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Qualitative And Quantitative Study Of Phytoconstituents, Antimicrobial And Antiinflammatory Activity Of Extract Of *Plumbago Indica*

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ABSTRACT: Phytochemical study revealed the presence of several compounds in the roots of Plumbago indica. Carrageenan-induced paw edema can be used to screen for anti-inflammatory agents. The study found that oral administration of the plant reduced hyperalgesia and inflammation.In humans, this nociceptor sensitization usually leads to clinical conditions known as hyperalgesia defined as an increased response to a painful stimulus or allodynia described as pain evoked by non-noxious stimuli. In our study, we have proven that oral administration of aqueous extract of Plumbago indica 30 min before Carrageenan was in grade to reduce hyperalgesia and allodynia significantly at 6 h post-Carrageenan..Edema and pain in the hind paw of animals as a result of Carrageenan -induced inflammation usually limit their motility and cause trouble in using their hind paw. During a Carrageenan -induced acute inflammation event, paw tissue loses normal muscle architecture and shows important amassing of infiltrating inflammatory cells and increased inter-fiber space during physical observation..During our study, we found that oral administration of Plumbago indica decreased infiltrating inflammatory.

1. INTRODUCTION

1.1 Herbal medicine

Herbal medicine therapies have long been a vital source of first-rate healthcare all over the world. Plants have provided a plentiful source of useful and safe medicinal therapies since ancient times. Despite this, conventional pharmaceuticals are still used by about 80% of the world's population. Herbal medicines are finished, classified medicinal preparations that contain living components, aerial or underground a portion of vegetation or other plant materials, or a combination of them, whether or not in the crude country or as plant arrangements. Plant

compounds mixed with chemically defined energetic chemicals, as well as chemically characterized distant aspects of plant life, aren't deemed herbal medication therapies [1].

Herbal medication treatments are still a big business in the US pharmaceutical industry, worth billions of dollars. Approximately 1500 botanicals are offered as dietary supplements; formulations are not subjected to rigorous toxicity testing by the Food and Drug Administration (FDA) to ensure their safety and efficacy. The Indian natural medication business is worth over $1 billion, with plant-based crude drug exports worth around $100 million. Herbal medicine's present market potential in Europe and the United States is estimated to be between $ 80 and $250 billion [2-3].

The ayurvedic notion was recognised and evolved in India between 2500 and 500 BC. The exact meaning of Ayurveda is "science of lives," since it is an ancient Indian system of health treatment that focused on man and his ailment. It is stated that people with good health have a metabolically well-balanced metabolism. The practise of Ayurveda therapies was organised into eight parts and three hundred and eighty chapters, with 314 flowers designated as pharmacological treatments in India [4].

Ayurveda was the name given to the medicinal knowledge of the Indian subcontinent four thousand years ago. In India, Ayurveda continues to be an essential tool for medical and pharmacological therapy. Plant alkaloids are the most common active ingredients in Ayurvedic medicines. Many Ayurvedic medications now include pharmacologically active ingredients, and their utility in drug therapy is being determined. Only a small percentage of plants are used in traditional medicines, as stated in the introduction. The Indian subcontinent is home to a large number of medicinal plants that are used in traditional medical treatments [5].

Although almost 15000 medicinal flowers have been reported in India [6] traditional tribes use just 7,000-7,500 plant life to treat certain illnesses [7-9]. For extraordinary ailments, therapeutic plant life is indexed in many indigenous structures such as Siddha (600), Ayurveda (700), Amchi (600), Unani (700), and Allopathy (30) plant species [10]. According to various estimates, 17,000 species of medicinal plants have been identified, with about 3,000 species being used in the medical field [11]. Chemical concepts derived from herbal assets have become a lot easier to come by, and they've helped a lot with the development of new medicinal flora capsules [12-13]. Saponins, tannins, alkaloids, alkyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids, and phorbol esters all contribute to the valuable medicinal properties of various plants [14].

Among them some are act as synergistic and beautify the bioactivity of other compounds. Artemisinin produced via Artemisia annua plant may be very powerful in opposition to *Plasmodium falciparum, P. Vivax* and also drug resistant parasite. The most important active components of *Artemisia annua* are sesquiterpenoid lactone endoperonides named artemisinin and artemisinic acid. For more than century quinine, an alkaloid acquired from the bark of diverse species of cinchona timber has been used in the treatment of Malaria and interestingly changed into one of the first sellers used for the treatment of amoebic dysentery.

Reserpine remoted from uncooked plant extract of *Rauwolfia serpentina* is used as tranquilizer and in control of high blood pressure. From 2000 years the powdered root of *Rauwolfia serpentina* has been utilized in remedy of intellectual infection in India. Although synthetic drugs are regularly used in treatment of positive ailment however a great interest and self assurance on plant remedy turned into found [15]. Indian Vedas describe the massive use of natural products and aqueous extract of various plant parts for curing distinctive illnesses. Maximum 30 % of root a part of medicinal plant is utilized in distinctive practices in assessment to other plant elements [16].

1.2 Inflammation and anti-inflammatory interest

Inflammation is a part of the complicated biological reaction of vascular tissues to harmful stimuli, including pathogens, damaged cells or irritants. It is characterized via redness, swollen joints, joint pain, its stiffness and lack of joint characteristic. Inflammation is presently treated via NSAIDs. Unfortunately these capsules motive elevated danger of blood clot ensuing in heart assaults and strokes [17]. Inflammation is a normal, protective reaction to tissue damage caused by physical trauma, noxious chemical compounds or microbiological marketers.

1.2.1 Types of Inflammation

There are mainly two types of inflammation which are as follows:

1.2.1.1 Acute inflammation

It is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes.

1.2.1.2 Chronic infection

It is related to infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis [18].

Inflammation is a commonplace scientific situations and rheumatoid arthiritis (RA) is a persistent debilitating autoimmune disease that affects approximately 1% of the population in evolved nations [19-20]. The classic symptoms of inflammation are neighborhood redness, swelling, pain, warmness and loss of characteristic [21].

Inflammation is a stereotyped reaction, inherent to vascularized tissues, which has the goal of reestablishing tissues homeostasis. The inflammatory process has cell and humoral additives, such as leucocytes (neutrophils, macrophages, eosinophils, mast cells and lymphocytes) and the humoral proteolytic structures (complement, kinins and coagulation), respectively. These components paintings synergistically and concurrently, inflicting vascular changes and leukocyte recruitment to the lesion [22].

Leucocytes (to begin with neutrophils), start to phagocytose micro organism and mobile particles, performing a number one clearance of the lesion. The top of neutrophil recruitment is accompanied with the aid of the

appearance of macrophages into the tissue, which phagocytose the final cell and bacterial residues, consisting of apoptotic neutrophils [23]. At the same time, lymphocytes can be activated in the lymph nodes via antigen-imparting cells (e.g., dendritic cells) from the tissue, initiating the manufacturing of antibodies by B cells and the migration of T helper lymphocytes to the inflamed web site. Following the path, stromal and parenchymal cells multiply and reconstitute the tissue, at the same time as most of the remaining macrophages and lymphocytes go away through the lymphatics.

Inflammation is critical for the survival of the host, however is observed by its classical cardinal symptoms rubor, calor, tumor and dolor (redness, warmth, tumor and pain), which can be the main motive of patient discomfort, specially after surgical methods. This impels health professionals to prescribe anti inflammatory drugs, a practice that must be constrained to the shortest duration viable following the patient's lesion or surgical intervention.

