANTS :

- 20g of plant powder material was soaked with 200ml of ethanol in a sealed container for 3days.
- Then the mixture was filtered through a Whatman no. 1 filter paper.
- Crude extract was obtained by evaporating the solvent in a water bath at low temperature (40-50ºC) and stored in a refrigerator at 4ºC-8ºC.
- Paste from of the extract obtained was subjected to screening test.

MACERATION PLANT EXTRACT

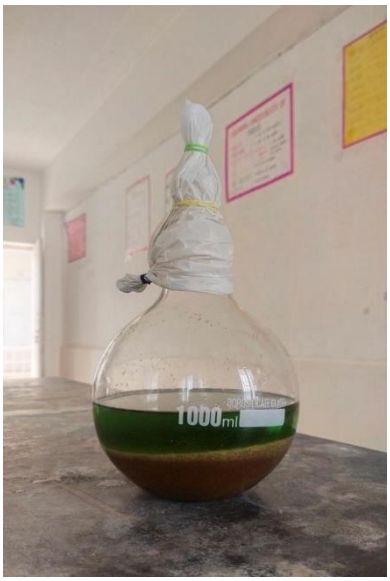

FIGURE:5

PLAN OF WORK

STEP 1:

Selection of Plants

STEP 2:

Collection, authentication and preparation of Pedalium murex L extract.

STEP3:

Extraction of aerial parts of Pedalium murex L. using solvent

STEP4:

Preliminary Phytochemical analysis tests from the extract

STEP 5:

Evaluation of anti- inflammatory activity of the extract using the protein denaturation method.

STEP 6:

Analyze the results and to provide Conclusion

PRELIMINARY PHYTOCHEMICAL INVESTIGATION

The different qualitative chemical tests were performed for establishing profile of extract for their chemical composition. The following tests are performed on extract to detect the various phytoconstituents present in them.

1. Detection of Alkaloids:

Solvent free extract, 50mg were stirred with few ml of dil. Hydrochloric acid and filtered. This filtrate is carefully with various alkaloidal reagents as follows:

A. Mayer's test:

To a few ml of filtrate, a drop or two of Mayer's reagents are added to the sides of the test tube. A white or creamy precipitate indicates the test as positive.

B. Hager's test:

To a few ml of filtrate, 1 or 2 ml of Hager's reagent are added. A prominent yellow precipitate indicates the as positive.

C. Wagner's test:

To a few ml of filtrate, few drops of Wagner's reagent are added by the side of test tube. Reddish- brown precipitate confirms the test as positive.

D. Dragendorff's test:

To a few ml of filtrate 1 or 2 ml of Dragendroff's reagent are added. A prominent brown precipitate indicates the test as positive.

Detection of carbohydrates:

The extract (100mg) is dissolved in 5ml of water and filtered. The filtrate is subjected to the following tests.

A. Molisch's test:

To 2ml of filtrate, two drop of alcoholic solution of α-naphthalene added, the mixture is shaken well and 1ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

B. Fehling's test:

One ml filtrate is boiled on water bath with 1ml each of Fehling's solution A and B. A red precipitates the presence of sugar.

C. Barfoed's test:

To 1ml of filtrate, 1ml of Barfoed's reagent is added and heated on a boiling water bath for 2min. Red precipitate indicates presence of sugar.

D. Benedict's test:

To 0.5ml of filtrate, 0.5ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2min. A characteristic-coloured precipitate indicates the presence of sugar.

Test for Glycosides :

Borntrager's test for Glycosides:

50mg of Extract is hydrolysed with concentrated Hydrochloric acid for 2h on a water bath, filtered. To 2ml of filtered hydrolysis, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

Modified Borntrager's test:

Drug in powdered form is added with ferric chloride and the mixture is heated and filtrated. To the filtrate benzene is added, leads to the formation of two layers. Benzene layer is separated and to the benzene the layer strong ammonia solution is added. The ammonical layer present beneath the benzene layer shows pink to red color.

Legal's test:

50mg of the extract were dissolved in the pyridine, sodium nitroprusside solution is added and made alkaline using 10%sodium hydroxide. Presence of glycoside is indicated by pink colour.

3. Detection of saponins:

The extract (50mg)is dilute with distilled water and made up to 20ml. The suspension is shaken in a graduated cylinder for 15min. A 2cm layer of foam indicates the presence of saponins.

Detection of phenolic compounds and Tannins:

A. Ferric chloride test:

The extract (50mg) is dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution is added. A dark green colour indicates the presence of phenolic compounds.

