PHARMACOGNOSTIC AND PHYTOCHEMICAL ANALYSIS OF SELECTED MEDICINAL PLANTS USED IN AYURVEDA FOR THE TREATMENT OF ARTHRITIS

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CERTIFICATE

This is to certify that the dissertation titled "**PHARMACOGNOSTIC AND PHYTOCHEMICAL ANALYSIS OF SELECTED MEDICINAL PLANTS USED IN AYURVEDA FOR THE TREATMENT OF ARTHRITIS**" is an authentic record of work carried out by **AISWARYA RAJU** under the supervision and guidance of **Dr. LIZA JACOB**, Associate Professor, Department of Botany & Centre for Research, St. Teresas's College (Autonomous), Ernakulam in partial fulfilment of the requirement for the Master's Degree of Science in Botany.

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INTRODUCTION

Plants are considered as one of the most important sources of medicines. Among the 2,50,000 higher plant species reported in the world, more than 80,000 species are being used as medicinal. The medicinal plants are extensively utilized throughout the world and are not only a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of world population.

In India, approximately 3000 plant species are known to have medicinal properties and being used in our traditional systems of medicines, viz. Ayurveda, Yunani, Siddha, Homeopathy etc. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. (Jain *et. al.*,1968).

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, flavonoids etc. About 61% of new drugs developed between 1981 and 2002 were based on natural products and the have been very successful, especially in the areas of infectious diseases and cancer. The pre-historic period in India, works of Charaka and susrutha namely charakasamhitha were additions of knowledge about medicine. Charakasamhitha deals with about 700 drugs, a few of which are indigenous to India. Today there are about 2000 drugs in use (Sharma,1995).

There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities and their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and future studies. In the development of human culture medicinal plants have played an essential role, for example religions and different ceremonies. Among the variety of modern medicines, many of them are produced indirectly from medicinal plants, for example aspirin. Many food crops have medicinal effects, for example garlic. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.

The medicinal effects of plants are due to secondary metabolite production of the plants. Keeping this consideration there have been increased waves of interest in the field of research in natural product chemistry. This interest can be due to several factors, including therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural compounds as biochemical probes, the development of novel and sensitive techniques to detect biologically

active natural products, improved techniques to isolate, purify, and structurally characterise these active constituents, and advances in solving the demand for supply of complex natural product. The importance of traditional medicine has also recognized by WHO and has created strategies, guidelines and standards for botanical medicines. For the cultivation, processing of medicinal plants and the manufacture of herbal medicines. For the cultivation, processing of medicinal plants and the manufacture of herbal medicines agro-industrial technologies need to be applied. Medicinal plants are resources of new drugs and many of the new drug and many of the modern medicines are produced indirectly from plants (Clark,1996).

PHARMACOGNOSY

Pharmacognosy (the science of biogenic or nature-derived pharmaceuticals and poisons) has been an established basic pharmaceutical science taught in institutions of pharmacy education for over two centuries. Over the past 20 years though it has become increasingly important given the explosion of new drugs, phytomedicines (plant medicines), nutraceuticals and dietary supplements – all of which need to be fully understood, tested and regulated

During the past 50 years there have been tremendous advances in chemical and biological techniques of analysis that have transformed research in pharmacognosy. The PSE has regularly held symposia of relevance to pharmacognosy and some of these are briefly reviewed in the area of natural products from higher plants. These symposia have charted the developments that link pharmacognosy with phytochemistry and illustrate the application of increasingly more sophisticated analytical techniques to the discovery of biologically active compounds. Plants have yielded clinical drugs, either as natural product molecules, or as synthetic modifications (Phillipson *et. al*.,2007).

The term pharmacognosy as a constituent scientific discipline of pharmacy has been in use for nearly 200 years, and it refers to studies on natural product drugs. During the last half of the 20th century, pharmacognosy evolved from being a descriptive botanical subject to one having a more chemical and biological focus. At the beginning of the 21st century, pharmacognosy teaching in academic pharmacy institutions has been given new relevance, as a result of the explosive growth in the use of herbal remedies (phytomedicines) in modern pharmacy practice, particularly in western Europe and North America.

In turn, pharmacognosy research areas are continuing to expand, and now include aspects of cell and molecular biology in relation to natural products, ethnobotany and phytotherapy, in addition to the more traditional analytical method development and phytochemistry.

Herbal medicines are complex compounds with multiple synergistic mechanisms of action that modulate (patho) physiological functions. Pharmacognosy is the study of medicine derived from natural sources that include plants, animals, and microorganisms, and the scope of the field depends on knowledge about the safety, purity, and efficacy of complex multi compound products. Herbal pharmacognosy is the application of this science specifically to traditional herbal medicine sources. Traditional medicines, particularly herbal medicine, remain the primary source of medicine in many countries and cultures globally. Although the root of this field is within traditional medicine, there is increased scientific focus on herbal pharmacognosy in recent years for novel therapeutic molecules.

Modern pharmacognosy includes the application of molecular, genomic, and metabolomic techniques, providing a significant increase in knowledge on the biological and clinical applications of herbal medicines. Secondary plant metabolites serve numerous roles in plant biology, including innate immunity, defence against herbivores and pathogens, antioxidant activity, and attraction of pollinators for cellular communication. These compounds have been used by humans throughout recorded and pre-recorded history as various commodities, including pigments, condiments, nutrition sources, and medicines.

The oldest form of traditional Asian medicine is Ayurveda, which is basically Hindu in origin and which is a sort of art-science-philosophy of life. In this respect it resembles traditional Chinese medicine, and like TCM has influenced development of more practical, less esoteric forms of medicine. In 20^{th} century one of the most important events that influenced the use of medicinal plants in the Western world in last century was the serendipitous discovery of the antibacterial properties of fungal metabolites such as benzylpenicillin, by Florey and Fleming in 1928 at St. Mary's Hospital (London). These natural products changed forever the perception and use of plant – derived metabolites as medicines by both scientists and the lay public. Another important development came with the advent of synthetic chemistry in the field of pharmacy. Many of these studies involved compounds that were synthesized because of their potential as colouring material.

A large number of natural products or their derivatives were introduced as medicines, including many anti-cancer agents, the anti-malarial agent artemisinin and the anti-dementia medication

galantamin. Numerous examples of drugs which are natural products, their derivatives or a pharmacophore based on a natural product have given rise to such compounds, as well as the biochemical basis of many important illnesses. This has opened up new opportunities and avenues for drug development (Wallis,2002).

The systematic study of herbal remedies offers pharmacognosy groups an attractive new area of research, ranging from investigating the biologically active principles of phytomedicines and their mode of action and potential drug interactions, to quality control, and involvement in clinical trials (Kinghorn *et. al*., 2001).

Arthritis is the swelling and tenderness of one or more joints. The main symptoms of arthritis are joint pain and stiffness, which typically worsen with age. The most common types of arthritis are Osteoarthritis and Rheumatoid arthritis.

It is a symptomatic disorder of chronic joint inflammation followed by swelling and pain. It occurs due to malfunctioning of the immune system or, from the family background or, from some injuries of joints in childhood. It can affect the cartilages and bones places around the affected joints and the internal organs like eyes, heart and lungs. Arthritis usually observed in the hand, feet or, wrist of the human body. Phytochemicals are most helpful in the treatment of arthritis, which are very much effective in inflammatory, autoimmune and infectious diseases.

Arthritis is especially of two types, rheumatoid arthritis (RA) and osteoarthritis (OA). RA is an autoimmune disease followed by chronic inflammation. This type of arthritis is happening due to hyperplasia of synovial membrane, which causes large-scale bone destruction around the joints. Some symptoms are there like pain, stiffness, restricted movement etc. with cardiovascular, skeletal and physiological disorders. There are some medications such as nonsteroidal anti-inflammatory drugs (NSAID's) and steroids, which can control RA. But these NSAID's have some side effects such as such as gastric ulceration and acute renal failure.

Women are more affected by RA rather than men. In another side OA is a disease of articular cartilage i.e., protective tissue presents at the end of the joints, which is wearing down day by day. It is causes joint pain and disability in movement followed by formation of osteophyte, joint space narrowing and chronic synovial inflammation. It also affects entire synovial joint like synovium, meniscus, ligaments etc (Bhattacharya *et.al.,* 2020)

Age is the most powerful risk factor for osteoarthritis (OA) in the United States. It is estimated that 68% of individuals older than 55 years have radiographic evidence of OA. The US is

growing older--the over-65 age group represented only 4% of the population in 1900, but accounted for 12.4% in 1988, and is projected to account for 22% by the year 2030. As the age of our population has increased, so has the prevalence of arthritis. About 43 million individuals (1 in 6) have arthritis, and most are older than 45 years. By the year 2020, 59.4 million persons in the US will be affected by arthritis, thus increasing chronic disability and costs by more than 25%. The annual cost to society in medical care and lost wages is currently estimated to be $65 billion, and is projected to escalate to $95 billion by the year 2000. Physicians who provide care for the increased number of patients with arthritis in the 21st century must be aware of improved therapeutic modalities to reduce arthritis related disabilities, hospitalizations, and complications related to therapy, to minimize the risk of adverse drug reactions, and to preserve function (Elders 2000).

PHYTOCHEMISTRY

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients. They protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson et al., 1998; Mathai, 2000).

Recently, it has been clearly shown that they also have roles in the protection of human health, when their dietary intake is significant (Samrot et al., 2009). Till date over 4,500 phytochemicals have been reported and are classified on the basis of their protective functions, and physical and chemical characteristics, amongst these about 350 phytochemicals have been studied in detail (Koche et al., 2010). Phytochemicals accumulate in different parts of the plants, such as in the root, stem, leaf, flower, fruit and seed. Many phytochemicals, particularly the pigment molecules like anthocyanins and flavonoids, are often concentrated in the outer layers of the various plant parts like leaves and fruits of vegetables. However, the levels of these phytochemicals vary from plant to plant depending upon the variety, climatic growing conditions. These compounds have biological properties such as antioxidant activity, anti-microbial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Hamburger & Hostettman, 1991).

The exact classification of phytochemicals has not been given so far, because of their diverse forms and structures. Classically, the phytochemicals have been classified as primary or secondary metabolites, depending on their role in plant metabolism. Primary metabolites include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary metabolites are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics and glucosides (Hahn, 1998; Ramawatet. al., 2009).

Phenolic Compounds

Phenolic compounds represent the largest category of phytochemicals and are most widely distributed in the plant kingdom. Phenolics are hydroxyl group (–OH) containing class of chemical compounds where the (–OH) group is bonded directly to an aromatic hydrocarbon group. Being a secondary metabolite, they have an important role as defence compounds. Phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes. The three most important groups of dietary phenolics are flavonoids, phenolic acids and polyphenols (Schofield et al., 2001).

Flavonoids

Flavonoids are the largest group of plant phenols and also the most studied one. They are polyphenolic compounds that are ubiquitous in nature and occur as aglycones, glucosides and methylated derivatives. The flavonoids appear to have played a major role in successful medical treatments in ancient times, and their use has persisted up to now. Most flavonoids occur naturally associated with sugar in conjugated form and within any one class, may be characterized as monoglycosidic, diglycosidic etc. Flavonoids have gained recent attention because of their broad biological and pharmacological activities. The flavonoids have been reported to exert multiple biological properties including anti-microbial, cytotoxic, anti-inflammatory and anti-tumour activities (Dai & Mumper, 2010).

Tannins

Chemically, it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers. It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin etc.),

alkaloids, nucleic acids and minerals. On the basis of their structural characteristics, it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins and condensed tannins. Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly diseases such as AIDS and cancers (Schofield et al., 2001).

Alkaloids

Alkaloids are natural products that contain heterocyclic nitrogen atoms and are always basic in character. The name of alkaloids derives from the 'alkaline' nature and it was used to describe any nitrogen-containing base. Almost all the alkaloids have a bitter taste. Alkaloids are significant for the survival of plant because they ensure their protection against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrents) and also against other plants by means of allelopathy (Molineux et al., 1996). The use of alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization. Alkaloids have many pharmacological activities including anti-hypertensive effects (many indole alkaloids), antiarrhythmic effect, anti-malarial activity (quinine), and anti-cancer actions (dimeric indoles, vincristine, vinblastine).

Terpenoids

This class comprises natural products which have been derived from five-carbon isoprene units. Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things and therefore considered as the largest group of naturally occurring secondary metabolites (Elbein et al., 1999). Many of these are commercially interesting because of their use as flavours and fragrances in foods and cosmetics.