The motive for this is the mechanism of motion of NSAIDS, which is the inhibition of the enzyme cyclooxygenase (COX) which takes part inside the synthesis of seasoned-inflammatory lipid mediators called prostanglandins and thromboxanes. Ironically, the identical mediators that result in the preliminary section and symptoms of inflammation are folks who will take part and stimulate the expression of different enzymes that synthesize mediators liable for the decision of irritation, or in other phrases, its cease. For instance, prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2) induce the expression of the enzyme 15-lipoxygenase (15-LOX) in its lively shape in leucocytes, which catalyzes a step in the manufacturing cascade of a potent pro-resolving mediator named lipoxin A4 [24].

Lipoxin A4 is a member of a group of lipid mediators of decision that includes resolvins, protectins and the aspirin-brought about analogs of those training. It does now not have immunosuppressive residences, in assessment, it turns on precise cellular mechanisms, consisting of the stimulation of non-phologistic recruitment of monocytes (with out elaborating pro-inflammatory mediators), activation of macrophage phagocytosis of microorganisms and apoptotic cells, boom in phagocyte exit thru the lymphatics, expression of antimicrobial molecules and inhibition of in addition neutrophil and eosinophil infiltration [25].

Another exciting decision pathway, whose discovery created quite a few controversy in this area of studies, is the motion of prostaglandins at the resolution section of inflammation [26]. It turned into tested that COX is expressed also at that point, correlating with the manufacturing of PGD2, prostaglandin 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2 - the non-enzymatic degradation made of PGD2) and currently, prostaglandin F2a (PGF2a). While statistics confirming PGF2a decision residences is restricted, PGD2 and 15d-PGJ2 have properly set up anti inflammatory and pro-resolving results on inflammation fashions, such as Promotion of leukocyte apoptosis, macrophage clearance from infected sites, and control of cytokines and chemokines that adjust leukocyte trafficking . These results are mediated by means of activation of DP1 receptor via PGD2 and inhibition of

nuclear aspect γB (NFγB) activation via peroxisome proliferator-activating receptor γ (PPAR γ) via 15d-PGJ2 [27].

Considering all this statistics, the extended or behind schedule use of anti-inflammatory tablets, by blocking the manufacturing of prostaglandins and the further synthesis of pro-resolving mediators, ought to cause a postpone in tissue recovery or even establishment of a continual lesion. Some measures can be taken to avoid harming the patients, which includes decreasing the prescription and use of NSAIDS to the smallest duration essential for symptom remedy, for instance, edema and ache. In addition, the selection of a medicinal drug with little anti-inflammatory hobby but nonetheless suitable analgesic effect, including acetaminophen (Paracetamol), dypirone and diclofenac, or even codeine-NSAIDs combined drugs, ought to additionally be taken into consideration.

Special interest need to also accept to determine precisely the degree of the inflammatory method encountered inside the affected person earlier than the administration of any form of anti inflammatory drug. Knowing the degree of irritation, the choice of applying pills with anti inflammatory interest or simplest analgesic pastime is less difficult. Proceeding this way, it is much more likely that the pharmacological intervention will not interfere with the natural path of inflammation and resolution, consequently, growing the performance in patient treatment and recuperation.

1.3.1 Non-steroidal anti-inflammatory tablets (NSAIDs)

Non-steroidal anti-inflammatory tablets (NSAIDs) have been the corner stone of ache management in sufferers with osteoarthritis and other painful situations. In the USA an envisioned five% of all visits to a doctor are associated with prescriptions of non-steroidal anti inflammatory tablets and they are most of the most usually used drugs [28-29]. In 2004, rofecoxib, advertised as a cyclo-oxygenase-2 (COX- 2) selective inhibitor, turned into withdrawn from the market after the outcomes of a randomised placebo managed trial confirmed an accelerated chance of cardiovascular occasions associated with the drug. This locating becomes confirmed in other trials and a cumulative meta-analysis [30-31]. Since then debate has surrounded the cardiovascular safety of cyclo-oxygenase-2 selective inhibitors, observed by comparable concerns approximately conventional non-steroidal anti inflammatory drugs [32]. More lately, america Food and Drug Administration determined against the approval of etoricoxib because of its insufficient hazard-benefit profile [33].

1.3 Treatment of anti-inflammatory

1.3.3.1 Mechanism of movement of NSAIDs

Traditionally, the analgesic motion of non-steroidal anti-inflammatory drugs (NSAIDs) has been defined on the basis of their inhibition of the enzymes that synthesis prostaglandins. However, it's miles clear that NSAIDs exert their analgesic impact now not best through peripheral inhibition of prostaglandin synthesis however also

thru a ramification of other peripheral and vital mechanisms. It is referred to now that there are 2 structurally wonderful varieties of the cyclo-oxygenase enzyme (COX-1 and COX-2). COX-1 is a constitutive member of normal cells and COX-2 is precipitated in inflammatory cells. Inhibition of COX-2 activity represent the maximum in all likelihood mechanism of action for NSAID-mediated analgesia, whilst the ratio of inhibition of COX-1 to COX-2 by means of NSAIDs ought to decide the probability of detrimental outcomes. In addition, a few NSAIDs inhibit the lipoxygenase pathway, which may additionally itself bring about the manufacturing of algogenic metabolites. Interference with G-protein-mediated signal transduction by way of NSAIDs can also shape the basis of an analgesic mechanism unrelated to inhibition of prostaglandin synthesis [34].

1.3.3.2 Immune Selective Anti-Inflammatory Derivatives (ImSAIDs)

Leukocyte (white blood cell) activation and transmigration are the earliest and most essential occasions that arise in the course of irritation. Leukocytes, which participate in all inflammatory processes, are essential in host defense but immoderate and inappropriate activation can bring about worsening of pathology. Granulocytes, particularly, launch a spectrum of materials which amplify the inflammatory cascade as illustrated in pancreatitis, sepsis, allergies, ischemic reperfusion harm, trauma, hepatitis, and so forth. It is properly widely wide-spread that modulating the excessive activation and migration of leukocytes is a new goal for pharmaceutical improvement [35-36]. In precis, the modern anti inflammatory repository is deficient for a spread of inflammatory and critical care warning signs which advise to be happy by means of the ImSAIDs.

ImSAIDs constitute a completely new technology of biologically derived immuno-modulating anti inflammatory molecules. ImSAIDs are not steroids or NSAIDs and act through a mobile mode of movement that inhibits infection triggered with the aid of the innate immune reaction. ImSAIDs have interaction with co-stimulatory cellular floor receptors to inhibit immoderate activation and infiltration of leukocytes without compromising the immune machine. This upstream technique reduces the release of seasoned-inflammatory cytokines, digestive enzymes, and oxidative burst merchandise thereby inhibiting inflammatory amplification and keeping collateral tissue [37].

1.4 Plants as natural anti-inflammatory agents

Unlike modern allopathic drugs which are single active components that target one specific pathway, herbal medicines work in a way that depends on an orchestral approach. A plant contains a multitude of different molecules that act synergistically on targeted elements of the complex cellular pathway [38]. Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions [39]. The use of herbal medicines becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. India with its

biggest repository of medicinal plants in the world may maintain an important position in the production of raw materials eitherdirectly for crude drugs or as the bioactive compounds in the formulation of pharmaceuticals and cosmetics etc [40].

