PHYTOCHEMICAL AND PHARMACOGNOSTIC ANALYSIS OF SELECTED MEDICINAL PLANTS OF RUTACEAE

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JAYASREE V.J

REG NO: AM20BOT011

DEPARTMENT OF BOTANY AND CENTRE FOR RESEARCH

ST.TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

KOCHI- 682011

2020-2022

CERTIFICATE

This is to certify that the dissertation entitled,"**PHYTOCHEMICAL AND PHARMACOGNOSTIC ANALYSIS OF SELECTED MEDICINAL PLANTS OF RUTACEAE**" is an authentic record of the research work carried out by **JAYAREE V J** (AM20BOT011) under the supervision and guidance of **Dr.LIZA JACOB**, Associate Professor, Department of Botany and Centre for Research, St. Teresas's College (Autonomous), Ernakulam in partial fulfilment of the requirements for the Master's Degree of Science in Botany.

Liza

Dr.Liza Jacob

Supervising Teacher and Head

Department of Botany and Centre for Research

St.Teresa's College (Autonomous)

Ernakulam

St.Teresa's College (Autonomous) Ernakulam DEPARTMENT OF BOTANY

Examiners

1)...K. Madhusoothanan 13/6/22.

2)...Dr. Stephy Siqueira 13/6/2022.

PLACE: ERNAKULAM

DATE: 24-5-2022

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INTRODUCTION

Plants are pivotal part of our planet. Because of the existence of plants, earth is called a green planet. Plants are one of the two major groups of living organisms that are an essential entity to the function of the biosphere. Plants can be found in all known parts of the earth, in all shapes and sizes. They include the green algae, mosses, ferns, vines, grasses, bushes, herbs, flowering plants and trees. The importance of plants in the food chain dates back to ancient times. The first humans gathered wild plants for food. As settlements developed, food crops were cultivated, leading to selection of high-yielding cultivated varieties to feed the growing populations. Plants are found in natural ecosystems such as rain forests, and also in agricultural areas and urbanized settings. They are an essential part of our daily lives providing food, clean air, and important ecosystem functions (Fernando, 2012).

Plants containing inborn potentially active ingredients used to cure disease or relieve pain are called medicinal plants. Plants play a therapeutic and restorative role in protecting human beings from the adverse effects of diseases and other complications, thus considered to have a beneficial role in healthcare system (Srivastava *et. al*., 2011; Nair *et. al*., 2003).

Plants are very useful source of various bioactive compounds which have direct or indirect use in the treatment of various human ailments. From the time immemorial, human civilizations have been exploring and using various plants and plant products to cure the deadly diseases. In India Rig-Veda was believed to be the oldest repository of human traditional knowledge about medicinal usage of plants which was written during 4500 to 1600 BC. (Dogra et. al., 2015)

The therapeutic potential of plant products can be traced back to over five thousand years ago as there is evidence of its use in the treatment of diseases and for revitalizing body systems in Indian, Egyptian, Chinese, Greek and Roman civilizations. In India, plants of therapeutic potential are widely used by all sections of people both as folk medicines in different indigenous systems of medicine like Siddha, Ayurveda, and Unani and also as processed product of pharmaceutical industry. India has about 4.5 million plant species and among them estimated only 250,000-500,000 plant species, have been investigated phytochemically for biological or pharmacological activity. The bioactive constituents or plants extracts may be uses for treatment of various diseases and these would be used as a new formulation for the novel drugs discovery in pharmaceutical industries.

Medicinal and aromatic plants can play an important role in the subsistence livelihood enhancement rural people, especially women in an environmentally sustainable manner while

maintaining the biodiversity of these natural products. Today according to the World Health organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs.

Medicinal Plant is of the great of the health of individual and communities. The medicinal value of plants lies in some chemical active substances that produce define physiological action on the human body. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Secondary metabolites or phytochemicals from plants have eminent pharmacological activities such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic. These secondary metabolites protect the cells from the damage caused by unstable molecules known as free radicals. There are growing interests in using natural antimicrobial compounds, especially extracted from plants, for the preservation of foods. There is therefore the need to search for plants of medicinal value (Neelam Bamola et. al., 2017).

The contribution of medicinal plants to modern medicine:

The factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. Medicinal plants have been an integral part of the Chinese, Indian and Arabian ancient culture as medicine and their importance even dates back to the Neanderthal period. In spite of this, we discerned about history and traditional uses of medicinal plant by common people. In the 18th century knowledge about plant derived drugs expanded, but attempts to identify the active ingredients from plants were unsuccessful. Another achievement in the field of medicinal plants was the development of methods to study the pharmacological effect of natural products and vegetable extracts. Claude Bernard (1813-1878), who conducted detailed studies on the pharmacological effects of curare (a drug and arrow poison used by the American Indians of the Amazon), is considered one of the first scientists in this field. The 20th century saw the integration of ethno botanical, pharmacological and phytochemical studies, a process that had taken many and many years, but which allowed the development of a new approach to the study the significance of medicinal plants in pharmaceutical field (Rajasekar, 2018)

Significant increase in medicinal plants usage has been recorded continuously both for traditional users and pharmaceutical industry. Medicinal plants provide opportunities for biological screening, methods useful for the industry and trends in the pharmacological investigations of natural products. Plants are the natural and most easy accessible source of therapeutically active biological principles, thus there is a dire need to screen out plant for development of new drugs. For this purpose plants have been assayed widely but still large

number of them has not arrived to the conventional health care system. Therefore, search for new drugs from microorganisms, fungi, plants and animals must be persistent and these can be the sources of innovative and prevailing restorative agents for newer, safer and accessible drugs. Now a day, due to advancement of modern and new sophisticated methods, plant scientists are taking more in trust in exploring new drugs from natural and biologically active compounds of the plants, which could be serve as inexhaustible resources for pharmaceutical industries (Esimone *et. al.*, 2005).

PHYTOCHEMISTRY

Phytochemistry (from the Greek word phyto, meaning plant) is the study of the chemicals produced by plants, particularly the secondary metabolites, synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, ultraviolet exposure and environmental hazards. Phytochemicals are simply plant-derived chemicals. The proper understanding of phytochemical is essential for drug discovery and for the development of novel therapeutic agents against major diseases. The study of phytochemicals has been instrumental in the discovery of new plant natural products which are of commercial values in various industries such as the traditional and complementary medicine systems, pharmaceutical industries, and dietary supplement industries. (Egbuna et. al., 2018)

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's color, aroma and flavour. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics. In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions.

These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Saxena et. al., 2013).

In resent year Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Saranraj et. al., 2016)

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. The name of alkaloids derives from the "alkaline" and it was used to describe any nitrogen-containing base. Alkaloids are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi. Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores and also against other plants by means of allelopathically active chemicals. In general the alkaloids may be therapeutic in small doses and in special formulation but can poison or even kill otherwise (Mamta Saxena et. al., 2013).

The carbohydrates or saccharides ('hydrate of carbon') of general formula Cn(H2O)n are mostly sweet compounds (hence the term sugar) are found abundantly in higher terrestrial plants, fungi, and seaweed and consist of compounds such as sugars, starch, and cellulose. Polysaccharides of higher plants possess immunostimulatory, anti-complementary, anti-inflammatory, hypoglycemic, and anti-viral activities Carbohydrates in general, are essential constituents of all living organisms and are associated with a variety of vital functions, which sustain life (Saranraj et. al., 2016)

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenol (C6H5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants. They are plant secondary metabolites, and 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.