Saponin

Most members of this group form stable foam in aqueous solutions such as soap, hence the name 'saponin'. Chemically, saponins, as a group, include compounds that are glycosylated steroids, triterpenoids and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives. The main triterpene aglycone is a derivative of oleanane (Bohlmann et al., 1998). Many saponins are known to be anti-microbial, to inhibit mould, and to protect plants from insect attack. Saponins may be considered a part of plants' defence

systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or Phyto protectants.

Phytochemicals play an important role in the routine healthcare systems worldwide. The major classes of phytochemicals like alkaloids, phenolics, terpenoids and tannins have potential to prevent diseases and act as anti-microbial, anti-inflammatory, anti-oxidant, anti-cancerous, detoxifying agent, immunity potentiating agent and neuropharmacological agent. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals are found to be multifunctional.

Phytochemicals play an important role in the routine healthcare systems worldwide. The major Classes of phytochemicals like alkaloids, phenolics, terpenoids and tannins have potential to prevent diseases and act as anti-microbial, anti-inflammatory, anti-oxidant, anti-cancerous, detoxifying agent, immunity-potentiating agent and neuropharmacological agent. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals are found to be multifunctional. There is, however, much scope for further systematic research in screening Indian medicinal plants for their phytochemicals and assessing their potentiality as crude drug or drug components.

HISTOCHEMISTRY

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. The knowledge about the use of medicinal plants has been acquired through centuries and such plants are still valued even today. Medico scientist practicing allopathy and research minded Vaidya's, Hakims have contributed valuable knowledge regarding efficacy of reputed medicinal plants indigenous to India. Establishment of herbal forms in well selected localities will excise scientific control over the cultivation of medicinal herbs.

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissues in the stem and root, tubers, rhizomes and corn. Starch and proteins are the principal ergastic substances of the protoplast. Tannin is the heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves (xylem) of many plants (Kadam et.al., 1996). Saponins are the rare occurrence. Fats are widely

distributed in the plant body and they probably occur in small amount in every plant cell. Fats are common reserve material in seeds, spores and embryos in meristematic cells. Glucosides are the degradation product of the carbohydrates. Alkaloids are the degradation product of protein.

Histochemistry is a powerful technique for localization of trace quantities of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as protein, lipid, starch, phytin and minerals such as calcium, potassium and iron in rice grains (Krishnan et al.2001; Krishnan and Dayanandan 2003).

SOURCE PLANT

Glycosmis pentaphylla Corr. is a genus within the tribe Clauseneae of the Citroideae subfamily of the Rutaceae. Its range of distribution is centred in south and southeast Asia and extends to south China and Taiwan as well as to New Guinea and north Australia. And there are still many taxonomic problems at the species level that remain to be solved.

The plant is often used in traditional medicine, both on its own and as an ingredient of various medicinal mixtures. Several alkaloids and amides that have been isolated from the plant are reported to have biological activities. Glycozolidol, a carbazole alkaloid isolated from the roots, is active against some gram-positive and gram-negative bacteria. Leaf and stem bark extracts have been shown to have a healing effect upon damaged liver tissue. Extracts of the root bark have been shown to exhibit significant activity in the treatment of diarrhoea. An ethanol extract was found to be more effective at lower dosages than an aqueous extract. A decoction of roots and leaves is taken for intestinal trouble. An infusion of leaves and roots is given after childbirth as a protective medicine. The leaves are considered appetitive, stomachic and an infusion of roasted leaves is prescribed for women after delivery as an appetizer. In traditional Indian medicine, the plant is used to treat diarrhoea, coughs, rheumatism, anaemia, and jaundice.

Vitex negundo Linn. (Verbenaceae) is a woody, aromatic shrub growing to a small tree. It commonly bears tri- or penta-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. It thrives in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Sri Lanka, Thailand, Malaysia, eastern Africa and

Vitex negundo L. (Verbenaceae) is a hardy plant, flourishing mainly in the Indian subcontinent. All parts of the plant, from root to fruit, possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. It is interesting to note that a single plant species finds use for treatment of a wide spectrum of health disorders in traditional and folk medicine; some of which have been experimentally validated. The plant is a component of a number of commercially available herbal formulations and has also shown potential as an effective bio-control agent. (Vishwanathan et.al.,2010).

Calotropis gigantea L. is a tall shrub with yellowish-white bark, and oblong thick leaves and purplish or white flowers in the family Asclepiadaceae. In ancient ayurvedic medicine the plant Calotropis gigantea is known as "Sweta Arka" and *Calotropis procera* as "Raktha Arka". Both of them are often similar in their botanical aspects and also have similar pharmacological effects. *Calotropis gigantea* L. is drought resistant, salt tolerant to a relatively high degree, grows wild up to 900 meters (msl) throughout the country and prefers disturbed sandy soils with mean annual rainfall: 300-400 mm. Through its wind and animal dispersed seeds, it quickly becomes established as a weed along degraded roadsides, lagoon edges and in overgrazed native pastures. It has a preference for and is often dominant in areas of abandoned cultivation especially disturbed sandy soils and low rainfall. It is assumed to be an indicator of over cultivation.

Moringa oleifera Lam, is a natural as well as cultivated variety of the genus *Moringa* belonging to family Moringaceae. It is one of the richest plant sources of Vitamins A, B {1,2,3,6,7}, C, D, E and K. The vital minerals present in *Moringa oleifera* Lam include Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc. It has more than 40 natural anti-oxidants. The leaves, pods, seeds, gums, bark and flowers of *Moringa oleifera* Lam are used in more than 80 countries {including Pakistan} to relieve mineral and vitamin deficiencies, support a healthy cardiovascular system, promote normal blood-glucose levels, neutralize free radicals {thereby reducing malignancy}, provide excellent support of the body's anti-inflammatory mechanisms, enrich anaemic blood and support immune system. It also improves eyesight, mental alertness and bone strength. It has potential benefit in malnutrition, general weakness, lactating mothers, menopause, depression and benefits, nutritional value, therapeutic use osteoporosis. It is also used to make an efficient fuel, fertilizer and livestock feed. *Moringa oleifera* Lam is an edible extremely safe plant. Its tree could easily and cheaply

be cultivated and grown in Pakistan. We need to explore therapeutic, nutritional and benefit of this gift of nature reported to be one of the world's most useful trees.

OBJECTIVES OF THE PRESENT STUDY

- To study the pharmacognostic aspects of the plants *Glycosmis pentaphylla* Corr., *Vitex negundo L., Calotropis gigantea* L., *Moringa oleifera* Lam. used in the treatment of disease arthritis.
- To determine the important phytochemical constituent in *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam.
- To study the histochemical characters of *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam.
- To study the anatomical features of *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam.

REVIEW OF LITERATURE

Medicinal plants used against various inflammatory biomarkers for the management of rheumatoid arthritis were studied by (Shareen Singh 2020). This study highlights the properties of different plants which acts against arthritis. *Moringa oleifera* Lam was one among that. The various plant parts like seed and leaf possess antioxidant and anti-inflammatory property. The ethanolic extract of moringa leaves proved to be potent anti-arthritis at a dose of 250 mg/kg and tends to inhibit the inflammatory paw oedema in both acute and chronic phases of CFA-induced arthritic model. There was significant inhibition of production of nitric oxide (NO) by macrophage cells, reduction in the levels of cytokines (IL-1, IL-6 and TNF-alpha) and also the inhibition of autacoid (COX2 and PGE2) involvement in inflammation. Hence, the effective results of various studies concluded that the plant tends to possess potential beneficial medicinal effects for the treatment of arthritis.

Anti-inflammatory activity was studied on ethanolic extract of leaves of *Glycosmis pentaphylla* Corr by (Prawej Ansari 2015). The study was carried out with the ethanolic extract of leaves of *Glycosmis pentaphylla* Corr. This study investigated the blood corpuscular protective power of this plant as it used as blood tonic in Chinese traditional medicine. By the phytochemical screening, it showed both flavonoids and steroids. This study shows that *G. pentaphylla* Corr has property of pain or inflammation healing and from phytochemical analysis they found the presence of alkaloid and steroid, so it could also have anti-coagulation property as well and it seems as an ideal anti-inflammatory agent.

Pharmacological studies on *Glycosmis pentaphylla* Corr. whole plant was studied by (Kishore Sarkar 2013). In this study ethanolic extract of whole plant of *Glycosmis pentaphylla* Corr. was pharmacologically investigated to survey and assess the antioxidant, analgesic and antibacterial activities. In scavenging assay by DPPH, a stable radical, the extract showed a significant inhibition of scavenging activity of DPPH radical in concentration dependent manner, where IC50 value of the extract was 32µg/mL which was comparable to the IC50 value of the standard ascorbic acid, 16 µg/mL. In antibacterial activity test performed by disc diffusion method, the extract showed activity against the bacterial strains including *S. aureus, S. dysenteriae, S. paratyphi and S. Typhi* at the dose of 250 µg/disc and 500µg/disc. The results suggest that the ethanol extract of *G. pentaphylla* Corr could be a potential source of antioxidant, analgesic and antibacterial activity and demands for further pure compound isolation to identify the underlying mechanism.

A study was conducted on *Glycosmis pentaphylla* Corr. by (Natarajan et.al. 2020) to isolate Potential Bioactive Arborine and Skimmianine Compounds for Controlling Multidrug-Resistant *Staphylococcus aureus*. The isolation and structural characterization of bioactive compounds were carried out using various chromatographic techniques (TLC, column, HPLC, and LC-MS) and spectral studies such as FT-IR, CHNS analysis, ^{1}H-NMR, and ^{13}C-NMR. The antimicrobial potential of isolated compounds was assessed according to the standard methods. The isolated compounds were identified as arborine and skimmianine, which exhibited a significant antibacterial effect with the lowest MIC and MBC values against MDR *S. aureus* and *in vitro* kinetic and protein leakage assays supported the antimicrobial activity. This study concludes that the isolated arborine and skimmianine compounds from *G. pentaphylla* Corr. harbor a strong antibacterial activity against MDR *S. aureus* and may be used as alternative natural drugs in the treatment of MDR *S. aureus*.

A study on 14 species of Thai *Glycosmis* in "Thai Plant Names, Tem Smitinand, Revised Edition". Were conducted by (Stone, 1985). Thai *Glycosmis* species are still poorly known. The actual number of existing species of Glycosmis in Thailand was not previously enumerated. *G. pentaphylla* Corr. represents the most widespread and variable species within this genus. Morphological characteristic is various in pair and form of foliage, size and position of inflorescence (Stone, 1985).

In vitro mechanistic and in vivo anti-tumour studies of *Glycosmis pentaphylla* Corr (Retz.) DC against breast cancer was studied by (Shoja et.al. 2016). The present study is aimed at elucidating the effect of *Glycosmis pentaphylla* Corr (Retz.) DC on the key markers of apoptosis, metastasis and angiogenesis, in vitro. This study also evaluated the effect of fractions in vivo in DMBA-induced mammary tumour model. This study showed that the fractions induced apoptosis in breast cancer cells through the intrinsic/mitochondrial apoptotic pathway. The fractions were also able to inhibit the metastatic and angiogenic markers, MMP-9 and HIF-1α. Anti-tumour studies in DMBA-induced mammary model in Sprague Dawley rats also showed favourable results.

Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo L.* was studied by (Dharmasiri et.al. 2006). This study confirmed the oral anti-inflammatory, analgesic and antihistamine properties of mature fresh leaves (MFL) of *Vitex negundo* L. (Verbenaceae) claimed in the Ayurveda medicine by orally treating a water extract of the leaves to rats. This study concludes that the fresh leaves of *Vitex negundo* L. have anti-inflammatory

and pain suppressing activities possibly mediated via PG synthesis inhibition, antihistamine, membrane stabilising and antioxidant activities. The antihistamine activity can produce the anti-itching effect claimed in Ayurveda medicine.

Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo* L. was studied by (Praveen Kumar et.al. 2010). This study identified the phytochemicals present in the *vitex negundo* L. leaves and also evaluated the total phenols, total flavonoids and antioxidant activity of the leaf extract. Total phenol was carried out by Folin ciocalteu method. The antioxidant activity was evaluated by DPPH method and the leaves of *v. negundo* L. showed 23.21 mg/100 of ascorbic acid equivalent antioxidant capacity. The GC-MS study also carried out and it showed the presence of phytochemicals like 4H -Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6-methyl.