1. ***Achillea millefoliumLinn.*** **(Asteraceae)**

Achillea millefolium Linn**. (Asteraceae)**

Achillea millefolium L. is a perennial herb native to Europe and highly recognized in traditional medicine for its anti-inflammatory properties. The plant has been traditionally used externally for treatment of wounds, burns, swollen and irritated skin. Studies have shown two classes of secondary metabolites, isoprenoids and phenolics, contribute mainly to the anti-inflammatory properties[41].Aqueous and alcoholic extracts of A. millefolium are used in traditional medicine internally in treatment of gastro-intestinal and hepato-biliary disorders and as an antiphlogistic drug. The topical anti-inflammatory activity of sesquiterpenes is caused by inhibition of arachidonic acid metabolism. The three flavnoids present in the crude extract and enriched in flavnoid fraction are rutin, aspigenin-7-O-glucoside and luteolin-7-Oglucoside. The crude plant extract and two fractions enriched in the dicaffeoyquinic acids and the flavnoids inhibit human neutrophil elastase as well as the matrix metalloproteinases, which are associated with anti-inflammatory process in vitrostudies [42].

2. ***Aconitum heterophyllum*** **(Valeraneaceae)**

Aconitum heterophyllum

A.heterophyllum is a plant which is commonly known as 'Ativisha' or 'Patis' in Ayurveda. It is used for the treatment of diseases of nervous system, digestive system, fever and rheumatism. The ethanolic extract of root

of A.heterophyllum contains alkaloids, glycosides, flavnoids and sterols. It has been reported that plants with these chemical classes of compounds possess potent antiinflammatory effects through inhibition of prostaglandin pathways. The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation. The weight of the wet cotton pellets correlates with the amount of granulomatous tissue. The administration of A.heterophyllum extract has been observed to inhibit the weight of wet cotton pellet in a dose dependent manner and the higher dose of A.heterophyllum exhibited inhibition of inflammation very close to the inhibitory effect of diclofenac sodium. In literature it has been reported that ethanolic root extract of A.heterophyllum has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism [43].

3. ***Adhatoda vasica*** **(Acanthaceae)**

Adhatoda vasica **(Acanthaceae)**

Adhatoda vasica L. is an indigenous herb belonging to family Acanthaceae. The plant has been used in the indigenous system of medicine in worldwide as herbal remedy for treating cold, cough, whooping cough, chronic bronchitis, asthma, sedative expectorant, antispasmodic, anthelmintic, rheumatism and rheumatic painful inflammatory swellings. The drug is employed in different forms such as fresh juice, decoction, infusion and powder. It is also given as alcoholic extract and liquid extract or syrup [44].This plant contains alkaloids, tannins, flavnoids, terpenes, sugars and glycosides[45]. The anti-inflammatory potential of ethanolic extract has been determined by using carrageenan-induced paw edema assay, formalin-induced paw edema assay in albino rats. The ethanolic extract of Adhatoda vasica produced dose dependent inhibition of carrageenan and formalin-induced paw edema [46].

4. ***Bacopa monnieri Linn.*** **(Scrophulariaceae)**

***Bacopa monnieri* Linn**

The Bacopa monnieriis a creeping, glabrous, succulent herb, rooting at nodes and habitat of wetlands and muddfy shores [47]. Earlier, itis used as a brain tonic to enhance memory development, learning and concentration. The plant has also been used in India and Pakistan as a cardio tonic, digestive aid and to improve respiratory function in cases of bronchoconstriction. The plant possesses anti-inflammatory activity on carrageenan-induced rat paw edema and it has shown 82% edema inhibition when compared to indomethacin. Bacopa monnieri also significantly inhibited 5lipoxygenase (5-LOX), 15 (LOX) and cyclooxygenase-2 (COX-2) activities. Bacopa monnieri possesses significant anti-inflammatory activity that may well be relevant to its effectiveness in the lealing of various inflammatory conditions in traditional medicine. The antiinflammatory activity of Bacopa monnieri is due to the triterpenoid and bacoside present inthe plant. The ability of the fractions containing triterpenoids and bacosides inhibited the production of proinflammatory cytokines such as tumour necrosis factor –alpha and interleukin-6. This was tested using lipopolysaccharide activated peripheral blood mononuclear cells and peritoneal exudates cells in vitro. So, Bacopa monnieri has the ability to inhibit inflammation through modulation of proinflammatory mediator release [48].

5. ***Cassia fistula L.* (Caesalpiniaceous)**

Cassia fistula L.

Cassia fistula tree is one of the most widespread in the forests of India. The whole plant possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice. The bark extracts of Cassia fistula possess significant anti-inflammatory effect in the acute and chronic anti-inflammatory model of inflammation in rats. Reactive oxygen species (ROS) generated endogenously or

exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process. ROS play an important role in pathogenesis of inflammatory diseases. The main constituents responsible for antiinflammatory activity of Cassia fistula are flavnoids and bio-flavnoids [49].

6. ***Daphne pontica Linn.*** **(Thymelaeaceae)**

Daphne pontica **Linn**

Daphne species are supposed to have anti-cancer activity since the the time of AD 2nd century. Flavonoids constituents like daphnodorins were isolated from the roots of Daphne pontica which was reported to have antitumour activity.Several Daphne species have been used against inflammatory disorders. Daphne pontica have been used for the treatment of rheumatic pain and inflammatory ailments. The extracts inhibits the production of PGE2 and IL-1ß [50].

7. ***Emblica officinalis*** **(Euphorbiaceae)**

Emblica officinalis

Emblica officinalis is a tree growing in subtropical and tropical parts of China, India, Indonesia and Malay peninsula. It has been used for antiinflammatory and antipyretic activities in these areas. In the recent studies, the anti-inflammatory activity was found in the water fraction of methanol extract of plant leaves. The effects of fraction were tested on the synthesis of mediators of inflammation such as leukotriene B4, platelet activating factor (PAF) and thromboxane. The water fraction of methanol extract inhibited migration of human PMNs in relatively low concentrations [51].

8. ***Garcinia mangostana Linn.*** **(Guttiferae)**

Garcinia mangostana Linn.

The fruit rinds of Garcinia mangostana have been used as a traditional medicine for the treatment of trauma and skin infections. The xanthones, α- and γ-mangostins are major bioactive componds found in the fruit hulls of mangosteen. The xanthones exhibits their biological effects by blocking inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). It was reported that two mangostins decrease prostaglandins (PGE2) levels through inhibition of COX-2 activity and NO production. It is reported that α-mangostin shows a more potent inhibition of PGE2 release than either histamine or serotonin [52].

9. ***Lantana camara Linn.*** **(Verbenaceae)**

Lantana camara **Linn.**

The aerial parts of many species of Lantanaare widely used in folk remedies like cancer and tumours. A tea prepared from leaves and flowers were taken against fever, influenza and stomachache. The other uses of plant shows antimalarial, anti-bacterial and anti-diarrhoeal activities. From the studies it has been reported that aqueous extract of Lantana camara leaves is highly effective and safe for the treatment of hemorrhoids. It has been reported that aqueous extract of Lantana camaraleaves has promising analgesic, anti-inflammatory and anti-hemorrhoidal activities [53].