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks. The flavonoids appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. Flavonoids have gained recent attention because of their broad biological and

pharmacological activities in these order Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Mamta Saxena et. al., 2013).

Steroids are merely modified triterpenes and are widespread in both animal and plant kingdoms and many microorganisms. The saponins have attracted much attention in recent years because of their varied biological properties, some of which are deleterious, but many of which are beneficial to human health. They are plant glycosides, which have the property of forming a soapy lather when shaken with water. Saponins exhibit divergent antimicrobial, anti-inflammatory, antibiotic, hemolytic analgesic, hypoglycemic, anthelmintic and cytotoxic activities (Saranraj et. al., 2016).

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, etc. In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours, and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Mamta Saxena et. al., 2013).

PHARMACOGNOSTIC ANALYSIS

The word pharmacognosy is derived from the Greek word *pharmakon*-drug and *gnosis*-knowledge. The term pharmocognosy was first time coined by the Austrian physician Schmidt in 1811. Pharmacognosy is the study of medicines derived from natural sources, mainly from plants which may further lead to development of new drug.

During pharmacognostic investigations, physico-chemical analysis also considered as important parameter in evaluation and identification of crude drug. Macroscopic and microscopic analysis is necessary for the detection of adulterants, contaminants of herbal drug and for assessing quality before going for further study (Smitha, 2022)

Pharmacognosy is the study of medicines derived from natural sources. The study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources is the definition given by The American Society of Pharmacognosy. It is the

oldest of all pharmacy sciences. Pharmacognosy is related to both botany and plant chemistry "Phytochemistry", and its history entitles it to be regarded as parent of both. Pharmacognostical evaluation represents valuable information regarding the morphology, microscopical and physical characters of crude drugs and thus gives the scientific information regarding the purity and quality of crude drugs.

In broad sense pharmacognosy is defined as the scientific and systematic study of physical, chemical and biological characters of crude drugs including their history, cultivation, collection and preparation for the commerce. The study of the action of the drugs is known as pharmacology (Pandey and Chadha, 1996). The word pharmacology is derived from Greek words pharmacon (an active principle) and logos (a discourse or treatise). It is the science that deals with drugs. It consists of detailed study of drug, particularly their actions on living animal's organ and tissue. The objective of pharmacology is mainly to provide such scientific data, using which one can choose a drug treatment of proven efficacy and safety from various options available, so suit the patient (Vijayalakshmi, 2014).

Generally the crude drugs are obtained from plants. But a limited number of drugs are also obtained from animal and mineral origin. The entire plant or part of plant is used as crude drug. The medicinal plants contain substances that produce specific physiological action in the human body. The important substances are alkaloids, compound of carbon, hydrogen, oxygen and nitrogen. Besides these glycosides, essential and fatty oils, resins, gums, mucilages, tannin etc. are of large use (Agarwal and Ghosh 1985). These active principles may be present in the storage organs of the plants viz. roots, seeds leaves, bark, wood etc. these compounds have anticancerous, anti-malarial and antidysenteric properties (Khory and Nanabhai 1999)

Thousands of plants known and unknown, that yield medicines drugs of great use to man are known as medicinal plants. The branch of science that deals with the study of drug plants, their history, collection, selection, identification, preservation, extraction and preparation of drugs from the plant is called pharmacognosy (Pandey, 2001)

In recent years, rapid development in pharmacology and Phytochemistry has enormously expanded the boundaries of the subject. This expansion of the knowledge has been increased waves of interest in the field of research in natural products chemistry. This level of interest can be attributed to several factors, including unmet 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 characterize these active constituents, and advances in solving the demand for supply of complex natural products (Rajasekar, 2018).

HISTOCHEMICAL STUDIES

Histochemistry is an essential analytical tool interfacing extensively with plant science. It is the branch of histology dealing with the identification of chemical components of cells and tissues Plant cell structures are translucent unless they are stained. Histochemistry allows the identification and localization, at the cellular level, of biomolecules and organelles in different types of cells and tissues, based on the use of specific staining reactions and imaging. The product of the reaction can be noticed with specific colors and this localization of a particular compound is termed 'qualitative histochemistry'. Histochemical techniques are also widely used for the in vivo localization of promoters in specific tissues, as well as to identify specific cell wall components such as lignin and polysaccharides (Vaishali Yadav et. al., 2021).

Histochemical analysis provides a best tool for the botanical identification and standardization of crude drugs. Histochemistry is also known as Cytochemical localization technique, it is a powerful tool in the hands of plant scientists. Generally for histochemical analysis, qualitative and quantitative tests of certain primary constituents or metabolites are done by employing widely accepted methodologies proposed by authentic workers. These metabolites include starch, protein, lipids, conjugated polysaccharides and phenols. These compound develop characteristic coloration based on the dye utilized, thereby making it possible to locate them in the plant tissue depending on the purpose of study. 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 groups of phenol derivatives, usually related to glucosides Tannins are particularly abundant in the leaves (xylem) of many plants. 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 (Momin, 2011).

Botanical Histochemistry, although relatively now, is a fast developing area of research, combining histology and analytical biochemistry. Histochemical technique helps us to

identify and localize biochemical substances of the cell wall, cytoplasm and nucleus in the cells and tissues.

In the present work histochemical tests were carried out to localize and identify the nature of primary metabolites present within the foliar epidermis, specifically the stomata complex. These biochemical compounds are present in every cell of the plant body, but are more readily available and easily observed in the epidermal tissue of the leaves.

SOURCE PLANT

The Rutaceae are the family, having the synonyms like Rue or Citrus or Orange family, are the largest family of flowering plants which occupy a place in the seventh order Sapindales of the phylum Angiospermae. Rutaceae family, comprising of about 150 genera and over 1500 species, are widely distributed in warm temperate regions and to some extend in tropical areas. India represents about twenty-five genera and bygone eighty species of this family. This family is of tremendous commercially and economically important and is popularly known as the sole source of the citrus fruits of commerce such as lemons, limes, oranges, pomelos, etc. Despite of its economic importance, it has traditionally and conventionally been known in popular medicine. The ubiquitous role of flora in the regimen and remedy of sickness and illness is epitomized by their implementation in all the vital system of medicine heedless of the underlying philosophical premise. Antique literature such as Rig-Veda, Yajurveda, Atharvaveda, Charaka Samhita and Sushruta Samhita also describe the use of plants for treatment of various health problems. Phytonutrients or Phytochemicals are the natural bioactive compounds that are produced by the plants and beneficial for the human wellness and fitness. Rutaceous members harmonize and accumulate in their cells, a great sort of phytochemicals, which have well built in capacity to alter the body's reaction to allergens, viruses, fungus and many more. The important genera of this family include Aegle, Citrus, and Murraya etc. These fruits of this genus are the important and major source of Vitamin C as an important part of everyone's diet (Panda et. al., 2019)