Phytochemical and Pharmacological Profile of *Vitex negundo* L. was studied by (Cheng-Jian Zheng et.al. 2015) This article reviewed all the chemical constituents and pharmacological properties of *Vitex negundo* L. (Verbenaceae) (VN). Total of 120 compounds isolated from VN can be divided mainly into four classes: flavonoids, lignans, terpenoids and steroids. The crude extracts and purified compounds of VN exhibited promising bioactivities, including anti-nociceptive, anti-inflammatory, anti-tumour, anti-oxidant, insecticidal, antimicrobial, anti-androgenic, anti-osteoporotic, anti-cataract, hepatoprotective and anti-hyperglycaemic activity. All the reported data lead to a conclusion that VN has convincing medicinal potential.

An In Vitro study of antibacterial activity on aqueous extract of *Calotropis gigantea* L. was conducted by (Gaurav Kumar et.al. 2010). In this study the leaves of *Calotropis gigantea* L. were screened for the antimicrobial activity against clinical isolates of bacteria. The aqueous extract of the *C. gigantea* L. was studied for its antagonistic activity against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Micrococcus luteus* and *Klebsiella pneumoniae*. In vitro antimicrobial activity was performed by well diffusion method in MH agar. The extract showed significant effect on the tested organisms. The extract showed maximum zone of inhibition against E. coli (17.6±1.15), whereas, lowest against K. pneumoniae (12.6±1.52). Crude extract showed maximum relative percentage inhibition

against B. cereus (188.52 %) and lowest relative percentage inhibition against M. luteus (24.92 %).

Gaurav Parihar& Neelam Balekar (2016) had studied the anti-inflammatory activity of latex of *Calotropis gigantea L.* Different parts of the plant showed the presence of various phytochemicals containing cardiotonic agents such as calotropin, calotropagenin, calotoxin, calotropagenin and voruscharine, steroids, di and triterpenes such as stigmasterol, β-sitosterol, flavonoids, polyphenolic compounds, and various newer reported hydrocarbons and proteins. This study concludes that it has the potential to be used as an antiarthritic agent. The latex (DL) of the plant *C. procera* has been reported to exhibit potent anti-inflammatory activity against carrageenan and formalin that are known to release inflammatory mediators.

Analgesic activity of *Calotropis gigantea* L. flower was studied by (Pathak 2011). In this study the alcoholic extract of the flowers of *Calotropis gigantea* L. was administered orally and explored for its analgesic activity in chemical and thermal models in mice. In acetic acid induced writhing test, an inhibition of 20.97% and 43.0% in the number of writhes was observed at the doses of 250 and 500 mg/kg, respectively. In the hot plate method, the paw licking time was delayed. The analgesic effect was observed after 30 min of dose administration which reached its maximum after 90 min.

A Study on Wound healing activity of *Calotropis gigantea* L. root bark in rats was conducted by (Pradeep et.al. 2009). This study investigated the effects of *Calotropis gigantea* L. root bark on wound healing activity in rats by excision, incision and dead space wound healing models in rats. Wistar albino rats of either sex weighing between 180 and 200 g were topically treated with extract formulated in ointment by using simple ointment BP as base. Rats of standard groups were treated with 5% Povidone iodine ointment topically. Topical application of *Calotropis gigantea* L. in excision wound model increased the percentage of wound contraction. Scar area and epithelization time were decreased. In incision wound and dead space wound breaking strength of wounds and hydroxyproline was increased.

Phytochemical and cytotoxic studies on the leaves of *Calotropis gigantea was studied by (*Khang Guyen et.al. 2017). A new lignan, 9′-methoxypinoresinol , and two new glycosylated 5-hydroxymethylfurfurals, calofurfuralside A , and calofurfuralside , together with nine known compounds have been isolated from the active fractions, $CHCl_3$ and EtOAc fractions of the

leaves of *Calotropis gigantea* L. Their structures were elucidated based on NMR and MS data. Among the isolated compounds, compounds **1** and **9** exhibited potent cytotoxicity against PANC-1 human pancreatic cancer cell line under the normoglycemic condition with IC_{50} values of 3.7 and 3.3 μM, respectively. 9′-Methoxypinoresinol (**1**) significantly inhibited the colony formation of PANC-1 cells in a concentration-dependent manner.

Adak et.al. (2006) evaluated the anti-inflammatory activity of *Calotropis gigantea* L. (AKANDA) in various biological system. The anti-inflammatory activity was evaluated using carrageenin-induced kaolin -induced rat paw oedema for acute and cotton-pellet granuloma, adjuvant-induced arthritis model for chronic inflammation. Antipyretic activity was carried out using yeast induced pyresis method. Phenyl quinone induced writhing method in mice was used for analgesic activity. Test compounds exhibited variable anti-inflammatory activity and peak activity of the test compounds were reached at 2 h. Alkaloid fraction possesses comparatively high initial anti-inflammatory activity. The residual anti-inflammatory activity of alkaloid fraction of *Calotropis gigantea* L. suggests either a greater protein binding nature of the compound there by providing a slow released pool of active drug molecule in the system or non-available of possible bioactive metabolites to retain the activity profile relation.

Health benefits of *Moringa oleifera* Lam. was documented by (Abdull Razis et.al. 2014). This study revealed that *Moringa oleifera* Lam contains essential amino acids, carotenoids in leaves, and components with nutraceutical properties, supporting the idea of using this plant as a nutritional supplement or constituent in food preparation. An important factor that accounts for the medicinal uses of Moringa oleifera is its very wide range of vital antioxidants, antibiotics and nutrients including vitamins and minerals. This study concludes that almost all parts from Moringa can be used as a source for nutrition with other useful values.

Nutritional characterization of Moringa *(Moringa oleifera Lam.*) leaves was studied by (Moyo et.al. 2011). The objective of the study was to assess the nutritional value of *Moringa* leaves of the South African ecotype. Proximate and Van Soest methods were used to determine the nutritional value of *Moringa leaves*. The dried leaves had the following mineral contents: calcium, phosphorus, magnesium, potassium, sodium, sulphur, zinc, copper, manganese, iron and selenium. Vitamin E had the highest concentration of 77 mg/100 g than beta-carotene, which had 18.5 mg/100 g in the dried leaves. The fibre content was neutral detergent fibre (NDF) (11.4%), acid detergent fibre (ADF) (8.49%), acid detergent lignin (ADL) (1.8%) and (acid detergent cellulose (ADC) (4.01%).

Phytochemistry and Pharmacology of *Moringa oleifera* Lam was studied by (Birendra Kumar 2010). This study discusses the phytochemical composition, medicinal uses & its pharmacological activity. The present data suggest that the Methanolic and aqueous extract of root and bark, methanolic extract of leaves and flowers and ethanolic extract of seeds of *Moringa oleifera* Lam showed anti-inflammatory activity. In-vitro anti-inflammatory activity from the hot water infusions of flowers, leaves, roots, seeds and stalks or bark of *Moringa oleifera* Lam using carrageenan-induced and the extract was pharmacologically evaluated.

Determination of flavonoids by LC/MS and anti-inflammatory activity in *Moringa oleifera* Lam. was documented by (Coppin et.al. 2013). In this study twelve flavonoids were identified, including quercetin and kaempferol glucosides and glucoside malonates as major constituents and *Moringa oleifera* Lam was found to exhibit anti-inflammatory activity of constituents-rich varieties.

Manoj Kumbhare et.al. (2011) documented the Anti-Inflammatory and Analgesic Activity of Stem Bark of *Moringa Oleifera* Lam. The analgesic activity of stem bark of *Moringa oleifera* Lam. carried out using acetic acid-induced writhing in mice and tail flick test in rats. The anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema and cotton pellet-granuloma formation in rats. Petroleum and methanolic extracts of *Moringa oleifera* Lam. was used. Treatment with Methanol extract showed significant inhibition of carrageenan induced rat paw edema. Maximum inhibition was observed at 400 mg/kg dose as compared to the control. So, the result obtained indicates that *Moringa oleifera* Lam. has analgesic and anti-inflammatory activities that supports the folk medicinal use of the plant.

MATERIALS AND METHODS

The present study was carried out using selected plants used for the treatment of arthritis.

Glycosmis pentaphylla Corr. is an evergreen shrub or small tree growing up to 5 metres tall which belongs to the family Rutaceae.

Vitex negundo L. is shrubs or trees rarely woody lianas. Stem and branches obtusely quadrangular. It belongs to the family Verbenaceae.

Calotropis gigantea L. is a large shrub growing to 4m tall. It has clusters of waxy flowers that are either white or lavender in colour. This belongs to the family Asclepiadaceae.

Moringa oleifera Lam is a fast-growing, drought -resistant tree of the family Moringaceae, native to the Indian subcontinent. Common names include moringa, drumstick tree.

The following were the parameters of the study:

1. PHARMACOGNOSTIC ANALYSIS

1.1 ORGANOLEPTIC EVALUATION

1.2 MICROSCOPIC STUDIES

1.2.1 STOMATAL TYPE AND STOMATAL INDEX

1.2.2 PALISADE RATIO

1.2.3 VEIN -ISLET NUMBER

1.2.4 VEIN- TERMINATION NUMBER

1.2.5 ANATOMY

2. HISTOCHEMICAL STUDIES

2.1 STARCH
2.2 POLYPHENOL
2.3 LIPIDS

3. PHYTOCHEMICAL ANALYSIS

1 PHARMACOGNOSTIC ANALYSIS

The main features studied are follows:

1.1 Organoleptic evaluation

organoleptic evaluation includes the study of the nature of the powdered leaf drug, for this, the colour, taste, and texture of the drug were noted.

1.2 Microscopic studies

1.1.1 Determination of stomatal type and stomatal index

The term stomatal index was first introduced by (Salisbury 1932). The percentage proportion of the ultimate divisions of the epidermis of a leaf which have been converted in to stomata is termed the stomatal index. For determining the stomatal index, the fresh leaves of the plant were taken. From the lower surface of the leaves, epidermal peels were taken. It was then stained with saffranine. The peel was placed in a glass slide and a drop of glycerine was put over it and covered with a cover glass without air bubbles. The slide was examined under the compound microscope. After this type of stomata, number of epidermal cells and stomata in the field were noted. The stomatal index was calculated using the formula:

$$\text{Stomatal index} = \frac{S \times 100}{E + S}$$

Where,

S = Number of stomata per unit

E = Number of epidermal cells in the same area

1.1.2 Palisade ratio

Palisade ratio is defined as the average number of palisade cells beneath each epidermal cell. Fresh leaves of the plant were taken. Then they were boiled with trichloroacetic acid solution. The sample was then mounted in glycerine, and focused under high magnification. Then the focal length of the microscope was adjusted to see the palisade cells were counted from each field below four adjacent epidermal cell and the ratio was

determined by dividing the total number of palisade cells by 4. The average palisade ratio was taken.

Palisade ratio furnishes an important data for leaf drug evaluation, which can be successfully applied for the studies of several dicot leaves of medicinal importance.

$$\text{Palisade ratio} = \frac{\text{Number of palisade ratio}}{\text{Number of epidermal cells}}$$

1.1.3 Determination of vein-islet number

The word vein – islet is found for the minute area of photosynthetic tissues encircled by the ultimate divisions of conducting strands. Vein-islet number is defined as the number of vein-islet per square millimetre of the leaf surface midway between the midrib and the margin.

The method of determination of vein-islet number was put forth by (Levin 1929). Leaf pieces were put in trichloroacetic acid. When the leaf become transparent, they are mounted in glycerine. A camera lucida was attached to the microscope. A paper was placed and the vein islet was traced by looking through

1.1.4 Determination of vein termination number

Vein termination number is defined as the number of veinlet termination per square millimetre of leaf surface between midrib and margin. The method of determination of vein termination number was put forth by (Hall & Melville 1954). Leaf pieces were placed in trichloroacetic acid. When the leaves become transparent and clean, one of the pieces was mounted in glycerine. Camera Lucida drawings were made and the number of vein termination process within the 4cm X 4cm square counted and average vein termination number was determined.

1.1.5 Anatomy

Transverse section of leaf was taken and stained, mounted in glycerine on to a micro slide, examined under the microscope and photograph was taken.

2. HISTOCHEMICAL STUDIES

2.1 Starch – iodine-potassium iodide reaction

The fresh leaf epidermal peelings and stem section were treated with iodine-potassium iodide solution and mounted in the same. Presence of starch can be confirmed by the appearance of black grains.

2.2 Polyphenols -Toludine blue O Method

The peelings were stained in Toludine blue O for 5 minutes, washed in running water, retained in distilled water for some time and then mounted in glycerine. The polyphenols when present stained turquoise blue.

2.3 Lipids – Sudan Dye Method

The fresh peelings were kept in 50% ethanol for 10 minutes, washed in 50% ethanol and mounted in glycerine. The fats, oils, waxes and free fatty acids stained blue-black.