10. ***Lycopodium clavatum Linn.*** **(Lycopodiaceae)**

Lycopodium clavatum Linn.

Lycopodium clavatum commonly known as club moss has been reported to be used in wound healing effect. According to the study carried out by Orhan et al, four extracts prepared with petroleum ether, chloroform, ethyl acetate and methanol as well as the alkaloidal fraction from the aerial parts of Lycopodium clavatum using acetic acid-induced increase in capillary permeability assessment in mice revealed that only the chloroform extract and the alkaloid fraction displayed marked anti-inflammatory effect as compared to Indomethacin [54].

11. ***Mangifera indica Linn.*** **(Anacardiaceae)**

Mangifera indica Linn.

Mangifera indica grows in the tropical and subtropical region and its parts are commonly used in folk medicine for a wide variety of remedies. The plant Mangifera indica has been reported for various therapeutic uses in traditional medicines such as, a fluid extract or the infusion of the bark is used in monorrhagia, leucorrhoea, bleeding piles and in case of haemorrhage from the lungs. Idibs of the leaves calcined are used to remove warts of eyelids. Dried powdered leaves are used in diabetes. Dried flowers in decoction or powder are useful in diarrhea, chronic dysentery and gleet . The ethyl acetate and ethanol extracts of the roots of Mangifera indica has been reported to have considerable anti-inflammatory activity as compared with standard drug Diclofenac sodium. The phytochemical analysis revealed the presence of flavonoids. The flavonoids have potent anti-inflammatory activity by inhibiting prostaglandin synthesis [55].

12. ***Phyllanthus polyphyllus Linn.*** **(Euphorbiaceous)**

Phyllanthus polyphyllus Linn

It is a small shrub used in anti-inflammatory folk medicine in tropical and subtropical regions in India and Srilanka. Four compounds, one benzenoid and three arylnaphalide lignans isolated from whole plant showed growth inhibitory effect on production of NO and cytokines (TNF-α and IL . Since TNF-α and IL-12 were known as the main pro-inflammatory cytokines secreted during the early phase of acute and chronic inflammatory diseases, such as asthma, rheumatoid arthritis, septic shock. The use of Phyllanthus polyphyllus as anti-inflammatory remedy in traditional medicine may be attributed by these compounds [56].

13. ***Ricinus communis Linn.*** **(Euphorbiaceae)**

Ricinus communis Linn.

Ricinus communis Linn. is found almost everywhere in the tropical and subtropical regions of the world. Anti-inflammatory and free radical scavenging activities of the methanolic extract of Ricinus communis root was studied by llavarasan et alin Wistar albino rats. The methanolic extract exhibited significant anti-inflammatory activity in carrageenan-indiced hind paw edema model. The methanolic extract showed significant free radical scavenging activity by inhibiting lipid peroxidation. The obsereved pharmacological activity may be due to the presence of phytochemicals like flavonoids, alkaloids and tannins in the plant extract [57].

14. ***Sesbania sesban Linn.*** **(Leguminosae)**

Sesbania sesban Linn.

The genus Sesbania sesban contains about 50 spieces, the majority of which are annuals. The greatest greatest spieces diversity occurs in Africa with 33 spieces. Although the annual spieces have received attention, recent research has focused on perennial spieces. Of the perennial spieces, Sesbania sesban has shown potential. It is a small perennial tree with woody stems, yellow flowers and linear pods .According to the data from literature the phytochemical investigation of crude saponin extract revealed the presence of various constituents like terpenoidal and steroidal saponins, tannins and flavnoids which had been reported to have anti-inflammatory activity .This was proved by inhibition of carrageenan oedema by crude saponins extract. The crude saponin extract have been able to control the increase in Paw edema in early phase and also in late hours related to inhibition of prostaglandins release. Hence, it can besaid that the present anti-inflammatory activity of crude saponin extract might be due to its action on the early and latter phase of inflammation [58].

15. ***Sida cordifolia Linn.*** **(Malvaceae)**

Sida cordifolia Linn.

Sida cordifolia is a perennial subshrub of the mallow family Malvaceae. It has naturalized throughout the world and is considered an invasive weed in Africa, Australia, Hawaiian islands, New Guinea and French Polynesia. Sida cordifolia is used in folk medicine for the treatment of inflammation of the oral mucosa, blenorrhea, asthmatic bronchitis and nasal congestion . It has been investigated as an anti-inflammatory, for preventing cell proliferation and for encouraging liver growth [59].

16. ***Thespesia populnea*** **(Malvaceae)**

Thespesia populnea

The leaves and bark of Thespesia populnea are used to produce oil for the treatment of fracture wounds and as an anti-inflammatory poultice applied to ulcers and boils in southern India and Sri Lanka. Ethanolic extract of Thespesia populneashows anti-inflammatory activity in both acute and chronic models. The phytochemical studies indicated that the ethanolic extract of bark contains alkaloids, carbohydrates, proteins, tannins, phenols, flavonoids, gums & mucilage, saponins and terpenes [60].

AIM AND OBJECTIVE

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, reside in particular tissues and/or circulate in the blood. Inflammation may also happen in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration. Mostly, both the innate immune responses as well as the adaptive immune response are involved in the formation of inflammation.

Plumbago indica (Plumbaginaceae) named “agnichita” belonging to the family Plumbaginaceae. The family is sometimes referred to as the leadwort family or the plumbago family. Most species in this family are perennial herbaceous plants, but a few grow as shrubs. The plants have perfect flowers and are pollinated by insects. They are found in many different climatic regions, from arctic to tropical conditions, but are particularly associated with salt-rich steppes, marshes, and sea coasts. Plumbago is popularly known as “chittiramulam”, in Tamil and “white leadwort” in English. Plumbaginaceae is distributed as a weed throughout the tropical and subtropical countries of the world. The family Plumbaginaceae is composed of 10 genera and 280 species. The objective of the present study was to evaluate the anti- inflammatory activity of aqueous extract of *Plumbago indica* roots for scientific validation of the folklore claim of the plant.

PLAN OF WORK

1. Literature survey
2. Collection and identification of plant material

3. Extraction of plant material
    - *By maceration method*
4. Phytochemical screening of extract
5. Quantitative of bioactive constituents
6. *In vitro* anti-microbial activity of extract
    - *By well diffusion method*
7. Anti-inflammatory activity of extract

PLANT PROFILE

5.1 *Plumbago indica*

Plumbago indica (Plumbaginaceae) named “agnichita” belonging to the family Plumbaginaceae. The family is sometimes referred to as the leadwort family or the *plumbago* family. Most species in this family are perennial herbaceous plants, but a few grow as shrubs. The plants have perfect flowers and are pollinated by insects. They are found in many different climatic regions, from arctic to tropical conditions, but are particularly associated with salt-rich steppes, marshes, and sea coasts. *Plumbago* is popularly known as “chittiramulam”, in Tamil and “white leadwort” in English. Plumbaginaceae is distributed as a weed throughout the tropical and subtropical countries of the world.