Rutaceae is variable in their habit, being trees or shrubs, less often subshrubs or herbs, seldom lianas. The leaves are compound, impari or paripinnate, foliolate, or simple, alternate or opposite. Flowers are bisexual or unisexual, usually 3–5-merous, actinomorphic or rarely zygomorphic, hypogynous [or rarely perigynous]. `Perianth is in 2 series, with clearly differentiated calyx and corolla or sometimes in 2 irregular series or 1 series, with ± undifferentiated tepals. Sepals are distinct or connate to their full length. Petals are distinct

[or rarely coherent or connate for part of their length]. Stamens are usually as many as or 2 × as many as petals or sometimes more numerous; filaments distinct or sometimes coherent or connate for at least part of their length; anthers introrse or sometimes latrorse, longitudinally dehiscent. Disk are [rarely lacking] within androecium, nectariferous, flattened, annular, cup-shaped, pulvinate, or sometimes columnar, bell-shaped, conic, or hourglass- shaped. Gynoecium of 1–5 distinct 1-loculed carpels or 2, to many partially to completely connate carpels; placentation axile [very rarely becoming parietal]; ovules 1 to many per locule. Fruit are of 2–5 follicles [drupes or samaras] or a single follicle, capsule, or berry [or samara]. Seeds are with relatively large embryo; endosperm present and fleshy or lacking (Milton Groppo et. al., 2022).

Aegle marmelos Linn Correa belongs to family Rutaceae. It is commonly known as bael (Hindi), Kuvalam (Tamil and Malayalam) and Golden apple (English). It grows in the plains and submontane regions of India, wild in the sub- Himalayan tract and often planted. Bael is considered to be an auspicious tree by some Hindus and has been called Shiva druma (the tree of Shiva). It is a small deciduous thorny tree, up to 7.5 m in height. Wood yellowish white hard, flowers in axillary panicles, greenish white, fragrant, fruit hard-shelled, , greyish or yellowish, scented, pulp orange, sweet and gummy; seed numerous, oblong and compressed. The leaves of the tree used as a mild laxative, for fever, ophthalmia, deafness and inflammation. Decoction of the leaves is used as febrifuge, used for chronic diarrhoea or dysentery and irritation of the alimentary tract. The leaves are used for the treatment of inflammation, dyspepsia, mal-absorption, neurological diseases, edema, vomiting, and rheumatism asthma, hypoglycemia, febrifuge, hepatitis and analgesic. The fruits, bark, leaves, seeds, and roots of bael contain bioactive compounds such as coumarin, xanthotoxol, imperatorin, aegeline, and marmeline. These compounds can provide antidiabetic, anticancerous, antifertility, antimicrobial, immunogenic, and insecticidal activities. (Ulahannan et. al., 2008)

Murraya exotica L. (Rutaceae) are also known as *Murraya paniculata L*. It is an evergreen shrub plant, cultivated in Europe mainly as an ornamental pot plant for the fragrance flowers and glossy green leaves. It is a small tree with a spreading crown and short, often crooked, trunk; rather corky, fragrant. Leaves are glossy and darker above, gland-doted, base - rounded. The flowers are fragrant and are arranged in loose groups with white or cream-coloured petals. The fruit is an oval, glabrous, orange-red berry. *Murraya exotica L.*, is

known as a medicinal plant given that its leaves and roots have been traditionally used by the Chinese traditional medicine to treat stomach pains, stimulant, abortive and also used to treat dysentery, cuts, body aches, joint pain, diarrhea, venereal diseases toothache and body pains from injury or trauma and in India for treatment of diarrhea and dysentery. It has anti-diabetic, antioxidant, anti-nociceptive, anti-inflammatory, anti-diarrheal, oxytocic and anti-fertility properties (Diana et. al., 2018)

Ruta graveolens L. commonly known as Rue is an herbaceous perennial, originally native to the Mediterranean region .*Ruta graveolens L*, is a perennial, scented and glabrous herb or a sub-shrub. The stems become woody near the base, but remain herbaceous nearer the tips. Leaves are dissected pinnately into oblong or spoon shaped segments. They are somewhat fleshy and usually covered with a powdery bloom. The sea green foliage has a strong, pungent, rather unpleasant scent when bruised. The paniculate clusters of small yellow flowers appear in midsummer, held well above the foliage and often covering most of the plant. *R. graveolens L.* has been used in traditional medicines for the relief of pain, eye problems, paralysis, tremors, joint Pain, nervine disorders, rheumatism and dermatitis. The drug is also useful in the disorders of kidney, urinary bladder and helps regulate the function of these organs. It also relieves the back pain and chest pain. Extracts from *R. graveolens L.*, have been used as an antidote for toxins such as snake and scorpion venoms. In traditional system of medicine it is used as stimulant, emmenagogue, diuretic, abortefacient, and resolvent (Jinous Asgarpanah et. al., 2012).

AIM AND OBJECTIVES OF THE PRESENT STUDY

- To determine the important phytochemical constituents of *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L.
- To study the pharmacognostic aspects of the plant *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L.
- To study the anatomical features of the plant *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L.
- To study the histochemical aspects of these plants.

REVIEW OF LITERATURE

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Medicinal plants constitute a group of medicinally important crop which are of great value for export. A medicinal plant can be viewed as a synthetic laboratory as it produced and contains a number of chemical compounds. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, and antihepatotoxic compounds.

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (Rajeshwari Sivaraj et al., 2010)

As the present work deals with the pharmacognostic, phytochemical, anatomical and histochemical aspects of the plants *Aegle marmelos* Linn Correa, *Murraya exotica* L., and *Ruta graveolens* L. belonging to the family Rutaceae.

Phytochemical and pharmacological profile of leaves of *Aegle marmelos* Linn Correa was outlined by Narayan Yadav and. Chanotia (2009). In this study they suggested that most important pharmacological activity of the leaves of *Aegle marmelos* Linn Correa has been its antidiabetic activity and the leaf extract of this plant also exhibits the effect on metabolic enzymes involved in glucose metabolism.

Phytochemical profile and pharmacological activity of *Aegle marmelos* Linn Correa was documented by Bikash Manandhar et al., (2018). These studies have highlighted the medicinal activity and presence of potentially active drug components in *Aegle marmelos* Linn Correa which could be developed as novel drugs for treatment, prevention or relief of diabetes, cancer and various pathogenic infections.

Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos* Linn Correa was outlined by Farina Mujeeb et al.,(2014). The present study highlighted that the methanolic extracts of plant showed a significantly high

antibacterial, antioxidant and anti-inflammatory activities and plant has immense potential to be used in the area of pharmacology and as a prospective source of valuable drugs.

Comparative analysis of phytochemical constituents present in various parts of *Aegle marmelos* Linn Correa was attempted by Nirupamaa et al., (2012).This study proves that marmelosin is a coumarin and is found in all parts of the plant

Kuttan and Sabu (2004) studied on leaf extract of *Aegle Marmelos* Linn Correa on Alloxan induced diabetes and reported that used extract was enough capable to reduce oxidative stress by scavenging lipid peroxidation and enhancing certain anti-oxidant levels which causes lowering of elevated blood glucose level.

Tanmay Sarkar et al.,(2020) was highlighted by In-depth pharmacological and nutritional properties of bael. It has been observed that exploring the phytochemistry of bael reveals enormous phytochemicals especially alkaloids, coumarins, essential oils, phenols, flavonoids, and their extraordinary power as a natural magical remedy to various diseases and clinical trials have declared the non-toxic behaviour of bael which ensures the pharmacological application is extremely safe without any side effects.