3. PHYTOCHEMICAL ANALYSIS

(A) QUALITATIVE ANALYSIS

1.1 Detection of Alkaloids

For the preparation of plant extraction 1 gm of powdered sample of leaf was homogenized using 80% ethanol (10ml). The homogenate is centrifuged at 15000 rpm for 5 minutes and the supernatant was collected.

For the detection of alkaloids Mayer's, Wagner's, and Dragendroff's reagent was prepared.

(1) Mayer's reagent

It was prepared by dissolving 1.36g of Hgcl2 in 60 ml distilled water (Solution A) and 5g of KI dissolved in 10ml distilled water (Solution B). Both these solutions were mixed and diluted to 1000ml with distilled water. The extract was acidified with HCl before adding Mayer's reagent.

(2) Wagner's reagent

It was prepared by dissolving 1.27g of iodine and 2.9g of KI in 5ml distilled water and the solution were made up to 100ml.

(3) Dragendroff's reagent

It was prepared by dissolving 8g of bismuth sulphate in 20ml of Conc. HNO3(solution A) and 27.2g in 50ml distilled water (solution B). The solution was mixed, supernatant solution was decanted and made up to 100ml with distilled water.

After the preparation of reagents small amount of plant extract (only aqueous extract) was taken and tested with all the 3 reagents and the formation of precipitate shows the presence of Alkaloids or Absence of alkaloids.

1.2 Detection of saponins – foam test

1gm of powdered plant material life was taken in 20ml distilled water. It was shaken for 10 minutes. Formation of frothy solution indicates the presence of saponins.

1.3 Detection of Flavonoids – Alkaline reagent test

To a little of the sample of leaf powder add 2ml of NaOH followed by 2ml of dilute H2SO4. The yellow colour in NaOH changes when dilute acid is added. This shows presence of flavonoids.

1.4 Detection of Tannin – Ferric chloride test

Little of sample were mixed with water and filtered. The filtrate was mixed with 2ml of 5% Ferric chloride solution in a test tube. Formation of green, blue or black colour indicates the presence of tannin.

2.5 Detection of steroid – Salkowski test

The aqueous extract was treated with few drops of concentrated sulphuric acid. A red colouration in lower layer of the solution indicates the presence of steroid.

2.6 Detection of Bitters

1 gm of powdered sample were shaken with ethyl alcohol and then with ethyl acetate formation of green colour shows the presence of Bitter.

2.7 Detection of Protein

(1) Biuret test

A few drops of CUSO4 solution were added to 2ml of the test solution, aqueous extract of leaf powder followed the addition of 40% NaOH solution. It was mixed thoroughly and the colour was noted. Purple colour indicates the presence of Protein.

(2) Xanthoproteic test

Equal volume of test solution (aq. Extract) and Conc. HNO3(0.5ml) were mixed well and the solution was cooled to room temperature. The colour was noted. Yellow colour indicates the presence of protein. Again 40% NaOH was added to make the solution alkaline. The colour was noted again. Yellow colour changes to bright orange which indicates the presence of protein.

2.8 Detection of Resin

50% of HNO3 was added to a little of the powdered sample. Brown colour obtained indicates the presence of resins.

2.9 Detection of Carbohydrate

(1) Molisch's test

2 Drop of Molisch's reagent was added to 2ml of the test solution and was mixed well. 1ml Conc. H2SO4 was added along the sides of the test tube without shaking.

(2) Benedict's test

5ml of Benedict's reagent was added to 1ml of test solution and the test tube was kept in boiling water for 5 minutes.

(3) Fehling's test

1ml of Fehling 's reagent A was mixed with 1 ml of Fehling's reagent B to which a few drops of test solution are added and boiled.

2.10 Detection of Phenol

Two drops of 5% FeCl3 of the extract in a test tube. Presence of greenish precipitate indicated the presence phenolics.

(B) QUANTITAIVE ANALYSIS

ESTIMATION OF FLAVONOID

Sample preparation

A ground freeze dried sample of 0.5 g was weighed and flavonoid compound was extracted with 50ml of 80% aqueous methanol on an ultrasonic bath for 20mts. An aliquot 2ml of the extract was ultracentrifuged for 5mts at 14000rpm.

Total Flavonoid assay

Total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquot (1ml) of extract or standard solution of catechin (20,40,60,80,100) was added to 10ml volumetric flask containing 4ml of distilled water. To the flask was added 0.3ml 5% NaNO2. After 5 minutes 0.3ml of 10% of aluminium chloride was added. At the 6^{th} minute,2ml 1M NaOH was added and the total volume was made up to 10ml with distilled water. The solution was mixed and the absorbance was measured against prepared reagent blank at 510nm.

ESTIMATION OF TANNIN

For the estimation of tannins first indigo sulphonic acid was prepared. It was prepared by adding 1g indigo carmine in 25ml conc. Sulphuric acid in 100ml beaker. The indigo carmine was then dissolved completely in conc. Sulphuric acid. To this again 25ml of conc. Sulphuric acid was added and made up to 1L in volumetric flask using distilled water.

For titration, 0.1N potassium permanganate solution was taken in a burette and 25ml of the Indigo sulphonic acid was taken in 1L conical flask. It was then titrated and blank value was taken. Then 1g of the sample was taken in a conical flask with a rubber cork,100ml distilled water was added to it and shaken well for 10 minutes. It was filtered, 10ml of the sample solution was taken in 1L conical flask and 25ml indigo sulphonic acid was added to the solution

The above solution was titrated with 0.1N Potassium permanganate solution. End point was the change of blue colour to golden yellow colour then the burette reading was taken, 1ml of 0.1N KMnO4 is equal to 0.005647g of tannins.

Total tannin was calculated using the formula:

$$\text{Percentage of tannin} = \frac{\text{Burette reading} - \text{Blank reading} \times 0.005647 \times 10 \times 100}{\text{Weight of the sample}}$$

ESTIMATION OF PHENOLIC COMPOUNDS

1g of both leaf and bark (plant sample) were homogenized separately using 80% ethanol (25ml). the homogenate was centrifuged at 1000rpm for 20mts.The supernatant was collected and residue was again centrifuged and extract was collected. 0.2ml aliquot was taken from this extract in a test tube and 2.8ml distilled water was added. To this sample 0.5ml Folin cio calteau reagent was added and kept for 3 minutes. After that 2ml sodium bicarbonate was added. The test tube was placed in a boiling water bath for 1 mts. And optical density was measured against blank solution using colorimeter. Blank solution was prepared by taking 3ml distilled water. A standard graph was prepared using different concentration of sample and the concentration of phenol was obtained from the graph. It was expressed as mg phenol/100mg material. Phenol concentration of the plant was studied.

ESTIMATION OF CARBOHYDRATE

For the quantitative estimation of carbohydrate, the method adopted was that of Shirlaw and Gilchrist (1967). The method is based on colorimetric observations.

One gram each of Anthrone and Thyo urea was taken. 760ml of concentration H_2SO_4 was added to 240ml of distilled water. Anthrone and thiourea was dissolved in this. 200mg of oven dried and ground leaf sample was boiled for half hours with 20ml of distilled water in a 250ml conical flask. It was filtered and volume was made up to 50ml with distilled water. 1ml of the filtrate was pipetted in colorimetric tube and 10ml of anthrone reagent was added. After stoppering the tubes with rubber plugs then were kept in water bath and were cooled in running water. The coloured density developed was measured calorimetrically at 625nm.

The following equation was used for calculation:

$$\text{Mg. Carbohydrate/100gm of the sample} = \frac{Xa \times 20 \times 500}{\text{Reading the standard OD}}$$

Where Xa is the optical density of the sample

Preparation of standard carbohydrate solution

A Standard carbohydrate solution of 100.0ppm concentration was prepared by dissolving 100mg Glucose in distilled water and making it up to 1000ml.

OBSERVATIONS AND RESULTS

1.PHARMACOGNOSTIC ANALYSIS

1.2 ORGANOLEPTIC EVALUATION

Oven dried fine powder of these plants showed different colours. For *Glycosmis pentaphylla* Corr. & *Moringa oleifera* Lam leaf green colour, for *Vitex negundo L.* pale green, for *Calotropis gigantea* L. fainted green and for *Moringa oleifera* root white colour. The dried fine powder of *Glycosmis pentaphylla* Corr., *Vitex negundo L., Calotropis gigantea L., and Moringa oleifera* Lam leaf and root possess a characteristic odour, aromatic, odourless, aromatic and characteristic odour respectively. And fine powder of these plants tastes different. For *Glycosmis pentaphylla* Corr slightly bitter, for *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam *lea*f bitter and for *Moringa oleifera* Lam root pungent flavour.

1.3 MICROSCOPIC STUDIES

Glycosmis pentaphylla Corr, *Vitex negundo L., Moringa oleifera* Lam possess anomocytic stomata and *Calotropis gigantea* L. possess paracytic stomata. Stomatal index of *Glycosmis pentaphylla* Corr was found to be 10%. For *Vitex negundo L.* it was 6.06%. *Calotropis gigantea* L. shows 17.85% and for *Moringa oleifera* Lam 13.3%.

Palisade ratio of *Glycosmis pentaphylla* Corr was found to be 3.30. For *Vitex negundo L.* it was 2.7. *Calotropis gigantea* L. shows 1.25 and for *Moringa oleifera* Lam 2.3.

Vein islet number is defined as the number of vein islets per square nm of leaf surface midway between the midrib and margin and vein termination number is the number of veins per square mm of leaf surface midway between midrib and margin. Vein islet number and vein termination number for *Glycosmis pentaphylla* Corr was found to be 9 & 8. For *Vitex negundo L.* it was 23 & 7. *Calotropis gigantea* L. shows 10 & 18and for *Moringa oleifera* Lam 10 & 15.

1.4 ANATOMY

Transverse section of leaf of *Glycosmis pentaphylla* Corr shows three major regions namely epidermis, mesophyll and vascular region. Epidermis is the outermost protective part and it covers the whole of its upper and lower surfaces. It has two divisions upper & lower epidermis. Numerous small openings called stomata are found in the epidermis. Lamina and mesophyll are diagnostic characters. Palisade tissue is double layered. Mid rib has non-lignified phloem and lignified xylem. Vascular bundles are amphicribal surrounded by endodermis. Endarch type of xylem.

Transverse section of leaf of *Vitex negundo* L. shows three major regions namely epidermis, mesophyll and vascular region. Epidermis is the outermost protective part and it covers the whole of its upper and lower surfaces. It has two divisions upper & lower epidermis. Numerous small openings called stomata are found in the epidermis. Epidermis is single

layered with unicellular hairs. Followed by One to three layers of hypodermis. 4-8 layer of palisade cells are present. Endarch type of xylem.

Transverse section of leaf of *Calotropis gigantea* L. shows three major regions namely epidermis, mesophyll and vascular region. Epidermis is the outermost protective part and it covers the whole of its upper and lower surfaces. It has two divisions upper & lower epidermis. Upper and lower epidermis is with one row of elongated cells with cuticle and stomata. Numerous small openings called stomata are found in the epidermis. It is followed by 2-3 rows of mesophyll. Xylem and phloem are surrounded by parenchyma cells. Endarch type of xylem.

Transverse section of leaf of *Moringa oleifera* Lam shows three major regions namely epidermis, mesophyll and vascular region. Epidermis is the outermost protective part and it covers the whole of its upper and lower surfaces. It has two divisions upper & lower epidermis. Palisade cells is with one row of elongated cells and it have dense stellate crystals of oxalate. Endarch type of xylem.

2. PHYTOCHEMICAL ANALYSIS

(A) QUALITATIVE ANALYSIS

2.1 Detection of Alkaloids

For detecting alkaloid, the sample were treated with Mayer's reagent, Dragendroff's and Wagner's reagent. *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam *lea*f showed the presence of precipitate formation whereas *Moringa oleifera* Lam root showed the absence of precipitate formation.

2.2 Detection of Saponins

Detection of saponin was done by shaking the powder vigorously with water. The formation of froth indicated the presence of saponins. *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L. and *Moringa oleifera* Lam leaf & root showed the presence of saponin.

2.3 Detection of Flavonoids

For detecting flavonoid, the sample was treated with NaOH and dilute sulphuric acid. Yellow colour obtained showed discolouration on treatment with acid, indicated the presence of flavonoids. *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L., *and Moringa oleifera* Lam leaf & root showed the presence of flavonoids.

2.4 Detection of Tannins

For the detection of tannin, the filtered sample was treated with 5% ferric chloride solution in a test tube and the green colour indicated the presence of tannin in *Glycosmis pentaphylla* Corr, *Vitex negundo L.* and *Calotropis gigantea L.* and black colour indicated the presence of tannin in *Moringa oleifera* Lam leaf whereas *Moringa oleifera* Lam root showed the absence of tannin.