Scientific classification

Kingdom: Plantae

***Clade*:** Tracheophytes

***Clade*:** Angiosperms

***Clade*:** Eudicots

Order: Caryophyllales

Family: Plumbaginaceae

Genus: *Plumbago*

Species: *P. indica*

Origin and geographic distribution

Plumbago indica originates from India and South-East Asia, where it is widely used as a medicinal plant. It is cultivated as an ornamental throughout the tropics and in temperate regions in greenhouses. In tropical Africa, *Plumbago indica* is cultivated, sometimes as a medicinal plant, in countries with large populations of Indian immigrants: Kenya, Tanzania, Zimbabwe, Mozambique and Madagascar.

Vernacular names

- Indian leadwort, rose-coloured leadwort, scarlet leadwort (En).
- Plumbago de flor vermelha (Po).

Figure 5.1: Flowers and leaves of *Plumbago indica*

Uses

In eastern Africa, *Plumbago indica* is used medicinally in a similar way by the Indian population as it is traditionally used in India itself, where many households keep some plants in their backyard. Especially the root has many uses: it is acrid, vesicant, alterative, digestive, stimulant and a powerful abortifacient and oral contraceptive. High doses are dangerous and may cause death. An infusion of the roots is taken to treat dyspepsia, colic, cough and bronchitis.

- It is useful for the treatment of chronic respiratory problems.
- Chitrak is also useful for bronchitis and rheumatism.
- It works against tuberculosis.
- *Plumbago indica* is useful for asthma, cough and cold.
- It kills intestinal worms.
- The plant is useful for digestion and works against indigestion.
- In some countries, it is used for abortion.
- It is useful for the treatment of leucodermia.

- It is also helpful for skin diseases.
- The root helps to stimulate appetite.
- It cures the disease of the liver.
- The leaves of the plant are also helpful for the treatment of ringworm and scabies.
- It is a useful agent for enlargement of the spleen.
- It is anti-periodic and useful for obesity.
- *Plumbago indica* is useful for piles and reduces fever.
- It cures nausea, allergy and leucodermia.
- The powdered root is snuffed to get comfort from a headache.
- It also cures intestinal troubles.

MATERIALS AND METHODS

6.1 Collection of plant material

The roots of chosen plant were collected in the month of January 2022, from Shubham nursery Bhopal (M.P.). The roots were cleaned by tab water and a portion was dried at room temperature. The dried samples were ground and passed through a sieve (20 meshes). The powdered drugs were kept in sealed containers and protected from light until used. Another portion of root sample was used for maceration.

Figure 6.1: Collection of plant material

6.2 Extraction by maceration method

This is an extraction procedure in which coarsely powdered drug material, either roots or roots bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material.

The container is then closed and kept for at least three days [74]. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermo labile plant material.

48.6 gram of powdered roots of *Plumbago indica* was exhaustively extracted with water as a solvent for 36 h at room temperature (28 ± 2ºC) in closed air tight glass container with occasional shaking. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Figure 6.2: Extraction by maceration method

6.3 Phytochemical analysis [75-77]

Photochemical examinations were carried out extracts as per the following standard methods.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the occurrence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

6.4 Quantitative studies of phytoconstituents

In India, the ayurvedic system has features a numerous of such medicinal remedies on plants or plant products and the determination of their morphological, pharmacological or pharmacognostical characters can provide a better understanding of their active principle and mode of action. The phytochemicals which are present in the aqueous extract of *Plumbago indica* was determined and quantified by standard procedures.

6.4.1 Total phenol content estimation

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method [78].

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol

Preparation of Extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexes for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

6.4.2 Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminum chloride method [78].

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

6.5 *In vitro* antimicrobial activity

Media preparation (broth and agar media)

Composition of nutrient agar media;

Agar - 1.5 gms.

Beef extract - 0.3 gms.

Peptone - 0.5 gms.

Sodium chloride - 0.55 gms.

Distilled water - to make 100 ml

pH – 7

Composition of potato dextrose agar media;

Potato infusion - 20 gms.

Dextrose - 2 gms.

Agar - 1.5 gms.

Distilled water - to make 100 ml

pH – 7

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely. The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121^{o}C) for 15 minutes. After sterilization, the media in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use.

The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient and potato dextrose broth than incubated for 24 hours at 37ºC. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient and potato dextrose agar plates with loop full of microbes and further incubated for next 24 hours at 37ºC to obtain the pure culture and stored as stocks that are to be used in further research work.

Broth cultures of the pure culture of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient and potato dextrose broth and incubated at 37ºC for 24-48 hours [79]. A loop full was taken from these broths and seeded onto sterile nutrient and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture.

6.6 *In vivo* anti-inflammatory activity using Carrageenan-induced hind paw edema

6.6.1 Animals

The animals were maintained in colony cages at 25 ± 2 ºC, relative humidity 50-55% maintained under 12 h light and dark cycle (06 to 18 h light; 18 to 06 h dark). The animals were fed with standard animal feed (Hindustan Lever Ltd.) and water ad libitum. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional animal ethical committee. All the animals were acclimatized to the laboratory conditions prior to experimentation.

According to the method of [80] animals were subjected to sub plantar injection of 0.1 mL of λ-carrageenan (1% in NaCl 0.9%) into the right hind paw and were divided randomly into allocated groups (n=6).

Then, they were subjected to a gavage (Oral dose) with the following solutions; Group I: sterile saline 0.9% NaCl at 10 mL/kg;

Group II: Diclofenac sodium at 50 mg/kg as reference drug,

Group III: extract of *Plumbago indica* roots at 200 mg/kg;

Group IV: the same extract at 300 mg/kg.

Plumbago indica extract and Diclofenac, both dissolved in 0.9% NaCl, were administered by oral dose 1h following Carrageenan injection. The Extract was first evaporated under vacuum at room temperature to remove the methanol, and then the deposit was solubilizing in a 0.9% NaCl solution.

Allocated groups (n=6)

Groups	Drugs	Dose	No. of Animals
Group I	Sterile saline 0.9% NaCl	10 mL/kg (bw),p.o	6
Group II	Diclofenac sodium	50 mg/kg(bw),p.o	6
Group III	Aqueous extract of *Plumbago indica* roots	200 mg/kg(bw),p.o	6
Group IV	Aqueous extract of *Plumbago indica* roots	300 mg/kg(bw),p.o	6

Linear circumference of paw edema:

The circumference of paw was measured at h 1, 2, 3 and 4 after Carrageenan injection. Increases in the linear circumference of the right hind paw were taken as an indicator of paw edema. Percentage increase in edema (%IO) was estimated in terms of the difference in the zero time (C_0) linear circumference of the injected right hind paw, and its linear circumference at time t (C_t):

$$\%IO = \frac{Ct - C0}{C0} x100$$

Percentage inhibition:

Percentage inhibition of the inflammatory reaction produced by Carrageenan was calculated following formula:

$$\%Inhibition = \frac{(IOc - IOt)}{IOc} x100$$

Where IOc and IOt represented the mean increase in paw circumference in control and treated groups, respectively.

Statistical analysis: Differences between groups were determined using analysis of variance (ANOVA) followed by the post hoc Tukey test.

RESULTS AND DISCUSSION

7.1 % Yield of plant material

The moderately coarse powder of the roots of *Plumbago indica* was subjected to extraction by maceration method with water as a solvent. The obtained extracts were dried and weighed. The percentage yield of each plant was calculated as per standard method. The weighed extract of each plant drug was stored in desiccators for further use. The yield was found to be (21.55% w/w of crude drug) of aqueous extract with semisolid mass of *Plumbago indica*. Obtained results were recorded in table 7.1.