Amirtham (2016) had investigated preliminary phytochemical screening of different (benzene, chloroform) extract of *Aegle marmelos* Linn Correa. Benzene extracts showed the presence of alkaloid, emodin, phenolics, volatile, and absence of flavonoid, triterpenes, steroids, glycoside, and xanthoprotein. Chloroform extract showed the presence of xanthoprotein, carbohydrate, volatile oil, phenolics, emodin, alkaloid, and absence of flavonoid, and anthracene glycoside

Phytochemical and pharmacological review of maja (*Aegle marmelos* Linn Correa) was reported by Alfiah Putri Mulyaningsih, Rina Desni Yetti and Harrizul Rivai (2020). This literature supports the presence of several phytochemical constituents in plant that express their use for various therapeutic purposes and can be used to treat multiple disorders in humans, such as diabetes, liver toxicity, fungal infections, microbial infections, immunomodulators, antiproliferative, wound healing, and antifungal analgesic, anti-inflammatory, antipyretic, hypoglycaemic, anti-dyslipidemic.

Ali *et al*., (2004) report the 7-geranyloxycoumarin named marmenol isolated from the methanolic extract of *Aegle marmelos* Linn Correa the structure of marmenol were established with the help of NMR spectroscopy

Balakumar, *et al., (*2011) studied the antifungal activity of the leaves of *Aegle marmelos* was reported against clinical isolates of dermatophytes. *Aegle marmelos* Linn Correa leaf extracts and fractions were found to have fungicidal activity against *Trichophyton mentagrophytes, T.rubrum, Microsporum canis, M. gypseum, Epidermophyton floccosum.*.

Tremendous Pharmacological Values of *Aegle marmelos* Linn Correa was done by Asha Jhajhria, and Krishan Kumar (2016). The present study highlighted that the important pharmacological activity of the leaves of *Aegle marmelos* Linn Correa has been found to be its antidiabetic activity.

Rijamol et al., (2008) was highlighted by Antibacterial Action of Leaves of *Aegle marmelos* Linn Correa. This study suggested that High flavanoid, alkaloid and phenol content in the various extracts of the plant may be one of the reasons for their antibacterial activity.

Characterization of antioxidant and cytotoxic potential of Methanolic extracts of different parts of *Aegle marmelos* Linn Correa was outlined by Nusrat Jahan Bristy et al., (2017). In the current study was focused on the Preliminary phytochemical screening of the methanol extracts of ripe fruit, half-ripe fruit, leaf and seed of the plant *Aegle marmelos* Linn Correa and revealed the presence of alkaloids and flavonoids, Glucosides, saponins, Glycosides, tannins and carbohydrates.

Sugeng et al., (2001) had isolate the two alkaloid 4, 7, 8 – trimethoxyfuroquinoline (skimmianine) and aegeline from *Aegle marmelos* Linn Correa. It's confirmed by spectrometric method.

Siddique *et* al., (2010) had investigated the total phenolic and total flavonoid content using folin ciocatteu reagent in different part of *Aegle marmelos* Linn Correa., the leaves contain larger amount of total phenolic and total flavonoid content compared other parts of the plant.

Kothari, et al.,(2011) studied the preliminary phytochemical screening on different extract (ether, methanol, chloroform.) *Aegle marmelos* Linn Correa., methanol extract showed the presence of flavonoid. Phenolics, tannins, saponins, coumarin, sterols terpenoids, and the ether , and chloroform extract showed only presence of sterols and phenols

Amit pandey and Rashmi, Mishra, (2011) studied the preliminary phytochemical screening on different extract (ethanol, methanol, water, ethyl acetate) *Aegle marmelos* Linn Correa. It showed the presence of tannins, saponins, terpenoids, alkaloids, polyphenol

Antiplasmodial and Antipyretic Activity and Safety Evaluation of the Methanolic Leaf Extract of *Murraya exotica* (L.) were done by Arnold Donkor Forkuo et al., (2020). In this present study, an attempt was made to evaluate the phytochemical substances and antiplasmodial and antipyretic activity of methanolic leaf extract of *Murraya exotica* Linn.

Bishay *et* al., (1987) was highlighted by phytochemical study of leaves of *Murraya exotica* (L.) In this study they isolate six methoxylated flavones from the air-dried powdered leaves of *Murraya exotica L.*

Preliminary phytochemical, cytotoxic, thrombolytic and antioxidant activities of the methanol extract of *Murraya exotica* Linn., leaves was performed by Amina Khatun, Mahmudur Rahman & Shamima Jahan (2014). This study proves that *Murraya exotica Linn* possess potent anti-mutagenic and anti-oxidative activities.

Exploiting the nutritional requirement for growth, flower Production and phytochemical profile of *Murraya exotica* Linn was attempted by Adnan Younis (2015). In this study they mainly described that the growth potential and phytochemical profile of *M. exotica* (L.) under Faisalabad agro-climatic conditions.

Effect of fertilization regime on *Murraya exotica* Linn plants growth and bioactive compounds was outlined by Diana Vasca-zamfir et al.,(2019). In this present study, attempt was made to evaluate the effect of fertilization regime on *Murraya exotica* (L.) plants growth and on some bioactive compounds contained in its leaves.

Phytochemistry and pharmacological properties of *Ruta graveolens* L. was done by Jinous Asgarpanah and Roghaieh Khoshkam (2012). In this present study an attempt was made to evaluate the phytochemical substances in the plant parts of *Ruta graveolens* L.

Phytochemical profiling, antimicrobial and cytotoxicity studies of methanolic extracts from *Ruta graveolens* L. was highlighted by Suganthi et al.,(2011). In this work they identified more than 160 phytochemicals and cytotoxic activities against Hep2 cell lines.

Giresha et al., (2015) documented by phytochemical composition, antioxidant and *in-vitro* anti-inflammatory activity of ethanol extract of *Ruta graveolens* L. leaves. In this study they suggested the strong correlation between antioxidant activity and the phytochemical contents of the extracts.

Phytochemical Characterization, Antioxidant and Antimicrobial Properties of Agitated Cultures of Three Rue Species: *Ruta chalepensis, Ruta corsica,* and *Ruta graveolens L.* was documented by Agnieszka Szewczyk et al., (2022).It has been revealed that all rue cultures tested can be used to obtain furanocoumarins, Therefore, in vitro cultures of *Ruta* spp. can be proposed as an alternative source of obtaining these metabolites.

Traditional uses, Phytochemistry and Ethanopharmacology of *Ruta graveolens* Linn was outlined by Shamal Badhusha et al., (2020). In this study they highlighted the comprehensive analysed information on the botanical, chemical, and pharmacological aspects of R. graveolens Linn.

Teklit Gebregiorgis Amabye et al., (2015) were documented by Phytochemical Screening and Evaluation of Antibacterial Activity of *Ruta graveolens* L. It has been observed that the plant extracts showed good antibacterial and antifungal properties and Chloroform and methanol extracts showed more antibacterial activity than ethanol at lower concentration.

Comparative study of *Ruta graveolens* Linn from Traditional System of Medicine to Modern Pharmacology was attempted by Shabir Ahmad Parray et al.,(2012). In this study they highlighted the traditional use of *Ruta graveolens* Linn especially with reference to Unani system of Medicine, to the modern scientific reports.