2.5 Detection of Steroid

For steroid the sample was treated with few drops of concentrated sulphuric acid and the red colouration in the lower layer indicated the presence of steroid in *Glycosmis pentaphylla* Corr, *Vitex negundo L. and in Moringa oleifera* Lam root whereas *Calotropis gigantea L. and Moringa oleifera* Lam leaf showed the absence of steroid.

2.6 Detection of Bitter

Detection of bitter was done by shaking the powder with ethyl alcohol followed by the ethyl acetate. The green colour indicated the presence of bitter. *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam *lea*f showed the presence of bitter whereas *Moringa oleifera* Lam root showed the absence of bitter.

2.7 Detection of Protein

The sample solution showed the absence of protein, when it was treated with specific reagent of

2.8 Detection of Resin

When the extract was treated with 50% HNO3, brown colour was observed in *Glycosmis pentaphylla Corr., Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam leaf & root which indicated the presence of resin.

2.9 Detection of Carbohydrate

Presence of carbohydrate in extract was tested using Molisch's reagent, Benedict's reagent and Fehling's reagent. *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea Land Moringa oleifera* Lam leaf & root showed reddish violet zone at the junction of two liquids when treated with Molisch's reagent.

Glycosmis pentaphylla Corr, *Vitex negundo L., Calotropis gigantea L. and Moringa oleifera* Lam leaf & root showed orange red precipitate when treated with Benedict's reagent.

Glycosmis pentaphylla Corr, *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam leaf & root showed red precipitate when treated with Fehling's reagent. All the three tests indicate the presence of carbohydrate.

2.10 Detection of Phenol

For the detection phenol, two drops of 5% FeCl3 was added to the extract in the test tube. Presence of greenish colour indicated the presence of Phenol. *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L., *and Moringa oleifera* Lam leaf showed the presence of phenol whereas *Moringa oleifera* Lam root showed the absence of phenol.

(B) QUANTITATIVE ANALYSIS

ESTIMATION OF TANNIN

The result of tannin content shows that the *Glycosmis pentaphylla* Corr was found to be 14%. For *Vitex negundo L.* it was 67%. *Calotropis gigantea* L. shows 33% and for *Moringa oleifera* Lam leaf 50%

ESTIMATION OF PHENOLIC COMPOUND

Phenolic content of the *Glycosmis pentaphylla* Corr was found to be 2.52 µg/l. For *Vitex negundo L.* it was 2.03 µg/l. *Calotropis gigantea* L. shows 1.25 µg/l and for *Moringa oleifera* Lam leaf 1.51 µg/l.

ESTIMATION OF CARBOHYDRATE

Carbohydrate content of the *Glycosmis pentaphylla* Corr was found to be 0.24 mg/g. For *Vitex negundo L.* it was 1.16 mg/g. *Calotropis gigantea* L. shows 0.22 mg/g. For *Moringa oleifera* Lam leaf and root is 0.22 mg/g & 0.24 mg/g.

ESTIMATION OF FLAVONOID

Flavonoid content of the *Glycosmis pentaphylla* Corr was found to be 5.01mg/l. For *Vitex negundo L.* it was 4.00 mg/l. *Calotropis gigantea* L. shows 3.2 mg/l. For *Moringa oleifera* Lam leaf and root is 2.53 mg/l and 1.5 mg/l.

ESTIMATION OF CHLOROPHYLL CONTENT

Carbohydrate content of the *Glycosmis pentaphylla* Corr was found to be 1.19 mg/g. For *Vitex negundo L.* it was 1.10 mg/g. *Calotropis gigantea* L. shows 1.34 mg/g. For *Moringa oleifera* Lam leaf 1.52 mg/g.

PHARMACOGNOSTIC ANALYSIS

TABLE 1

ORGANOLEPTIC EVALUATION OF THE POWDERED DRUG

Sl. No.	NAME OF THE PLANT	PARTS USED	COLOUR	ODOUR	TASTE
1.	*Glycosmis pentaphylla* Corr	Leaf	Green	Characteristic	Slightly bitter
2.	*Vitex negundo L.*	Leaf	Pale green	Aromatic	Bitter
3.	*Calotropis gigantea* L.	Leaf	Fainted green	Odourless	Bitter
4.	*Moringa oleifera* Lam (leaf)	Leaf	Green	Aromatic	Bitter
5.	*Moringa oleifera* Lam (root)	Leaf	White	Characteristic	Pungent flavour

MICROSCOPIC STUDIES TABLE 2

Sl.no.	Name of the plant	Stomatal type	Stomatal index	Palisade ratio	Vein-islet number	Vein-termination number
1.	*Glycosmis pentaphylla* Corr	Anomocytic	10	3.30	9	8
2.	*Vitex negundo L.*	Anomocytic	6.06	2.7	23	7
3.	*Calotropis gigantea* L.	Paracytic	17.85	1.25	10	18
4.	*Moringa oleifera* Lam	Anomocytic	13.3	2.3	10	15

HISTOCHEMICAL STUDY OF *GLYCOSMIS PENTAPHYLLA* Corr

TABLE 3

SL.NO.	PRINCIPLE	TEST	RESULT
1.	Starch	Iodine potassium iodide test	Present
2.	Polyphenol	Toludine blue O test	Present
3.	Lipid	Sudan dye test	Present

HISTOCHEMICAL STUDY OF *VITEX NEGUNDO* LINN.

TABLE 4

SL.NO.	PRINCIPLE	TEST	RESULT
1.	Starch	Iodine potassium iodide test	Present
2.	Polyphenol	Toludine blue O test	Present
3.	Lipid	Sudan dye test	Present

HISTOCHEMICAL STUDY ON *CALOTROPIS GIGANTEA* L.

TABLE 5

SL.NO.	PRINCIPLE	TEST	RESULT
1.	Starch	Iodine potassium iodide test	Present
2.	Polyphenol	Toludine blue O test	Present
3.	Lipid	Sudan dye test	Present

HISTOCHEMICAL STUDY ON *MORINGA OLEIFERA* Lam

TABLE 6

SL.NO.	PRINCIPLE	TEST	RESULT
1.	Starch	Iodine potassium iodide test	Present
2.	Polyphenol	Toludine blue O test	Absent
3.	Lipid	Sudan dye test	Present

PHYTOCHEMICAL ANALYSIS OF *GLYCOSMIS PENTAPHYLLA* Corr

TABLE 7

SL.NO.	CHEMICAL CONSTITUENTS	PHYTOCHEMICAL TEST	OBSERVATION	RESULT	
1.	TEST FOR ALKALOID	MAYER'S TEST DRAGENDROFF'S REAGENT WAGNER'S REAGENT	Formation of brown colour precipitate	PRESENT	
2.	TEST FOR SAPONINS	FOAM TEST	The formation of froth	PRESENT	
3.	TEST FOR FLAVONOID	ALKALINE REAGENT TEST	yellow colour obtained showed decolouration on treatment with acid.	PRESENT	
4.	TEST FOR TANNIN	FERRIC CHLORIDE TEST	Greenish black colour is formed	PRESENT	
5.	TEST FOR STEROID	SALKOWSKI TEST	Red colouration in the lower layer of solution.	PRESENT	
6.	TEST FOR BITTER	BITTER TEST	No Green colour is formed	ABSENT	
7.	TEST FOR RESIN	NITRIC ACID TEST	Brown colour was observed	PRESENT	
8.	TEST FOR PROTEIN	XANTHOPROTEIC TEST	No Yellow colour obtained changes to bright orange on addition of 40% NaOH	ABSENT	
9.	TEST FOR CARBOHYDRATE	MOLISCH'S TEST	Reddish violet zone at the junction of liquids	PRESENT	
		BENEDICT'S TEST	Orange red precipitate		
		FEHLING'S TEST	Red precipitate		
10.	TEST FOR PHENOL	FERRIC CHLORIDE TEST	Greenish precipitate	PRESENT	

PHYTOCHEMICAL ANALYSIS OF *VITEX NEGUNDO* L.

TABLE 8

SL.NO.	CHEMICAL CONSTITUENTS	PHYTOCHEMICAL TEST	OBSERVATION	RESULT	
1.	TEST FOR ALKALOID	MAYER'S TEST DRAGENDROFF'S REAGENT WAGNER'S REAGENT	Formation of brown colour precipitate	PRESENT	
2.	TEST FOR SAPONINS	FOAM TEST	The formation of froth	PRESENT	
3.	TEST FOR FLAVONOID	ALKALINE REAGENT TEST	yellow colour obtained showed decolouration on treatment with acid.	PRESENT	
4.	TEST FOR TANNIN	FERRIC CHLORIDE TEST	Greenish black colour is formed	PRESENT	
5.	TEST FOR STEROID	SALKOWSKI TEST	Red colouration in the lower layer of solution.	PRESENT	
6.	TEST FOR BITTER	BITTER TEST	Green colour is formed	PRESENT	
7.	TEST FOR RESIN	NITRIC ACID TEST	Brown colour was observed	PRESENT	
8.	TEST FOR PROTEIN	XANTHOPROTEIC TEST	Yellow colour obtained changes to bright orange on addition of 40% NaOH	PRESENT	
9.	TEST FOR CARBOHYDRATE	MOLISCH'S TEST	Reddish violet zone at the junction of liquids	PRESENT	
		BENEDICT'S TEST	Orange red precipitate		
		FEHLING'S TEST	Red precipitate		
10.	TEST FOR PHENOL	FERRIC CHLORIDE TEST	Greenish precipitate	PRESENT	

PHYTOCHEMICAL ANALYSIS OF *CALOTROPIS GIGANTEA* L.

TABLE 9

SL.NO.	CHEMICAL CONSTITUENTS	PHYTOCHEMICAL TEST	OBSERVATION	RESULT	
1.	TEST FOR ALKALOID	MAYER'S TEST DRAGENDROFF'S REAGENT WAGNER'S REAGENT	Formation of brown colour precipitate	PRESENT	
2.	TEST FOR SAPONINS	FOAM TEST	The formation of froth	PRESENT	
3.	TEST FOR FLAVONOID	ALKALINE REAGENT TEST	yellow colour obtained showed decolouration on treatment with acid.	PRESENT	
4.	TEST FOR TANNIN	FERRIC CHLORIDE TEST	Greenish black colour is formed	PRESENT	
5.	TEST FOR STEROID	SALKOWSKI TEST	No Red colouration in the lower layer of solution.	ABSENT	
6.	TEST FOR BITTER	BITTER TEST	Green colour is formed	PRESENT	
7.	TEST FOR RESIN	NITRIC ACID TEST	Brown colour was observed	PRESENT	
8.	TEST FOR PROTEIN	XANTHOPROTEIC TEST	No Yellow colour obtained changes to bright orange on addition of 40% NaOH	ABSENT	
9.	TEST FOR CARBOHYDRATE	MOLISCH'S TEST	Reddish violet zone at the junction of liquids	PRESENT	
		BENEDICT'S TEST	Orange red precipitate		
		FEHLING'S TEST	Red precipitate		
10.	TEST FOR PHENOL	FERRIC CHLORIDE TEST	Greenish precipitate	PRESENT	

PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* Lam LEAF

TABLE 10

SL.NO.	CHEMICAL CONSTITUENTS	PHYTOCHEMICAL TEST	OBSERVATION	RESULT	
1.	TEST FOR ALKALOID	MAYER'S TEST DRAGENDROFF'S REAGENT WAGNER'S REAGENT	Formation of brown colour precipitate	PRESENT	
2.	TEST FOR SAPONINS	FOAM TEST	The formation of froth	PRESENT	
3.	TEST FOR FLAVONOID	ALKALINE REAGENT TEST	yellow colour obtained showed decolouration on treatment with acid.	PRESENT	
4.	TEST FOR TANNIN	FERRIC CHLORIDE TEST	Greenish black colour is formed	PRESENT	
5.	TEST FOR STEROID	SALKOWSKI TEST	No Red colouration in the lower layer of solution.	ABSENT	
6.	TEST FOR BITTER	BITTER TEST	Green colour is formed	PRESENT	
7.	TEST FOR RESIN	NITRIC ACID TEST	Brown colour was observed	PRESENT	
8.	TEST FOR PROTEIN	XANTHOPROTEIC TEST	No Yellow colour obtained changes to bright orange on addition of 40% NaOH	ABSENT	
9.	TEST FOR CARBOHYDRATE	MOLISCH'S TEST	Reddish violet zone at the junction of liquids	PRESENT	
		BENEDICT'S TEST	Orange red precipitate		
		FEHLING'S TEST	Red precipitate		
10.	TEST FOR PHENOL	FERRIC CHLORIDE TEST	Greenish precipitate	PRESENT	

PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* Lam ROOT

TABLE 11

SL.NO.	CHEMICAL CONSTITUENTS	PHYTOCHEMICAL TEST	OBSERVATION	RESULT	
1.	TEST FOR ALKALOID	MAYER'S TEST DRAGENDROFF'S REAGENT WAGNER'S REAGENT	No Formation of brown colour precipitate	ABSENT	
2.	TEST FOR SAPONINS	FOAM TEST	The formation of froth	PRESENT	
3.	TEST FOR FLAVONOID	ALKALINE REAGENT TEST	yellow colour obtained showed decolouration on treatment with acid.	PRESENT	
4.	TEST FOR TANNIN	FERRIC CHLORIDE TEST	No Greenish black colour is formed	ABSENT	
5.	TEST FOR STEROID	SALKOWSKI TEST	Red colouration in the lower layer of solution.	PRESENT	
6.	TEST FOR BITTER	BITTER TEST	No Green colour is formed	ABSENT	
7.	TEST FOR RESIN	NITRIC ACID TEST	Brown colour was observed	PRESENT	
8.	TEST FOR PROTEIN	XANTHOPROTEIC TEST	Yellow colour obtained changes to bright orange on addition of 40% NaOH	PRESENT	
9.	TEST FOR CARBOHYDRATE	MOLISCH'S TEST	Reddish violet zone at the junction of liquids	PRESENT	
		BENEDICT'S TEST	Orange red precipitate		
		FEHLING'S TEST	Red precipitate		
10.	TEST FOR PHENOL	FERRIC CHLORIDE TEST	No Greenish precipitate	ABSENT	

QUANTITATIVE ANALYSIS OF *GLYCOSMIS PENTAPHYLLA* Corr

TABLE 12

SL.NO.	PHYTOCHEMICAL	RESULT
1.	TANNIN	14%
2.	PHENOL	2.52µg/l
3.	CARBOHYDRATE	0.24mg/g
4.	FLAVANOID	5.01mg/l

QUANTITATIVE ANALYSIS OF *VITEX NEGUNDO* L.

TABLE 13

SL.NO.	PHYTOCHEMICAL	RESULT
1.	TANNIN	67%
2.	PHENOL	2.03µg/l
3.	CARBOHYDRATE	1.16 mg/g
4.	FLAVANOID	4.00 mg/l

QUANTITATIVE ANALYSIS OF *CALOTROPIS GIGANTEA* L.

TABLE 14

SL.NO.	PHYTOCHEMICAL	RESULT
1.	TANNIN	33%
2.	PHENOL	1.25µg/l
3.	CARBOHYDRATE	0.22mg/g
4.	FLAVANOID	3.2mg/l

QUANTITATIVE ANALYSIS OF *MORINGA OLEIFERA* Lam LEAF

TABLE 15

SL.NO.	PHYTOCHEMICAL	RESULT
1.	TANNIN	50%
2.	PHENOL	1.51µg/l
3.	CARBOHYDRATE	0.22mg/g
4.	FLAVANOID	2.53mg/l

QUANTITATIVE ANALYSIS OF *MORINGA OLEIFERA* Lam ROOT

TABLE 16

SL.NO.	PHYTOCHEMICAL	RESULT
1.	CARBOHYDRATE	0.24mg/g
2.	FLAVANOID	1.5mg/l

ESTIMATION OF CHLOROPHYLL CONTENT

TABLE 17

NAME OF THE PLANT	CHLOROPHYLL CONTENT
GLYCOSMIS PENTAPHYLLA Corr	1.19 mg/g
VITEX NEGUNDO L.	1.10 mg/g
CALOTROPIS GIGANTEA L.	1.34 mg/g
MORINGA OLEIFERA Lam	1.52 mg/g

PHYTOCHEMICAL ANALYSIS

QUALITATIVE ANALYSIS OF ALKALOID - FIG:1

Glycosmis pentaphylla Corr.

Vitex negundo L.

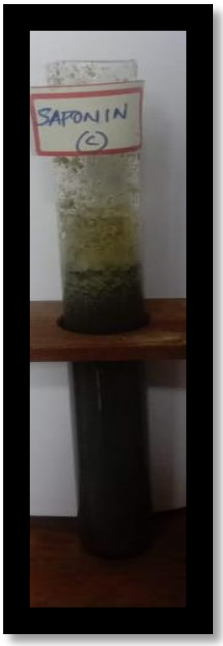
Moringa oleifera Lam. *leaf*

Calotropis gigantea L.

QUANTITATIVE ANALYSIS OF SAPONIN -FIG: 2

Glycosmis pentaphylla Corr.

Vitex negundo L.



Calotropis gigantea L.

Moringa oleifera Lam. *leaf*

Moringa oleifera Lam. root

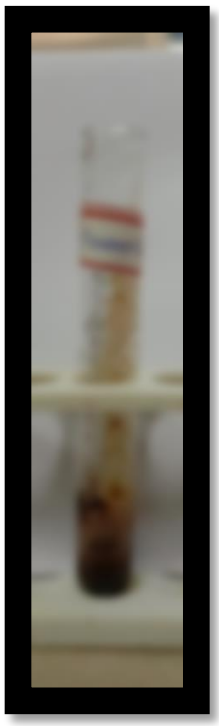
QUANTITATIVE ANALYSIS OF FLAVONOID -FIG: 3

Glycosmis pentaphylla Corr.

Vitex negundo L.

Calotropis gigantea L.

Moringa oleifera Lam. *leaf*



Moringa oleifera Lam. root

QUANTITATIVE ANALYSIS OF TANNIN – FIG: 4

Glycosmis pentaphylla Corr.

Vitex negundo L.

Calotropis gigantea L.

QUANTITATIVE ANALYSIS OF STEROID -FIG: 5

Glycosmis pentaphylla Corr.

Vitex negundo L.

Moringa oleifera Lam.root

QUANTITATIVE ANALYSIS OF BITTER -FIG: 6

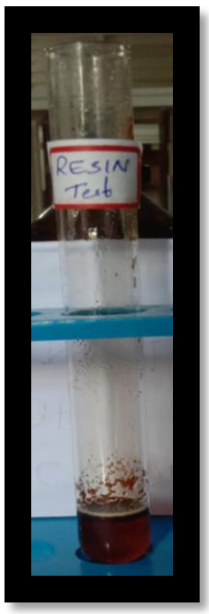
Vitex negundo L.

Calotropis gigantea L.

Moringa oleifera Lam. *leaf*

QUANTITATIVE ANALYSIS OF RESIN -FIG: 7

Glycosmis pentaphylla Corr.



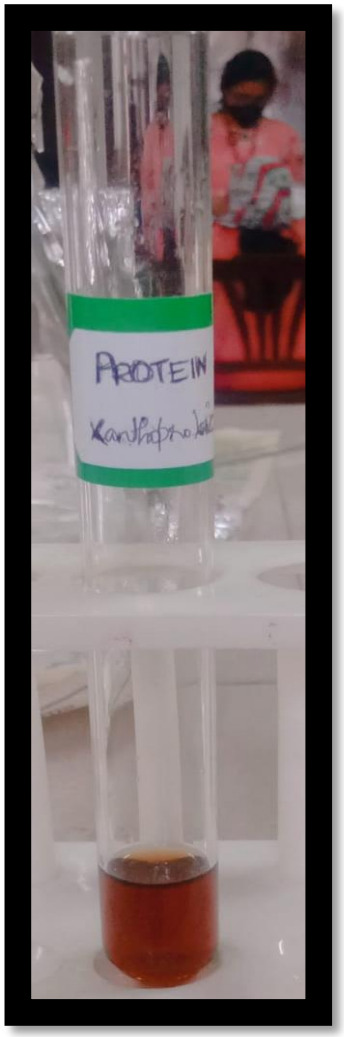
Vitex negundo L.

Calotropis gigantea L.

Moringa oleifera Lam. *leaf*

Moringa oleifera Lam. root

QUANTITATIVE ANALYSIS OF PROTEIN -FIG: 8



Vitex negundo L.

QUANTITATIVE ANALYSIS OF CARBOHYDRATE – FIG: 9

***Glycosmis pentaphylla* Corr.**

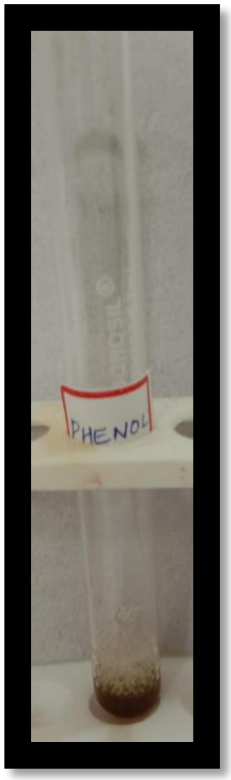
Vitex negundo L.

***Calotropis gigantea* L.**

Moringa* Lam. *oleifera leaf

***Moringa* Lam. *oleifera* root**

QUANTITATIVE ANALYSIS OF PHENOL FIG: 10



Glycosmis pentaphylla Corr.

Vitex negundo L.

Calotropis gigantea L.

Moringa oleifera Lam. *leaf*

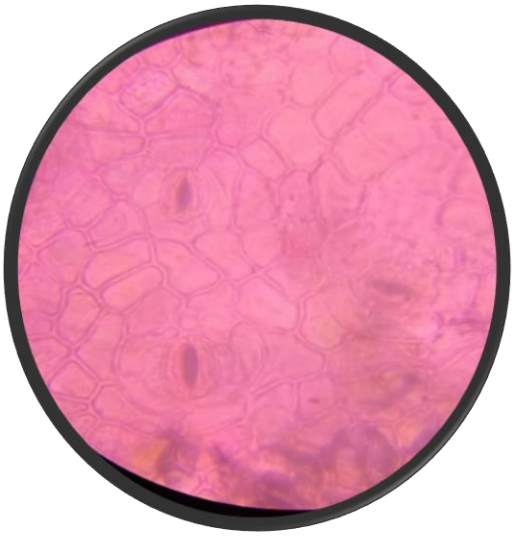
PHARMAGNOSTIC ANALYSIS

MICROSCOPIS STUDIES

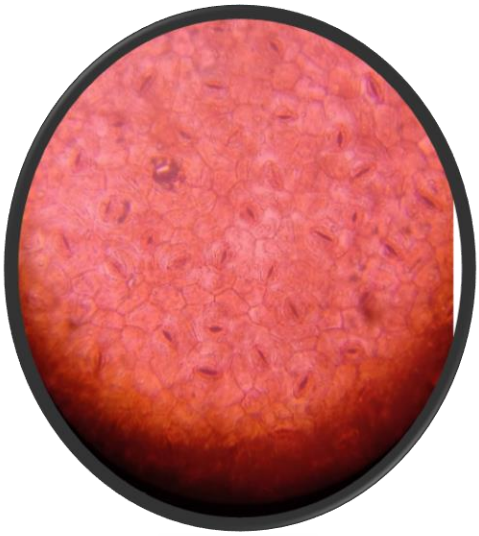
STOMATAL TYPE

FIG: 11

Glycosmis pentaphylla Corr.



Vitex negundo L.



Calotropis gigantea L.

Moringa oleifera Lam.

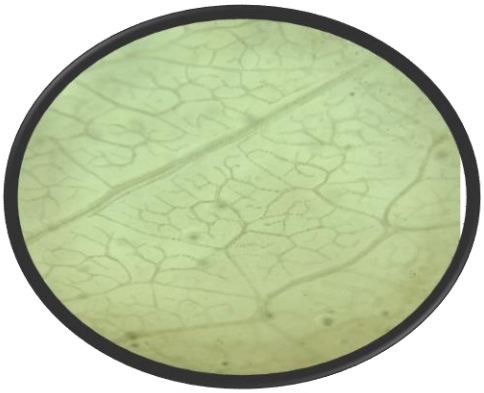
VEIN – ISLET NUMBER AND VEIN TERMINATION NIMUBER

FIG: 12

Glycosmis pentaphylla Corr.

Vitex negundo L.

Calotropis gigantea L.

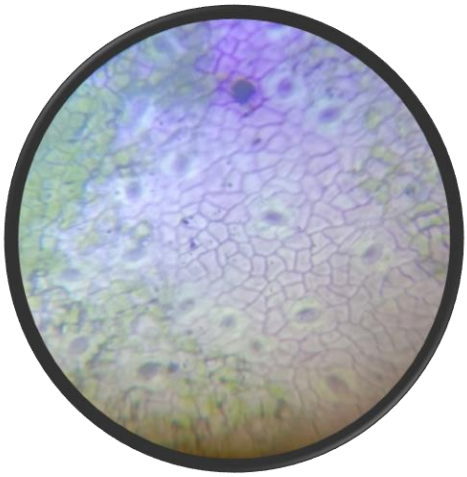


Moringa oleifera Lam.