Table 7.1: Extractive values obtained from *Plumbago indica* using aqueous solvent

S.N.	Solvent	Time of extraction	% Yield
1	Aqueous extract	36 hours	21.55%

7.2 Preliminary phytochemical screening of *Plumbago indica*

Table 7.2: Preliminary phytochemical screening of *Plumbago indica*

S.N.	Phytoconstituents	Test Name	Aqueous extract
1	Alkaloids	Mayer's Test	**Absent**
		Dragendorff's Test	Present
2	Glycosides	Raymond's Test	Present
		Killer Killani Test	Present
3	Carbohydrates	Molisch's Test	**Absent**
		Fehling's Test	**Absent**
4	Tannins	Vanillin- HCl Test	Present
		Gelatin Test	**Absent**
5	Flavonoids	Lead acetate	Present
		Shinoda Test	Present
6	Resins	Color detection with ferric chloride	**Absent**
		Turbidity Test	**Absent**
7	Steroids	Libermann- Bur chard Test	Present
		Salkowski Reaction	Present
8		Biuret Test	Present

	Proteins & Amino acids	Precipitation test	**Absent**
		Ninhydrin Test	Present
9.	Phenols	Ellagic Acid Test	Present

7.3 Quantitative study of bioactive compounds

7.3.1 Estimation of total phenolic content

Gallic acid is used as a standard compound and the total phenols were expressed as mg/100mg gallic acid equivalent using the standard curve equation: $y = 0.011x + 0.011$, $R^2 = 0.998$, Where y is absorbance at 765 nm and x is total phenolic content in the aqueous extract of *Plumbago indica*. The results were expressed as the number of equivalents of Gallic acid (mg/100mg of extract). The results were presented in Fig 7.1.

Table 7.3: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.135
2	20	0.247
3	30	0.364
4	40	0.474
5	50	0.581

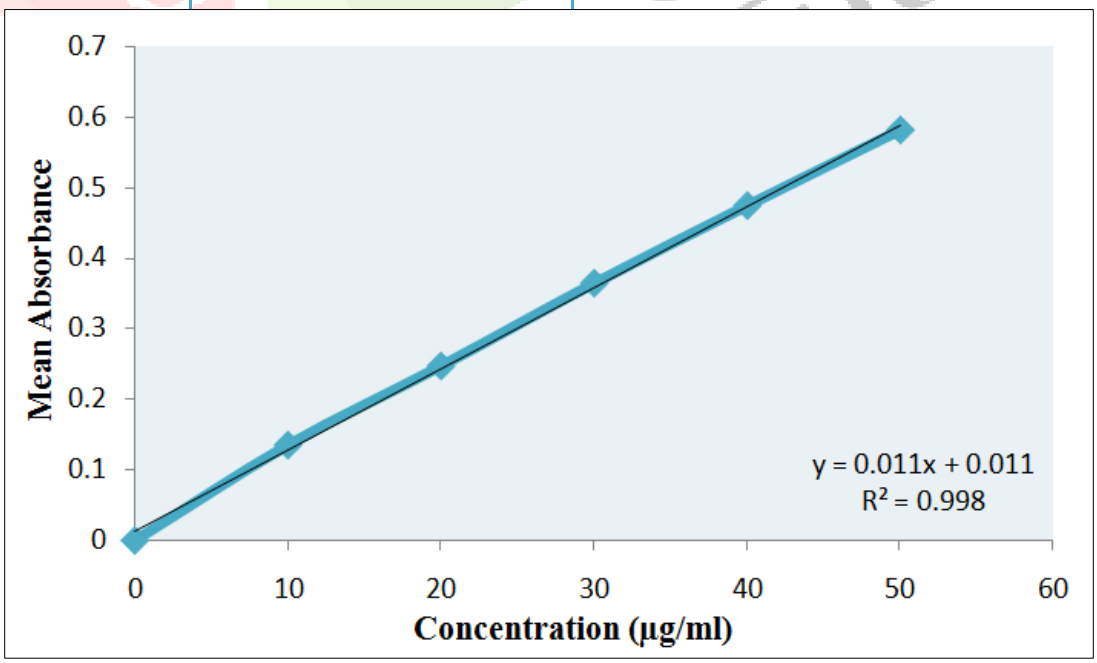

Figure 7.1: Graph of calibration curve of Gallic acid

7.3.2 Estimation of total flavonoids content

Flavonoids content was calculated from the regression equation of the standard plot (y=0.032x+0.018, R^2 =0.998) and is expressed as quercetin equivalents (QE) (fig. 7.2). Total flavonoids content was 0.784mg/100mg quercetin equivalent in aqueous extract of *Plumbago indica*. Flavonoids are the most common and widely distributed group of plant's phenolic compounds.

Table 7.4: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.191
2	10	0.348
3	15	0.514
4	20	0.652
5	25	0.812

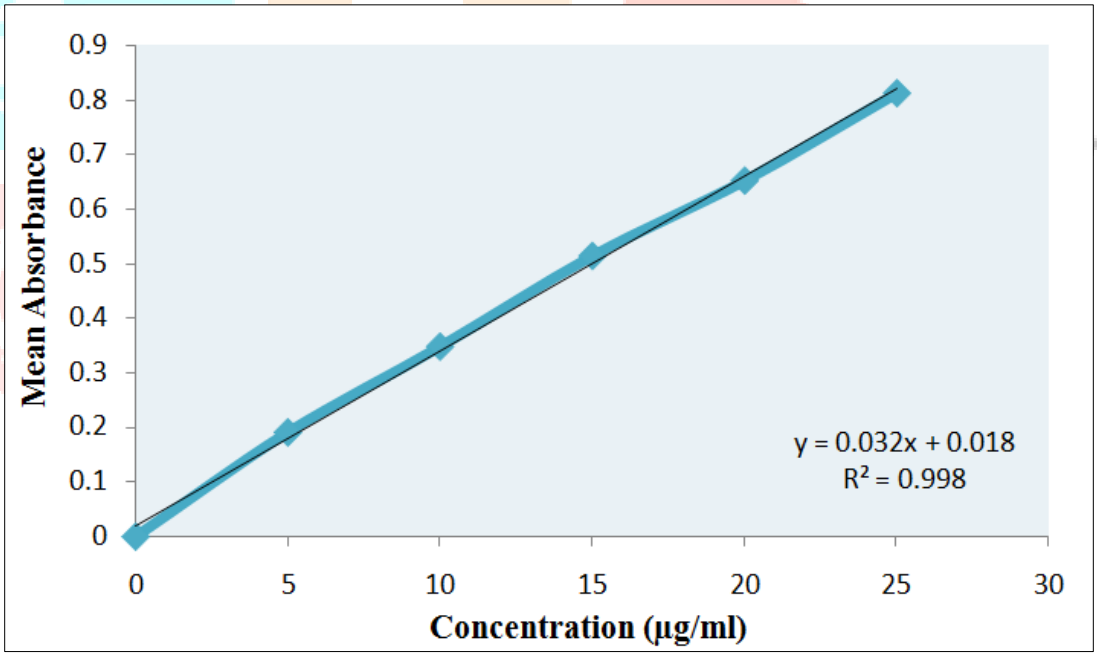

Figure 7.2: Graph of calibration curve of Quercetin

Table 7.5: Estimation of total phenolic and flavonoids content of *Plumbago indica*