B. Lead acetate test:

The extract (50mg) is dissolved in distilled water and to this, 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

C. Alkaline reagent test:

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Detection of Flavonoids:

Shinodas Test:

The extracts were dissolved in 5ml of alcohol and few fragment of magnesium ribbon and concentrated hydrochloric acid (drop wise) are added. If any pink or crimson colour develops, presence of flavonoids is confirmed.

Test for Terpenoids:

Salkowski Test:

Mix 5 ml of extract with 2ml of chloroform.

Carefully add 3ml of concentrated sulfuric acid to form a layer.

A reddish-brown coloration of the interface indicates the presence of terpenoids.

IN-VITRO ANTI-INFLAMMATORY ACTIVITY INHIBITION OF PROTEIN DENATURATION

PRINCIPLE:

The denaturation of egg albumin is induced by heat treatment and the absorbance is measured at 660nm… The agents that can prevent protein denaturation and the increments in absorbance of test sample with respect to control indicates stabilization of protein.

MATERIAL REQUIRED:

Acetyl salicylic acid, BSA, 10X PBS

PROCEDURE:

Denaturation of proteins is the main cause of inflammation. Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Sakat*et al*. with slight modification. 500 µL of 1% bovine serum albumin was added to sample(UR) (500, 250, 100, 50 and 10 µg/mL) of test sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control.

The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\text{Percentage inhibition} = (1- B/A) \times 100\%$$

Where B is the absorbance reading of the test sample, and A is the absorbance of control sample.

RESULT AND DISCUSSION PRELIMINARY PHYTOCHEMICAL TEST RESULT:

Preliminary Phytochemical test of the ethanolic extract revealed the Presence and absence of different primary and secondary metabolites.

Aerial parts of Pedalium murex L. were found to contain the presence of alkaloids, flavonoids, phenolic compounds.

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FIGURE 6

TABLE 1: RESULTS OF THE PHYTOCHEMICAL TEST

S.NO	CONSTITUENTS	ETHANOLIC EXTRACT OF Pedalium murex(L.) STEM
1	CARBOHYDRATES	+
2	PHENOLS	+
3	TANNINS	+
4	SAPONINS	+
5	GLYCOSIDES	+
6	TREPENOIDS	+
7	ALKALOIDS	+
8	STEROIDS	+
9	FLAVONOIDS	+
10	ANTHRAQUINONE	_

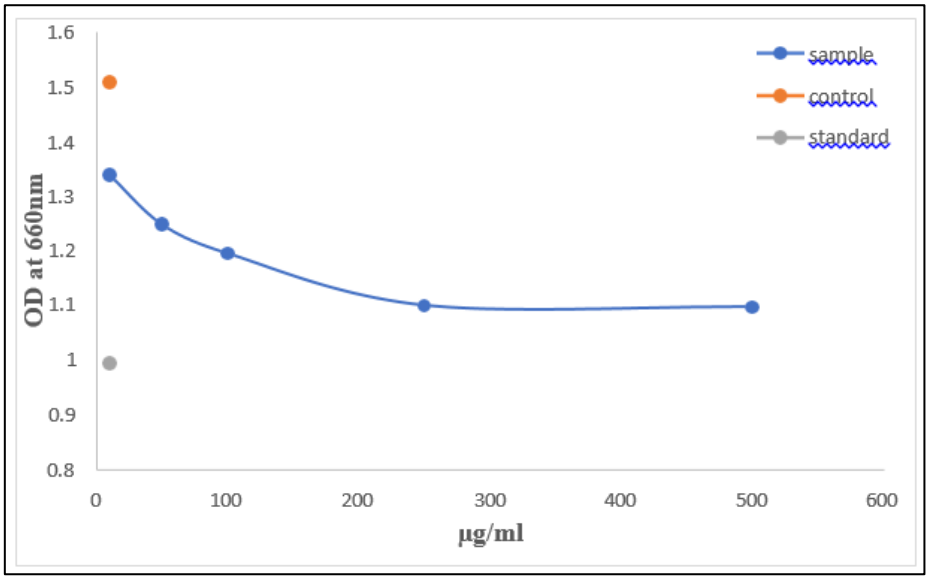
Present (+), Absent (-)

TABLE 2: THE ABSORBANCE VALUE OF PLANT EXTRACT CONCENTRATION AND STANDARD DRUG ACETYL SALICYLIC ACID

A. OPTICAL DENSITY Value at 660 nm

Control Mean OD value: 1.504

S.No	Tested sample concentration (µg/ml)	OD Value at 660nm (in Triplicates)		
1	Control	1.507	1.503	1.501
2	500 µg/ml	1.096	1.082	1.066
3	250 µg/ml	1.099	1.104	1.168
4	100 µg/ml	1.194	1.197	1.220
5	50 µg/ml	1.247	1.236	1.242
6	10 µg/ml	1.338	1.264	1.365
7	Standard	0.993	0.996	1.157