HISTOCHEMICAL STUDIES

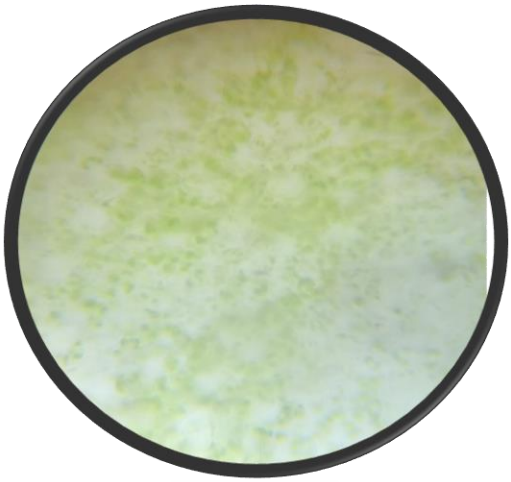
FIG: 13

1. *GLYCOSMIS PENTAPHYLLA* Corr.



polyphenol

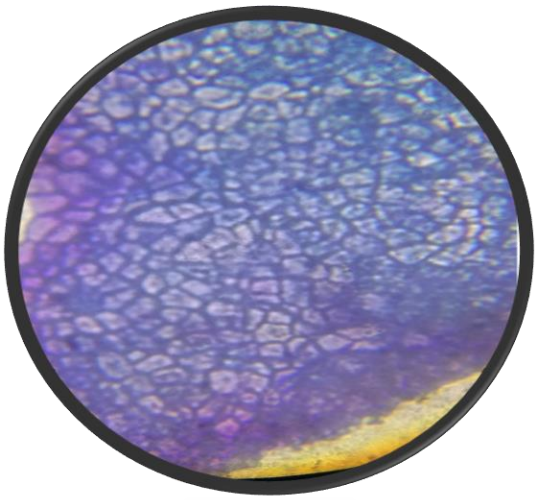
starch



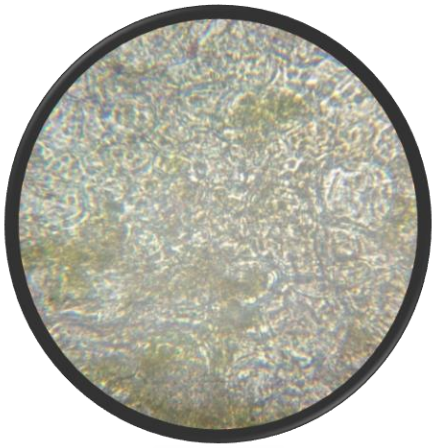
Lipids

FIG: 14

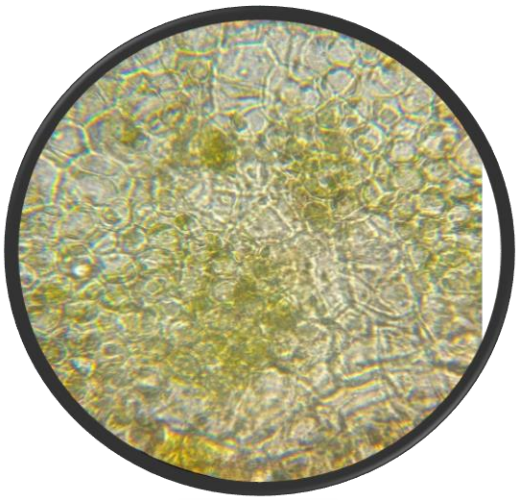
2. *VITEX NEGUNDO* L.



polyphenol



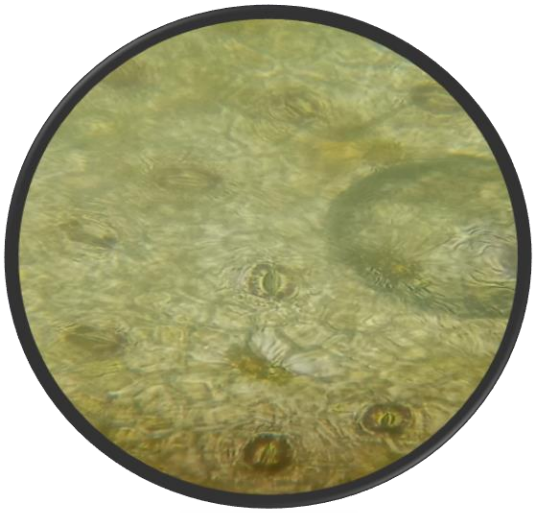
starch



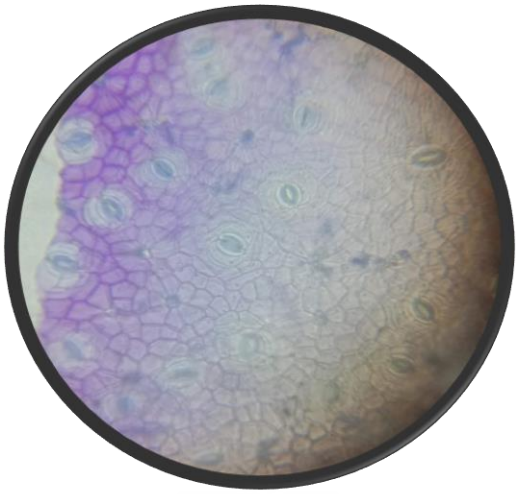
Lipids

FIG:15

3. *CALOTROPIS GIGANTEA* L.



starch

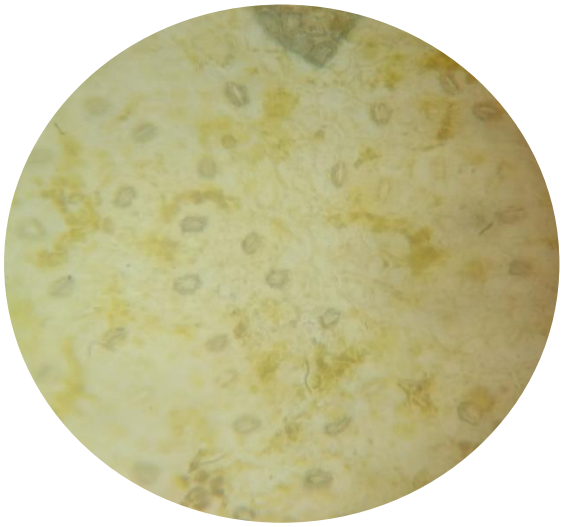


polyphenol

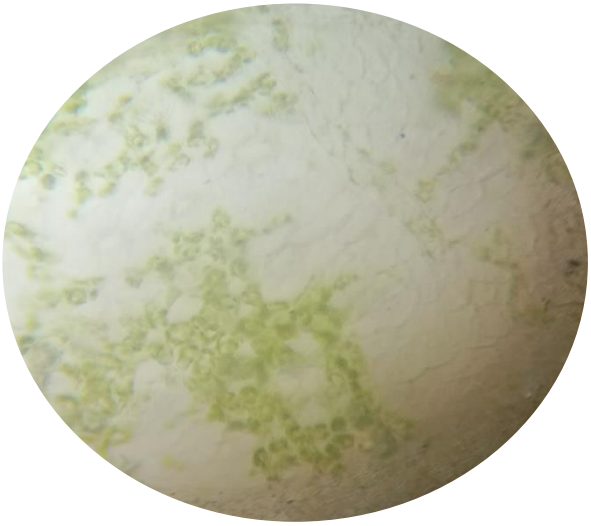
Lipids

FIG: 16

4. *MORINGA OLEIFERA* Lam



starch

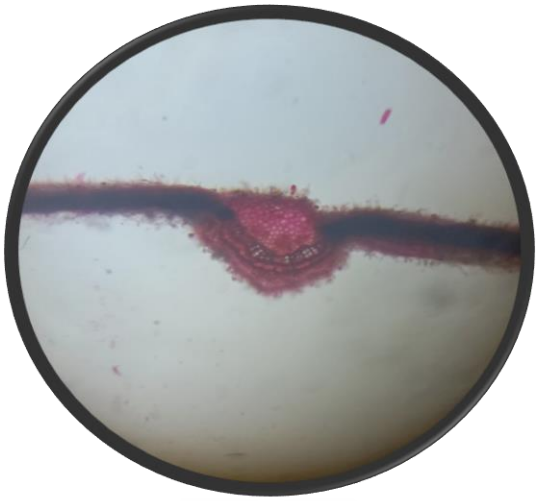


Lipids

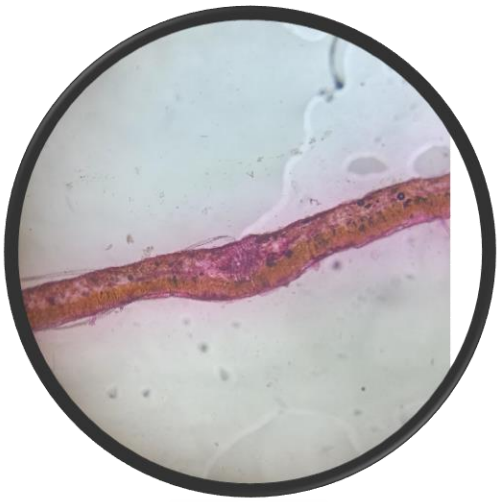
FIG: 17

ANATOMY

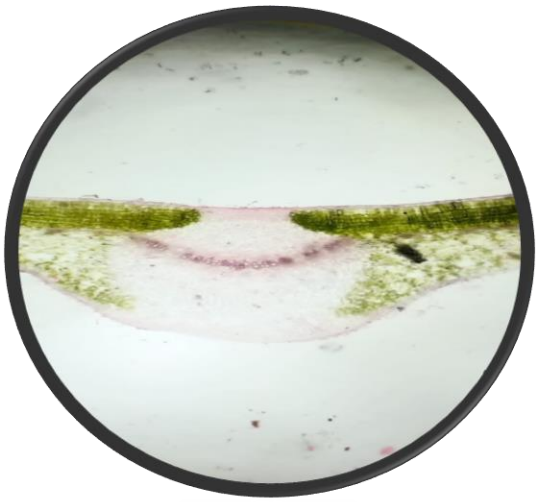
Glycosmis pentaphylla Corr.



Vitex negundo L.



Moringa oleifera Lam.



Calotropis gigantea L.

DISCUSSION

Medicinal plants constitute a group of industrially important crops which are great value for domestic use and for export. Plant based drugs are increasingly preferred in medical science. The forest areas have been the traditional source of medicinal plants and herbs and medicinal plants have curative properties due to the presence of various complex chemical substances of different composition.

The present study aimed to analyse the pharmacognostic, phytochemical and histochemical analysis of plants used for disease arthritis.

The pharmacognosy basically deals with standardization, authentification and study of natural drugs. It is closely involved with allied fields like phytochemical and toxicological screening of natural products. In pharmacognosy, organoleptic evaluation and microscopic studies are done. The organoleptic evaluation showed the dried finely powdered leaf of *Glycosmis pentaphylla* Corr & *Moringa oleifera* Lam leaf are green colour, for *Vitex negundo L.* pale green, for *Calotropis gigantea* L. fainted green and for *Moringa oleifera* Lam root white colour. The dried fine powder of *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam leaf and root possess a characteristic odour, aromatic, odourless, aromatic and characteristic odour respectively. *Glycosmis pentaphylla* Corr, *Vitex negundo L., Moringa oleifera* Lam possess anomocytic stomata and *Calotropis gigantea* L. possess paracytic stomata. Stomatal index of *Glycosmis pentaphylla* Corr was found to be 10%. For *Vitex negundo L.* it was 6.06%. *Calotropis gigantea* L. shows 17.85% and for *Moringa oleifera* Lam 13.3%.

Palisade ratio of *Glycosmis pentaphylla* Corr was found to be 3.30. For *Vitex negundo L.* it was 2.7. *Calotropis gigantea* L. shows 1.25 and for *Moringa oleifera* Lam 2.3.

Vein islet number is defined as the number of vein islets per square nm of leaf surface midway between the midrib and margin and vein termination number is the number of vein-per square mm of leaf surface midway between midrib and margin. Vein islet number and vein termination number for *Glycosmis pentaphylla* Corr was found to be 9 & 8. For *Vitex negundo L.* it was 23 & 7. *Calotropis gigantea* L. shows 10 & 18and for *Moringa oleifera* Lam 10 & 15.