Identification and Pharmacognosy analysis of *Ruta graveolens* Linn was outlined by Kannan et al., (2012). In the Present work they described the pharmacognostic characters of *R. graveolens* to differentiate it from *R. chalepensis*. It has been revealed that morphologically, *R. graveolens* can be identified with its non-fringed petals and blunted apices of fruit lobes.

Phytochemical Composition and Antioxidant Potential of *Ruta graveolens L*. in Vitro Culture Lines was documented by Renuka Diwan et al.,(2012). This study proves that Antioxidant activity of in vitro cultures was significantly higher compared to in vivo plant material.

MATERIALS AND METHODS

PLANT MATERIAL

The plants selected for the present study are *Aegle marmelos* Linn Correa., *Murraya exotica (*L.) and *Ruta graveolens* L. belonging to the family Rutaceae.

Aegle marmelos Linn Correa is a small genus of three species distributed in tropical Asia and Africa. *Aegle marmelos Linn Correa* is about 12-15m. Tall with short trunk, medium sized tree, soft, thick, flaking bark and sometimes spiny branches. The deciduous leaf originates singly, wide, pointed and the terminal one with a long petiole. Flowers are greenish white in color and have bisexual, actinomorphic, hypogynous. The calyx is gamosepalous, light green, five lobed pubescent and corolla with five petals and pale yellow color. The Fruits are yellowish green, with small dots and the pulp of dried fruits retains it yellow. There are numerous seeds, embedded in the pulp and cotton-like hairs on their outer surface. It can be used to treat multiple disorders in humans, such as diabetes, liver toxicity, fungal infections, microbial infections, immunomodulators, antiproliferative, wound healing, antifungal, analgesic, anti-inflammatory, antipyretic, hypoglycemia and anti-dyslipidemic.

Murraya exotica L. is distributed over the greater part of India and the Andaman Islands to an altitude of 1500 m. It is a small tree with a spreading crown and short, often crooked, trunk; rather corky, fragrant. Leaves alternate, imparipinnate, leaflets usually 3-5, ovate or elliptic-lanceolate or rhomboid, glossy and darker above, gland-doted, base - rounded. The flowers are fragrant and are arranged in loose groups, each flower on a pedicel. There are five (sometimes four) sepals and five (sometimes four) white or cream-coloured petals. The fruit is an oval, glabrous, orange-red berry. It has anti-diabetic, antioxidant, anti-nociceptive, anti-inflammatory, anti-diarrheal, oxytocic and anti-fertility properties

Ruta graveolens L. commonly known as Rue is an herbaceous perennial, originally native to the Mediterranean region .*Ruta graveolens L,* is a perennial, scented and glabrous herb or a sub-shrub. Stem is slender, smooth, pale glaucous green and reaches up to a meter in height. Leaves are alternate, gland-dotted, glaucous, compound, 2-3 pinnate. Leaflets are linear-oval or oblong. Inflorescence is terminal corymbose, irregularly dichotomous cymes. Flowers are regular bisexual, terminal ones are pentamerous and others are tetramerous. Petals are distinct, widely spreading, greenish yellow, wide and hooded at top, abruptly connected to narrow claw below, margin wavy and sometimes toothed. Fruits are dry, hard, roundish, 4-5

blunted lobed at top. *Ruta graveolens L.*, has been used in traditional medicines for the relief of pain, eye problems, rheumatism and dermatitis.

COLLECTION OF PLANT MATERIAL

Aegle marmelos Linn Correa and *Murraya exotica Linn* were collected locally from local areas of North paravoor (Ernakulam) whereas *Ruta graveolens L* is purchased from nursery of vaduthala (Ernakulam).The plants collected were identified botanically using taxonomical experts of Department of botany and Centre for Research, ST. Teresa's college ,Ernakulam (Autonomous). Fresh and tender leaves of selected plants were used for phytochemical analysis.

PREPARATION OF PLANT EXTRACT

The leaves of the selected plant were removed from the plants and then washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were crushed into powder with the help of a suitable grinder and stored in polythene bags for use.

For the preparation of plant extract, the plant powder was taken and mixed with distilled water and ethanol individually for preparing aqueous extract and ethanolic extract. The solution then filtered with the help of filter paper and filtered extract of the selected plant samples were taken and used for further phytochemical analysis

PHYTOCHEMICAL ANALYSIS

PHARMACOGNOSTIC ANALYSIS

- Morphological evaluation
- Organoleptic evaluation
- Foliar studies
    - Stomatal type and stomatal index
    - Palisade ratio
    - Vein- islet number
    - Vein termination number
- Anatomy

HISTOCHEMICAL STUDIES

PHYTOCHEMICAL ANALYSIS

Qualitative analysis

DETECTION OF ALKALOID

For the preparation of plant extract 1 gm of powder sample was homogenized using 80% ethanol (10 ml). The homogenate was centrifuged at 1500 rpm for 5 minutes and supernatant was collected.

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

Wagner's reagent

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

Dragendroff's reagent

It was prepared by dissolving 8 gm of Bismuth sub nitrate in 20 ml of conc. HNO_3 (solution A) and 27.2 g KI in 50 ml distilled water (solution B). The solution were mixed, supernatant solution was decanted and made to 100 ml with distilled water.

Mayer's reagent

It was prepared by dissolving 1.36 gm of $Hgcl_2$ in 60 ml distilled water (solution A) and 5 gm of KI dissolved in 10 ml 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.

After the preparation of reagents a small amount of plant extract was taken and tested with all the 2 reagents and the formation of precipitate shows the presence of alkaloids. Absence of precipitate shows the absence of alkaloids.

DETECTION OF SAPONINS- FOAM TEST

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

DETECTION OF FLAVANOID- ALKALINE REAGENT TEST

To a little of the sample (ethanolic extract) add 2 ml of NaOH followed by 2 ml of dilute H_2SO_4. The yellow color in NaOH changes when dilute acid is added. This shows the presence of flavonoid.

DETECTION OF TANNIN — FERRIC CHLORIDE TEST

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

DETECTION OF STEROID- SALKOWSKI TEST

The ethanolic extract of the three samples were treated with few drops of concentrated sulphuric acid. A red colouration in the lower layer of the solution indicates the presence of steroid.

DETECTION OF BITTERS

1 gm of powdered drug were shaken with ethyl alcohol and then with ethyl acetate. Formation of green colour shows the presence of bitters.

DETECTION OF RESIN

50% of HNO_3 was added to a little of the powdered drug sample. Brown colour obtained indicates the presence of resin.

DETECTION OF PROTEIN

Xanthoprotein test: equal volume of test solution (aqueous extract) and concentrated HNO_3 (0.5 ML) were mixed well and the solution was cooled at 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

DETECTION OF CARBOHYDRATE

Molisch's test: 2 drop of molisch's reagent was added to 2ml of the test solution and was mixed well. 1ml of concentrated H_2SO_4 was added along the sides of the test tube without shaking. The appearance of purple or violet ring confirms the presence of carbohydrate.

Benedict's test: 5 ml of Benedict's reagent was added to the 1 ml of test solution and the test tube was kept in boiling water bath for 5 minutes. The appearance of red precipitate confirms the presence of carbohydrate.