S. No	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Aqueous	0.538	0.784

7.4 *In vitro* antimicrobial activity of *Plumbago indica* extract

Table 7.6: Antimicrobial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	*Klebsiella pneumoniae*	15±0.57	24±0.47	27±0.5
2.	Fluconazole	*Candida albicans*	26±0.94	30±0.5	32±0.74

Figure 7.3: Photoplate of antimicrobial activity of standard drug against selected microbes

Table 7.7: Antimicrobial activity of *Plumbago indica* extract against selected microbes

S. No.	Microbes	Zone of Inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Klebsiella pneumoniae*	8±0.71	10±0.24	17±0.57
2.	*Candida albicans*	7±0.94	9.6±0.5	11±0.74

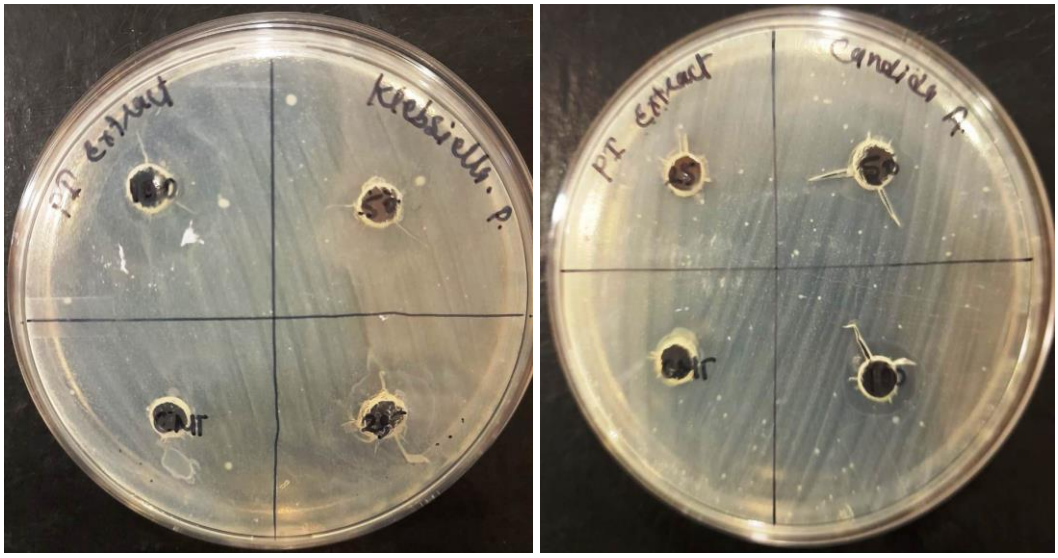

Figure 7.4: Photoplate of antimicrobial activity of *Plumbago indica* extract against selected microbes

7.5 Results of *in vivo* anti-inflammatory activity

Carrageenan-induced paw edema model has been used widely for the evaluation of anti-inflammatory activity of plant extracts. There are three different phases that appeared after carrageenan injection: the first phase (0–1.5 hours) involves the release of histamine and serotonin; the second phase (1.5–3 hours) the release of bradykinin and followed by the third phase (3-6 hours) involving the production of large amount of proinflammatory mediators such as prostaglandins (PGE2) and proinflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNFα); infiltration of neutrophils into the inflammatory site takes place.

One-hour after carrageenan injection, only 300 mg/kg dose of extract and Diclofenac sodium showed significant ($P < 0.05$) inhibition of paw edema compared to the negative control. This may be because the lower doses 200 mg/kg of the aqueous extract of *Plumbago indica* might not be able to achieve maximum plasma concentration at 1 h for first phase edema inhibition.

After 2 hours administration of Carrageenan, the 200 mg/kg and 300 mg/kg aqueous extract of *Plumbago indica* showed significant inhibition of paw edema. This could be because the lower dose was more effective to inhibit the release of bradykinin and prostaglandin.

Maximum percentage of inhibition of edema for the aqueous extract of *Plumbago indica* was observed after 4-hour injection of Carrageenan by 200, and 300 mg/kg oral dose of aqueous extract of *Plumbago indica* with their respective value 60.09% and 63.38%.

Table 7.8: Anti-inflammatory activity of aqueous extract of *Plumbago indica* on Carrageenan-induced paws edema

Treatment group	Dose (mg/kg)	The paw volume (ml), mean ± SEM				
		Basal	1 h	2 h	3 h	4 h
Negative Control	Sterile saline 0.9% NaCl	0.183±0.02	0.182 ± 0.007	0.204 ± 0.014	0.205± 0.02	0.215 ± 0.003
Diclofenac sodium	50 mg/kg, p.o	0.169 ± 0.007	0.137 ± 0.005	0.109± .003	0.090±0.006	0.072±0.005
***Plumbago indica* (Extract)**	200 mg/kg, p.o	0.195 ± 0.013	0.154 ± 0.004	0.130±0.008	0.101±0.008	0.087± 0.003
***Plumbago indica* (Extract)**	300 mg/kg, p.o	0.180 ± 0.013	0.149 ± 0.004	0.109±0.004	0.094±0.007	0.080±0.007

Note. Values are expressed as mean ± SEM (n = 6 mice in each group) and analyzed by one-way ANOVA followed by the post hoc Tukey test; a compared to the negative control; b compared to Diclofenac sodium; c compared to 200 mg/kg; 1 P < 0.05; 2 P < 0.01; 3 P < 0.001.

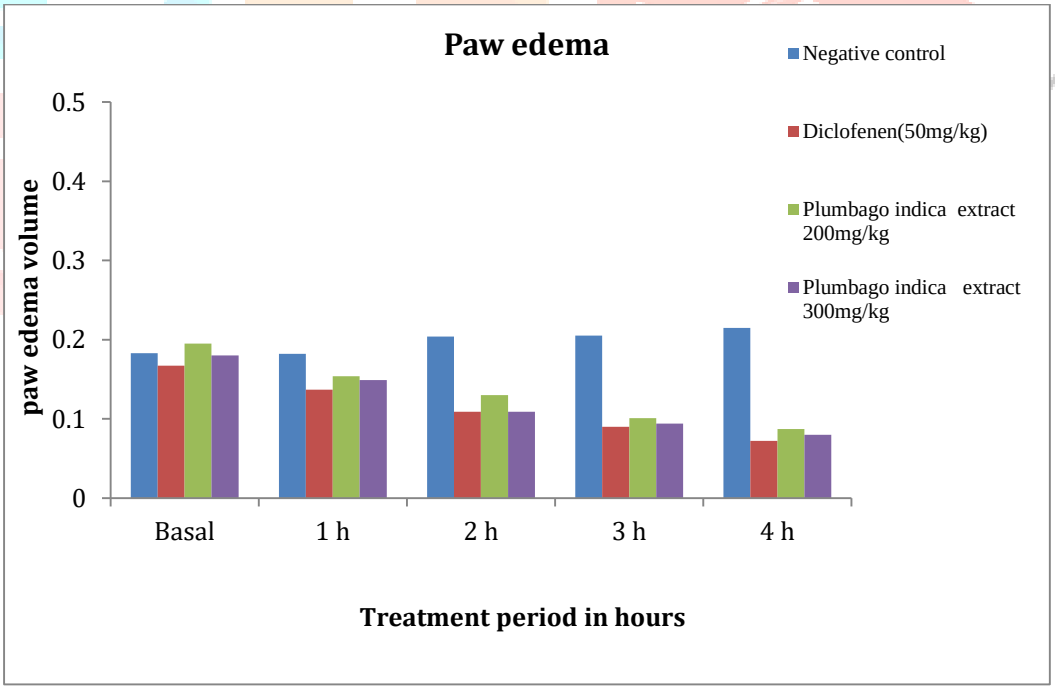

Figure 7.5: Anti-inflammatory activity of aqueous extract of *Plumbago indica* on Carrageenan-induced paws edema