The phytochemical detection of secondary metabolites is done by following standard protocol. The secondary metabolites like alkaloids, saponins, flavonoid, steroid, tannin, resin, carbohydrate and phenol are found in *Glycosmis pentaphylla* Corr.

Alkaloids, saponins, flavonoid, steroid, tannin, resin, bitter, protein, carbohydrate and phenol are found in *Vitex negundo L.*

Alkaloids, saponins, flavonoid, tannin, resin, bitter, carbohydrate and phenol are found in *Calotropis gigantea* L.

Alkaloids, saponins, flavonoid, tannin, resin, bitter, carbohydrate and phenol are found in *Moringa oleifera* Lam leaf.

Saponins, flavonoid, steroid, resin, carbohydrate and are found in *Moringa oleifera* Lam root.

Medicinal plants used against various inflammatory biomarkers for the management of rheumatoid arthritis were studied by (Shareen Singh et al.,2020). The current review retrieved that, various medicinal plants possess an active phytoconstituents with anti-inflammatory and antioxidant properties, which tends to be effective alternative approach over the synthetic drugs concerned with high toxic effects. So, the current available literature provides an evident data concluding that the active constituents like fatty acids, flavonoids, terpenes and sesquiterpene lactones attenuate the RA symptoms by targeting the inflammatory biomarkers involved in the pathogenesis of RA. This study evaluated the results of *Moringa oleifera* Lam plant extract comprising phytoconstituents like flavonoids, terpenoids, alkaloids and sterols embracing antioxidant and anti-inflammatory activity.

Anti- arthritic activity of leaves of *Calotropis gigantea* L. were studied by (Kalpana S. Patil et al.,2007). Petroleum ether (40-60°), ethyl acetate, ethanol and aqueous extract of *Calotropis gigantea* L. leaves were tested for various preliminary phytoconstituents and were screened for anti-arthritic activities using Freund's adjuvant arthritis in albino rats. The extracts were administered orally for 21 days and the mean changes in diameter of paw were noted at regular intervals. The changes in body weight were recorded daily. On 22nd day at the end of study blood was collected and haemoglobin content, total WBC count, differential WBC count, ESR and RBC were also estimated. Steroids, triterpenoids, glycosides, flavonoids, carbohydrates, proteins were found to be present in *Calotropis gigantea* L. leaves extracts as observed by the qualitative tests. The migration of leucocytes into the inflamed area is significantly suppressed

by the extracts as seen from the significant decrease in total WBC count. Due to the presence of steroids, triterpenoids, glycosides, flavonoids, carbohydrates, proteins, found in *Calotropis gigantea* L. leaves extracts, it is potentially used in anti-arthritic activity.

Potential investigation of anti-inflammatory activity and phytochemical investigations of ethanolic extract of *Glycosmis pentaphylla* Corr leaves were studied by (Pravej Ansari et al.,2015). This study evaluated that *G. pentaphylla* Corr has property of pain or inflammation healing and from phytochemical analysis found the presence of alkaloid and steroid, so it could also have anti-coagulation property as well. A preliminary study on the phytochemical analysis that performed showed the presence of alkaloid, flavonoid, steroid, saponin etc. Alkaloid, a nitrogenous group of phytochemicals that has wide diversity in classification and distribution, has good evidence of pain killing activity. Flavonoids have the hepatoprotective reputation as anti-oxidant Phyto agent. This shows why the plant has anti-inflammatory effect. The presence of flavonoid enhances the scope to find out its anti-oxidant property, it seems this would be an ideal agent as anti-inflammatory agent if properly modified.

A review of the important chemical constituents and medicinal uses of *Vitex* genus were documented by (Ajay et al.,2011). Phytochemical studies of *Vitex negundo* L. showed the presence of several types of compounds, such as volatile oils, lignans, flavonoids, terpenes (triterpenes, diterpenes, sesquiterpenes) and steroids. This plant contains many polyphenolic compounds, terpenoids, glycosidic iridoids and alkaloids. Due to the presence of flavonoids, steroid and alkaloids, the Ayurvedic medicine and its various recent experimental models conclude that the oral administration of the plant leaves have been claimed to have anti-inflammatory activity. In the present work the presence of phytochemicals such a flavonoid, steroid, alkaloid is confirmed hence it can use as an anti-arthritic agent.

Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* Lam plant were documented by (Garima Mishra et al.,2011). This article provides all necessary information regarding its phytochemical investigations, pharmacological actions and medicinal properties like anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhoea, eye and ear infections, fever, abnormal blood pressure, pain in joints, scurvy, semen deficiency, headaches and tuberculosis. It gives an account of all the data and reports which have been appeared to prove its medicinal and nutritional importance. A preliminary study on the phytochemical analysis that performed on leaves & roots showed the presence of flavonoid, sterol, glycosides which provide anti-

inflammatory activity. A Cáceres et al. (1992) reported anti-inflammatory activity from the hot water infusions of flowers, leaves, roots, seeds and stalks or bark of *Moringa oleifera* Lam using carrageenan-induced hind paw edema in rats. K.V. Sashidhara et al. (2009) from the roots of *Moringa oleifera* Lam isolated and characterized aurantiamide acetate 4 and 1,3-dibenzyl urea 5. Isolated compounds inhibited the production of TNF-alpha and IL-2.

A Review on Pharmacological and Phytochemical Profile of *Calotropis Gigantea Linn* were documented by (Gaurav et al.,2011). This study is a collective information concerning the ethnobotany, pharmacology, phytochemistry and biological activities of the *C. gigantea* L. Pharmacological screenings of C. gigantea revealed its medicinal potential and represents as a valuable medicinal plant with several medicinal properties such as antimicrobial activity, antimicrobial activity, Anti-inflammatory, Anti-pyretic activity Anti-diarrhoeal activity, Insecticidal activity and Wound healing activity. A preliminary study on the phytochemicals revealed the presence of alkaloids, cyanogenic, glycosides, phenolics, tannins 19, cardenolides 20, 21, flavonoids 22, terpenes 23, 24, sterols 25, Proteinases 26 and nonprotein amino acid 27 as major phytochemical groups. The present study also confirmed the presence of alkaloids, phenol, tannin and flavonoid.

Ethnomedicinal uses, phytochemistry, pharmacological activities and toxicological profile of *Glycosmis pentaphylla* Corr (Retz.) DC.: A review were documented by (Labony et al.,2021).

This review presented up-to-date information regarding the taxonomy, botany, distribution, ethnomedicinal uses, phytochemistry, pharmacology and toxicological profile of *G. pentaphylla* Corr.

Extensive phytochemical investigations of different parts of *G. pentaphylla* Corr have revealed the presence of at least 354 secondary metabolites belonging to structurally diverse classes including alkaloids, amides, phenolic compounds, flavonoids, glycosides, aromatic compounds, steroids, terpenoids, and fatty derivatives. A large number of *in vitro* and *in vivo* experiments have demonstrated that *G. pentaphylla* Corr had anticancer, antimutagenic, antibacterial, antifungal, anthelmintic, mosquitocidal, antidiabetic, antihyperlipidemic, anti-oxidant, anti-inflammatory, analgesic, antipyretic, anti-arsenicosis, and wound healing properties. So it can be suggested as a source of inspiration for the development of novel drugs, especially anticancer, antimicrobial, anthelmintic, and mosquitocidal agents. Moreover, bioassay-guided investigations into its diverse classes of secondary metabolites, especially the large pool of nitrogen-containing alkaloids and amides. The present study confirmed the

presence phytochemicals such as alkaloids, phenol, tannin, flavonoid, steroid, resin and carbohydrate. So, it can be suggested as a source for the development of novel drug of anti-inflammatory.

Phytochemical evaluation of *Vitex leuocoxylon, vitex negundo and vitex trifolia* were studied by (K Aditya et al.,2020). This study was conducted to investigate phytochemical evaluation of Vitex leuocoxylon, *Vitex negundo* L. and *Vitex trifolia.* The crude drug powder extracts of the leaves of the above plants were taken for the study. The Phytochemical Screening were done for the selected plants. Phenolic compounds, tannins, flavonoids, cardiac glycosides, and alkaloids were present in *Vitex leuocoxylon.* Alkaloids, flavonoids, carbohydrates, glycosides and tannins were present in *Vitex negundo*. Alkaloids, saponins, flavonoids, carbohydrates and anthraquinone glycosides were present in *Vitex trifolia*. The extracts and crude dried powders of *Vitex leuocoxylon*, *Vitex negundo* L. and *Vitex trifolia* were subjected to qualitative analysis for presence of chemical constituents. Alkaloids, flavonoids, carbohydrates, glycosides and tannins were present in *Vitex negundo* L. The present study also evaluated the presence of alkaloids, flavonoids, carbohydrates and tannin.

Presently investigation of the antiarthritic activity of the herbs leads to the development of many effective herbal therapies. Presence of Phytoconstituents like flavonoids, phenol, alkaloids, steroid indicates that it has potential anti-inflammatory activity. The information's about the plants or plant extracts which are discussed in the present study might be helpful for the further research on arthritis.

SUMMARY AND CONCLUSION

The world health organization (WHO) reveals that most percentage of the world's population relies on traditional remedies including plants as their primary health care aid. Medicinal plants are the oldest form of health care known to mankind. From the ancient times people are using different herbs or plants as the remedy for various diseases. Throughout the world several plants have been and are still used for medicinal properties. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, flavonoids, sterol, phenol, etc. about 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious diseases and cancer. Various plant parts including root, leaf, flower, bark, fruit and trees.

The plants selected for the present study are *Glycosmis pentaphylla* Corr, *Vitex negundo Linn., Calotropis gigantea Linn., Moringa oleifera* Lam. These are medicinal plants used for arthritis disease which had paid serious attention to pharmacognostic, phytochemical and histochemical studies were conducted.

In pharmacognostic studies organoleptic evaluation and microscopical analysis were carried out. The organoleptic evaluation showed the dried finely powdered leaf of *Glycosmis pentaphylla* Corr & *Moringa oleifera* Lam leaf are green colour, for *Vitex negundo L.* pale green, for *Calotropis gigantea* fainted green and for *Moringa oleifera* Lam root white colour. The dried fine powder of *Glycosmis pentaphylla* Corr, *Vitex negundo L., Calotropis gigantea* L. *and Moringa oleifera* Lam leaf and root possess a characteristic odour, aromatic, odourless, aromatic and characteristic odour respectively. *Glycosmis pentaphylla* Corr, *Vitex negundo L., Moringa oleifera* Lam possess anomocytic stomata and *Calotropis gigantea* L. possess paracytic stomata. Stomatal index of *Glycosmis pentaphylla* Corr was found to be 10%. For *Vitex negundo L.* it was 6.06%. *Calotropis gigantea* L. shows 17.85% and for *Moringa oleifera* Lam 13.3%.

Palisade ratio of *Glycosmis pentaphylla* Corr was found to be 3.30. For *Vitex negundo L.* it was 2.7. *Calotropis gigantea* L. shows 1.25 and for *Moringa oleifera* Lam 2.3.

Vein islet number is defined as the number of vein islets per square nm of leaf surface midway between the midrib and margin and vein termination number is the number of vein- per square mm of leaf surface midway between midrib and margin. Vein islet number and

vein termination number for *Glycosmis pentaphylla* Corr was found to be 9 & 8. For *Vitex negundo L.* it was 23 & 7. *Calotropis gigantea* L. shows 10 & 18and for *Moringa oleifera* Lam 10 & 15.

The phytochemical detection of secondary metabolites is done by following the standard protocol. The secondary metabolites like alkaloids, saponins, flavonoid, steroid, tannin, resin, carbohydrate and phenol are found in *Glycosmis pentaphylla* Corr.

Alkaloids, saponins, flavonoid, steroid, tannin, resin, bitter, protein, carbohydrate and phenol are found in *Vitex negundo L.*

Alkaloids, saponins, flavonoid, tannin, resin, bitter, carbohydrate and phenol are found in *Calotropis gigantea* L.

Alkaloids, saponins, flavonoid, tannin, resin, bitter, carbohydrate and phenol are found in *Moringa oleifera* Lam leaf.

Saponins, flavonoid, steroid, resin, carbohydrate and are found in *Moringa oleifera* Lam root.

In phytochemical study these plants showed the presence of secondary metabolites such as flavonoid, alkaloid, phenol, steroid which indicates that it has potential anti-inflammatory activity. Through screening of the literature available on these plants depicted the fact that these phytochemicals act as an anti-inflammatory agent. The information about the plants or plant extracts which are discussed here might be helpful for the further research on arthritis.

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