Fehling's test: 1 ml 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. The formation of red or brick red precipitate confirms the presence of carbohydrate.

DETECTION OF PHENOL

Two drops of 5% $Fecl_3$ is added to the extract in a test tube. Presence of greenish precipitate indicates the presence of phenol.

ASSAY OF ACTIVE INGREDIENTS

It include estimation of tannin, phenol content and carbohydrate

ESTIMATION OF TANNIN

For the estimation of tannins, first Indigo sulphonic acid was prepared. It was prepared by adding 1 gm Indigo carmine in 25 m1 concentrated sulphuric acid in 100 ml beaker. The indigo carmine was then dissolved completely in concentrated sulphuric acid. To this again 25m1 of concentrated sulphuric acid was added and it was made up to 1 litter in volumetric flask by using distilled water.

For titration, 0.1N Potassium permanganate solution was taken in a burette and 25 ml of the indigo sulphonic acid was taken in 1 litre conical flask. It was then titrated and blank value was taken .Then 1 gm of the sample was taken in a conical flak with a rubber cork, 100 m1 distilled water was added to it and shaken well for 10 minutes. It was filtered, 10m1 of the sample solution was taken in 1 litre conical flask and 25 ml Indigo sulphonic acid was added to the solution

The above solution was titrated with 0.1 N Potassium permanganate solutions. End point was the change of blue colour to golden yellow colour. Then the burette reading was taken, 1 ml of 0.1 N $Kmno_4$ is equal to 0.005647 gm of tannins. Total tannin was calculated using the formula.

Percentage of tannins = Burette reading - Blank reading X 0.005647 X 10 X 10O

Weight of the sample

ESTIMATION OF PHENOLIC COMPOUND

1 gm of both leaf and bark were homogenized separately using 80% ethanol (25m1). The homogenate was centrifuged at 1000rpm for 20 minutes. The supernatant was collected and residue was again centrifuged and extract was collected. 0.2 ml aliquot was taken from this extract in a test tube and 2.8 m1 distilled water was added. To this sample 0.5 m1 Folin cio-calteau reagent was added and kept for 3 minutes. After that 2 m1 sodium bicarbonate was added. The test tube was placed in a boiling water bath for one minute and optical density was measured against blank solution using colorimeter at 550nm. Blank solution was prepared by taking 3 m1 distilled water. A standard graph was prepared using different concentrations of sample and the concentration of phenol was obtained from the graph. It was expressed as mg phenol/ 100 mg material. Phenol concentration of leaf 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 observation

I gm each of Anthrone and Thiourea was taken. 760 ml of concentrated H_2SO_4 was added to 240m1 of distilled water. Anthrone and Thiourea were dissolved in this. 200 mg of oven dried and ground leaf sample was taken and was boiled for 1/2 an hour with 20 ml of distilled water in a 250 ml conical flask. It was filtered and volume was made up to 50 ml with distilled water. 1ml of the filtrate was pipetted in colorimetric tube and 10 ml of Anthrone reagent was added. After stoppering the tubes with rubber plugs then were kept in water bath at 100°C for 20 minutes. Tubes were removed from water bath and were cooled in running water. The color density developed was measured colorimetrically at 625nm.

The following equation was used for the calculation.

mg. Carbohydrate /100 gmof the sample = Xa x 20 x 500/reading the standard OD.

(2m1 of 100ppm glucose)

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 1000m1.

ESTIMATION OF FLAVANOID

Sample preparation: a ground freeze dried sample of 0.5g was weighed and flavonoid compound was extracted with 50 ml of 80% aqueous methanol on a ultrasonic bath for 20 minutes and aliquot 2ml of the extract was ultra-centrifuged for 5 minutes at 14000rpm

Total flavonoid assay: total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquot (1ml) of extract or standard solution catechin (20, 40, 60, and 80,100) mg\L was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5%$NaNo_2$. After 5 minutes 0.3 ml of 10% aluminium chloride was added .At the 6^{th} minute 2 ml 1M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm

ESTIMATION OF CHLOROPHYLL CONTENT

The method of Arnon (1949) was employed for the quantitative estimation of chlorophyll and carotenoid contents.

80% acetone was prepared. A preweighed (250mg) quantity of fresh leaf material was ground into a fine paste. 10 ml of 80% acetone was added into it. The extract was centrifuged and the green supernatant was obtained. Using small quantities of acetone the extract was centrifuged repeatedly till the lechate became colorless. The supernatant was taken together and was made up to 25m1 with 80% acetone. The extract was kept away from direct sunlight. The

optical density of the extract was read at 480,510,645, 652and 663 wave lengths. The samples were analysed in duplicates.

From the optical densities the chlorophyll contents were calculated using the formula:

1. Chlorophyll a (mg/gm) = 12.7 (D 663) - 2.69 (D.645) x V/1000 x W

2. Chlorophyll b (mg/gm) = 22.9 (D 645) - 4.68 (D.663) x V/ 1000 xW

3. Total Chlorophyll (mg/gm) = D 652 x 1000/34.5 x V/1000 x W

Where

D=Optical density

V=Final volume of 80% acetone (25m1).

W= Weight of sample taken (0.25gm)

The result obtained was compared with that of control**.**

PHARMACOGNOSTIC ANALYSIS

Morphological analysis

Morphological features of plants can be studied using standard parameters

Organoleptic evaluation

Organoleptic evaluation includes the study of the nature of the powdered leaf drug, for this, the color taste and textures of the drug were noted.

FOLIAR STUDIES

Determination of stomata type and stomata index

The term stomata index was first introduced by Salisbury (1932). The percentage of proportion of the ultimate divisions of the epidermis of a leaf which have been converted into stomata is termed the stomatal index. For determining the stomatal index the fresh leaves of the plant was taken. From the lower surface of these leaves, epidermal peels were taken. It was then stained with saffranin. Then the peel was placed in a clean 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 and the number of epidermal cells and stomata in the field were noted. Then the stomatal index was calculated using the formula

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

Where

S=Number of stomata per unit area

E= Number of epidermal cell in the same area

PALISADE RATIO

Palisade ratio is defined as the average number of palisade cells beneath each epidermal cells. Fresh leaves of the plants were taken. Then they were boiled with trichloro acetic 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 cells 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.

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

Determination of vein-islet number

The word vein islet is found for the minute area of photosynthetic tissue encircled by ultimate divisions of conducting strands. Vein islet number is defined as the number of vein-islet per square millimeter of the leaf surface mid-way between 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 leaves becomes transparent they were mounted in glycerine. A camera lucida was attached to the microscope. A paper was placed and the vein-islet was traced by looking through the microscope when the super imposed image of the leaf

portion and paper were seen at the same time. The number of vein-islets in each 4cnix4cm square was counted and the average vein-islet number was calculated.

Determination of vein termination number

Vein termination number is defined as the number of vein-let terminations per square millimeter of leaf surface between midrib and margin.

The method of determination of vein termination number was put forth by Hall and Melville (1954). Leaf pieces were placed in trichloroacetic acid.

When leaves become transparent and clean, one of the pieces was mounted in glycerine. Camera lucida drawings were made and the number of vein termination present within the 4cmx4cm square counted and the average vein termination number was determined.

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.