Table 7.9: Maximum percentage of inhibition of edema for the aqueous extract of *Plumbago indica* at 100 and 200 mg/kg

Treatment group	Dose (mg/kg)	% inhibition			
		1 h	2 h	3 h	4 h
Diclofenac sodium	50 mg/kg, p.o	24.64 %	48.05 %	57.38%	68.47 %
Plumbago indica (Extract)	200 mg/kg, p.o	16.35 %	37.33 %	51.21 %	61.25 %
Plumbago indica (Extract)	300 mg/kg, p.o	19.25 %	47.02 %	55.91 %	64.11 %

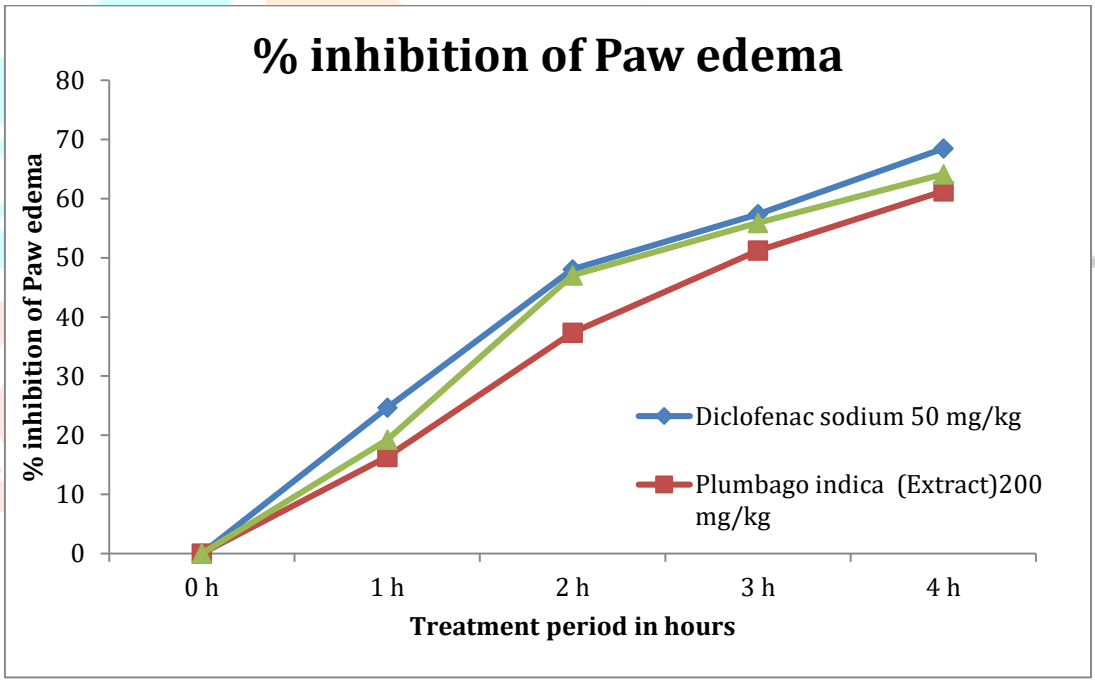

Figure 7.6: Maximum percentage of inhibition of edema for the aqueous extract of *Plumbago indica* at 200 and 300 mg/kg compare with negative control group and standard drug

8. SUMMARY AND CONCLUSION

The screening of phytoconstituents was done subjectively and quantitatively as a preliminary step. Using the maceration method, preliminary screening was carried out using water as solvents (Aqueous extract). *Plumbago indica* (Roots) were studied. Phytochemical study revealed the presence of several compounds in variable amounts in the Roots portions. Aqueous extract contained substantial levels of alkaloids, flavonoids, and phenols. Other phytochemicals found in *Plumbago indica* include tannins, saponins, quinones, and fats/oils, which are present in moderate levels. The percentage yields were calculated of the dried extracts. The percentage yield of dry extract of *Plumbago indica* roots was (21.55%).

In the results of qualitative photochemical analysis of aqueous extract of *Plumbago indica* roots. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve on various concentration in (µg/ml) 10, 20, 30, 40 and 50 shows the absorbance 0.135,0.247,0.364,0.474 and 0.581 respectively. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The total phenolic content in roots (mg/100mg) was found to be 0.538 mg/100mg dried extract.

Total flavonoids content (TFC) of roots *Plumbago indica* was calculated using calibration curve method. The different concentration showed the absorbance 0.191, 0.348, 0.514, 0.652 and 0.812 respectively for the concentration of 5, 10, 15, 20 and 25 µg/ml. Total flavonoid content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.032X + 0.018$, $R^2=0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance. The total flavonoid content in roots extract of *Plumbago indica*, aqueous extract showed the concentration of 0.784mg/100mg.

The antimicrobial activity of aqueous extract from roots of *Plumbago indica* was evaluated. The extract prevented effectively the growth of both bacteria and fungus. Ciprofloxacin and Fluconazole were selected as a standard drug. The results from the study suggest that the *Plumbago indica* extract show antimicrobial activity against *Klebsiella pneumonia* and *Candida albicans*. They could be used as alternatives to common antimicrobial agents for treatment of microbial infections.

Carrageenan-induced paw edema is a very sensitive and reproducible test used in the screening of new molecules with anti-inflammatory activities. Carrageenan-induced inflammation causes an acute and local inflammatory response that is advantageous for detecting orally active anti-inflammatory agents; therefore, it has significant prognostic value for anti-inflammatory agents acting through mediators of acute inflammation.

First step of acute inflammatory response is characterized by edema often formed because of exudation of fluid and plasma proteins.

In our work we found that edema formation was reduced significantly at 6 h post-CAR. Additionally, Carrageenan-induced paw edema leads to sensitization of primary sensory neurons, essentially event to inflammatory pain.

In humans, this nociceptor sensitization usually leads to clinical conditions known as hyperalgesia defined as an increased response to a painful stimulus or allodynia described as pain evoked by non-noxious stimuli.

In our study, we have proven that oral administration of aqueous extract of *Plumbago indica* 30 min before Carrageenan was in grade to reduce hyperalgesia and allodynia significantly at 6 h post- Carrageenan. Edema and pain in the hind paw of animals as a result of Carrageenan -induced inflammation usually limit their motility and cause trouble in using their hind paw. During a Carrageenan -induced acute inflammation event, paw tissue loses normal muscle architecture and shows important amassing of infiltrating inflammatory cells and increased inter-fiber space during physical observation. During our study, we found that oral administration of *Plumbago indica* decreased infiltrating inflammatory.

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