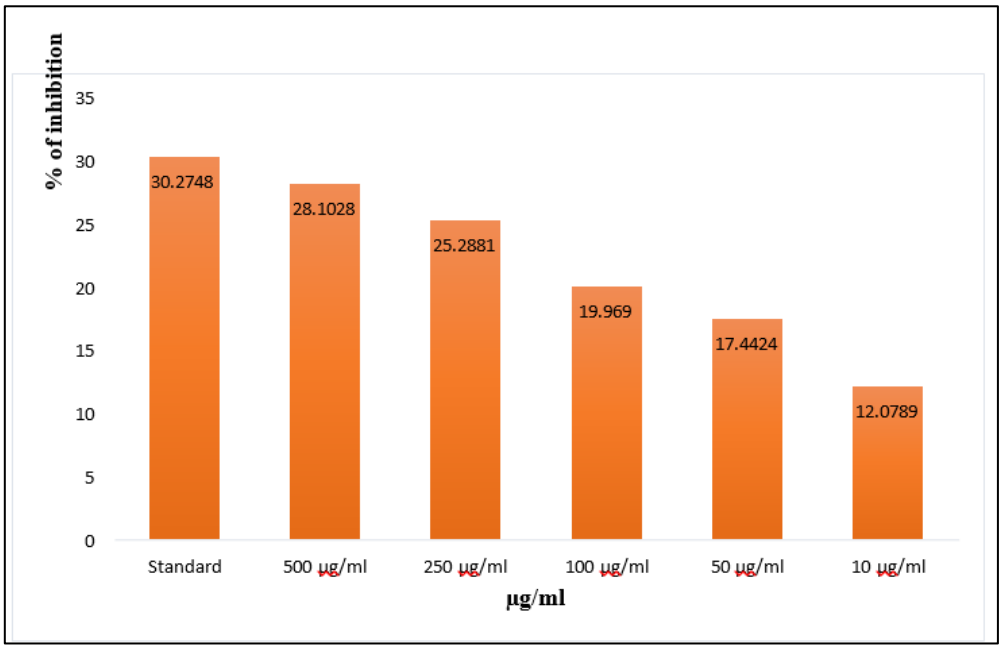
Graphical report for absorbance values

B. INHIBITION PERCENTAGE OF ALBUMIN DENATURATION (%)

TABLE 3: PERCENTAGE OF INHIBITION VALUE OF PLANT EXTRACT CONCENTRATION AND STANDARD DRUG

Percentage inhibition = (1-B/A) ×100%

S.No	Tested sample concentration	Inhibition percentage albumin Denaturation (%) (in triplicates)			Mean value (%)
1.	Standard	33.9761	33.7766	23.0718	30.2748
2.	500µg/ml	27.1277	28.0585	29.1 223	28.1028
3.	250µg/ml	26.9282	26.5957	22.3404	25.2881
4.	100µg/ml	20.6117	20.4 122	18.883	19.969
5.	50µg/ml	17.0878	17.8191	17.4202	17.4424
6.	10µg/ml	11.0372	15.9574	9.24202	12.0789



Graphical report for the percentage of inhibition

CONCLUSION

The phytochemical screening of ethanolic Extract of Pedalium murex (L.) Stem shows the positive results to alkaloids, flavonoids, Triterpenoids, carbohydrates, steroids, saponins, phenols. The strong presence of steroid in the extract may be responsible for the anti- inflammatory activity.

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological protein lose their biological function when denatured. Denaturation of tissue protein is one of the well documented causes of inflammatory and arthritic diseases. Production of auto antigen in certain arthritic and inflammatory diseases may be due to denaturation of protein in vivo. Hence by, prevention of protein denaturation may also help in preventing inflammatory conditions and the agents that can prevent protein denaturation therefore could be worthwhile for anti- inflammatory drug development.

The method of anti-denaturation of albumin was chosen to evaluate anti-inflammatory property of Pedalium murex (L.) stem. In anti-denaturation assay,the denaturation albumin is induced by heat treatment. It is the convenient method to check the anti-inflammatory activity. The results of the present study demonstrated the dose dependent inhibition of protein denaturation by ethanol extract. Since denaturation of tissue protein is one of the well documented causes of inflammatory and arthritic diseases, the ethanolic extract of Pedalium murex (L.) stem may have a significant anti-inflammatory activity.

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