HISTOCHEMICAL STUDIES

For the histochemical studies, free hand sections of the stem, leaf were taken treated with various stains for detection of primary and secondary Metabolites. The histochemical stains were prepared and the material was stained using it and was mounted in clean slide. Photomicrographs of these slides were taken with trinoclular microscope to which camera system was attached.

Starch — iodine- potassium iodide reaction (Johansen, 1940)

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

Polyphenols (Toludine blue 0 Method: 0 Brien et. al; 1964, Feder and Wolf, 1965; Cully, 1966)

The peelings were stained in toludine blue 0 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.

Lipids — Sudan Dye Method (Baker, 1947)

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.

OBSERVATION AND RESULT

PHYTOCHEMICAL ANALYSIS

Detection of Alkaloids

Presence of alkaloids in extract of *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L. was tested using Dragendroff's reagent, Mayer's reagent and Wagner's reagent. Formation of orange- red, green and brown colour precipitate indicated the presence of alkaloid. (TABLE 1-3), (FIGURE 1-3)

Detection of saponin

Detection of saponin was done by shaking the powder vigorously with water. The formation of froth indicated the presence of saponins. According to this study *Aegle marmelos* Linn Correa., and *Murraya exotica* L., showed the presence of saponin whereas saponin was absent in *Ruta graveolens* L. (TABLE 1-3), (FIGURE 4-5)

Detection of flavonoids

For detecting the flavonoid, the sample was treated with NaOH and dilutes H_2SO_4. Yellow color obtained showed discoloration on treatment with acid, which indicated the presence of flavonoids. *Aegle marmelos* Linn Correa., and *Ruta graveolens* L. indicated the presence of flavonoid whereas flavonoids are absent in *Murraya exotica* L. (TABLE 1-3), (FIGURE 6-7)

Detection of tannins

For detection of tannins the sample filtrate was treated with 5% ferric chloride solution in a test tube and greenish black colour confirmed the presence of tannin. According to this study *Aegle marmelos* Linn Correa., and *Murraya exotica* L., showed the presence of tannin whereas tannin was absent in *Ruta graveolens* L. (TABLE 1-3), (FIGURE 8-9)

Detection of steroids

For steroids, ethanolic extract of the sample was treated with few drops of concentrated sulphuric acid and red coloration in lower layer of the solution indicated the presence of steroid. All the three, *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L., showed the presence of steroids. (TABLE 1-3), (FIGURE 10-12)

Detection of bitters

Detection of bitters was done by shaking the powder with ethyl alcohol followed by ethyl acetate. The green colour obtained indicated the presence of bitters. All the three, *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L., showed the presence of bitters. (TABLE 1-3), (FIGURE 13-15)

Detection of resin

When the plant extract was treated with 50% of HNO_3, brown colour was observed which indicated the presence of resin. *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L. showed the presence of resin. (TABLE 1-3), (FIGURE 16-18)

Detection of protein

For detection of protein equal volume of extract and concentrated HNO_3 were mixed well. Yellow colour obtained changes to bright orange on addition of 40% NaOH which indicated the presence of protein. All the three, *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L., showed the presence of protein. (TABLE 1-3), (FIGURE 19-21)

Detection of carbohydrate

The presence of carbohydrate in extract was tested using Molisch's reagent, Benedict's reagent and Fehling's reagent. Reaction with Molisch's reagent showed reddish violet zone at the junction of two liquids, Benedict's reagent showed the orange red precipitate and Fehling's reagent showed red precipitate. *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L. showed the presence of carbohydrates. (TABLE 1-3), (FIGURE 22-24)

Detection of phenol

For phenols, Two drops of 5% $Fecl_3$ was added to the extract in a test tube. Presence of greenish precipitate indicates the presence of phenol. All the three, *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L., showed the presence of phenol. (TABLE 1-3), (FIGURE 25-27)

ESTIMATION OF TANNIN

The result of estimation of tannin content shows that the *Aegle marmelos* Linn Correa., has more tannin than *Murraya exotica* L. The tannin content of *Aegle marmelos* Linn Correa., was found to be 46 % and for *Murraya exotica* L., it shows 39 %.(TABLE 4-6)

ESTIMATION OF PHENOLIC COMPOUND

Phenolic content of the *Aegle marmelos* Linn Correa., was found to be 2.7µg/l. For *Murraya exotica* L., it shows 2.50µg/l. *Ruta graveolens* L., shows 2.92µg/l. (TABLE 4-6)

ESTIMATION OF CARBOHYDRATE

On estimating carbohydrate content, the conclusion obtained was carbohydrate content is slightly more in *Ruta graveolens* L., than other two plants. The carbohydrate content of *Aegle marmelos* Linn Correa., was 0.80mg/g. For *Murraya exotica* L., it was found to be 1.30mg/g. the carbohydrate content of *Ruta graveolens* L., was estimated as 1.45mg/g. (TABLE 4-6)

ESTIMATION OF CHLOROPHYLL CONTENT

The analysis of chlorophyll content shows that the quantity of chlorophyll is more in *Murraya exotica* L., and *Ruta graveolens* L., than *Aegle marmelos* Linn Correa. Chlorophyll content of *Aegle marmelos* Linn Correa., was found to be 3.62mg/g and for *Murraya exotica* L., shows 5.50mg/g. The chlorophyll content of *Ruta graveolens* L., was found to be 5.8mg/g. (TABLE 4-6)

ESTIMATION OF FLAVONOID

Flavonoid content of *Aegle marmelos* Linn Correa., was found to be 4mg/l and for *Ruta graveolens* L., it shows 4.37mg/l. (TABLE 4-6)

PHARMACOGNOSTIC ANALYSIS

MORPHOLOGICAL ANALYSIS

Aegle marmelos Linn Correa., is a small deciduous thorny tree. The deciduous leaves originate singly, ovate in shape, wide, reticulate venation; alternate and 3- foliate arrangement, pointed and the terminal one with a long petiole, dark green in colour with pinnate vernation. Flowers are greenish white in color. Flowers are in axillary panicles, The Fruits are yellowish green, with small dots. (FIGURE 28)

Murraya exotica L., are an evergreen shrub. Leaves alternate, spiral, imparipinnate, exstipulate, leaflets usually 3-5, ovate or elliptic-obovate or rhomboid, glossy and darker above, gland-doted, base – rounded, reticulate arrangement with pinnate vernation. The flowers are white or cream-coloured with terminal or axillary panicle. The fruit is an oval, glabrous, orange-red berry. (FIGURE 29)

Ruta graveolens L., is a perennial, scented and glabrous herb or a sub-shrub. The stems become woody near the base, but remain herbaceous nearer the tips. Leaves are dissected pinnately into oblong or spoon shaped segments, alternate, reticulate arrangement, gland-dotted, glaucous, and compound. Leaflets are linear-oval or oblong. They are somewhat fleshy and usually covered with a powdery bloom. The sea green foliage has a strong, pungent, rather unpleasant scent when bruised. The paniculate clusters of small yellow flowers held well above the foliage and often covering most of the plant. Inflorescence is terminal corymbose, irregularly dichotomous cymes. Fruits are dry, hard, and roundish. (FIGURE 30)

ORGANOLEPTIC EVALUATION

Dried fine powder of these plants showed different organoleptic characters. For *Aegle marmelos* Linn Correa., it was dark green in colour with characteristic odour, glabrous texture and bitter taste. For *Murraya exotica L.,* it was brownish green in colour with citrusy aroma, glossy texture and bitter taste. For *Ruta graveolens L.,* it was yellowish brown in colour with unpleasant odour, soft and glaucous texture with bitter taste. (TABLE 7-9)

FOLIAR STUDIES

The three plants possess different types of stomata. *Aegle marmelos* Linn Correa., possess paracytic type of stomata and for Murraya exotica L., it showed anisocytic type of stomata. The stomatal type of *Ruta graveolens L.,* was found to be anomocytic.

The stomatal index of the leaf is the ratio of the number of stomata to the total number of stomata and epidermal cells. Stomatal index of *Aegle marmelos* Linn Correa., was found to be 16.36. For *Murraya exotica L.,* it was found to be 26.08 and *Ruta graveolens* L., shows 27.90. (TABLE 10), (FIGURE 31-33)

The palisade ratio of the *Aegle marmelos* Linn Correa., was found to be 5.6 and for *Murraya exotica L.,* it showed 6.98. The palisade ratio of *Ruta graveolens L.,* was found to be 3.48. (TABLE 11)

Vein islet number is defined as the number of vein-islet per square millimeter of the leaf surface mid-way between midrib and the margin and Vein termination number is defined as the number of vein-let terminations per square millimeter of leaf surface between midrib and margin. Vein islet and vein termination number of *Aegle marmelos* Linn Correa., are 6 and8 and for *Murraya exotica L.,* it was found to be 8and 12. Vein islet and vein termination number of *Ruta graveolens L.,* are 7 and 10. (TABLE 12), (FIGURE 34-36)

ANATOMY

Anatomically the leaf of *Aegle marmelos* Linn Correa., is differentiated into epidermis, mesophyll and vascular tissue. Transverse section of *Aegle marmelos Linn Correa.,* leaf showed the presence of upper arched and lower epidermis. Both comprised of round to oval shaped cells. The Epidermis is single layered occasionally interrupted with sunken stomata on both surfaces and over lined by a thick layer of cuticle. Both upper and lower epidermal layers bear stomata. Each stoma has two guard cells and two subsidiary cells and they correspond to paracytic type. Numbers of stomata are high in upper epidermis compared to lower epidermis and both epidermis shown presence of covering trichrome. Below the upper epidermis and above the lower epidermis are seen strips of the collenchyma (3-4 layered). Mesophyll differentiated into palisade and spongy parenchyma Mesophyll cells include sclerenchyma and collenchyma fibres that proliferate from the sheath around the vein. Palisade tissues are many layered, which consists of closely, packed oval cell without much intercellular space. Spongy mesophyll is located below palisade mesophyll and comprises of leaf vascular tissue. Calcium oxalate crystals and Resin canal were evident in the mesophyll. The vascular bundle of the midrib is large, somewhat circular and collateral. Bundle sheaths are sclerenchymatous fibres. Midrib shown the presence of xylem and phloem arranged in an arc. Vascular bundles are radial and endarch. (FIGURE 37)

Transverse section of *Murraya exotica L.,* is differentiated into epidermis, mesophyll and vascular tissue. Epidermis is the outer most layer. It consists of abaxial and adaxial epidermis. The adaxial epidermis cells are larger than the adaxial epidermal cells. Epidermis is covered by thick cuticle. Epidermis is interrupted by anisocytic stomata. Mesophyll is differentiated into palisade tissue and spongy mesophyll. Palisade cells are multilayered, closely packed and vertically elongated chlorenchyma cells with little or no intercellular space. Spongy cells are thin walled, irregularly shaped and loosely arranged chlorenchyma

cells. Vascular region consists of vascular tissue. They are irregularly distributed in the spongy mesophyll. Vascular bundle is arched shaped, collateral, and endarch. (FIGURE 38)

Anatomically *Ruta graveolens L.,* is differentiated into epidermis, mesophyll and vascular tissue. Epidermis is the outermost layer. The upper and lower epidermis is single layered and covered with cuticle. Epidermis is interrupted with anomocytic stomata. Mesophyll is differentiated into double layered palisade cells and loosely arranged spongy cells. Rosette types of calcium oxalate are abundant, especially located in-between the palisade and spongy cells. Vascular bundle is v shaped with xylem on the upper side and phloem on lower side. (FIGURE 39)

HISTOCHEMICAL STUDIES

STARCH

When tested with iodine potassium iodide, starch grains were observed as black granules in guard cells and epidermal cells of the leaf peeling. Black granules were observed in *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L. (TABLE 13-15), (FIGURE 40-42)

POLYPHENOL

The peelings were stained in toludine blue. The polyphenols when present stained turquoise blue. All the three, *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L., shows the presence of polyphenol. (TABLE 13-15), (FIGURE 43-45)

LIPIDS

Presence of lipid was indicated by the presence of black granules in leaf peelings. Lipid were absent in *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L. (TABLE 13-15)

TABLE 1: DETECTION OF PHYTOCHEMICAL - *Aegle marmelos* Linn Correa.

SL NO.	CHEMICAL CONSTITUENTS	TEST	OBSERVATION	RESULT
1.	TEST FOR ALKALOID	Wagner's test	Formation of orange- red, green and brown colour precipitate	+
		Dragendroff's reagent		
		Mayer's reagent		
2.	TEST FOR SAPONIN	Foam test	formation of froth	+
3.	TEST FOR FLAVONOID	Alkaline reagent test	Yellow color obtained showed discoloration on treatment with acid	+
4.	TEST FOR TANNIN	Ferric chloride test	Greenish black colour is formed	+
5.	TEST FOR STEROID	Salkowski test.	Red coloration in lower layer of the solution	+
6.	TEST FOR BITTER	Bitter test	Green colour is formed	+
7.	TEST FOR RESIN	Nitric acid test	Brown colour was observed	+
8.	TEST FOR PROTEIN	Xanthoprotein test	Yellow colour obtained changes to bright orange on addition of 40% NaOH	+
9.	TEST FOR CARBOHYDRATE	Molisch's test	Reddish violet zone at the junction of two liquids	+
		Benedict's test	Orange red precipitate	
		Fehling's test	Red precipitate	
10.	TEST FOR PHENOL	Ferric chloride test	Greenish precipitate	+

TABLE 2: DETECTION OF PHYTOCHEMICALS - Murraya *exotica* L.

SL NO.	CHEMICAL CONSTITUENTS	TEST	OBSERVATIONS	RESULT
1	TEST FOR ALKALOID	Wagner's test	Formation of orange- red, green and brown colour precipitate	+
		Dragendroff's reagent		
		Mayer's reagent		
2.	TEST FOR SAPONIN	Foam test	formation of froth	+
3.	TEST FOR FLAVONOID	Alkaline reagent test	No discoloration on treatment with acid	--
4.	TEST FOR TANNIN	Ferric chloride test	Greenish black colour is formed	+
5.	TEST FOR STEROID	Salkowski test	Red coloration in lower layer of the solution	+
6.	TEST FOR BITTER	Bitter test	Green colour is formed	+
7.	TEST FOR RESIN	Nitric acid test	Brown colour was observed	+
8	TEST FOR PROTEIN	Xanthoprotein test	Yellow colour obtained changes to bright orange on addition of 40% NaOH	+
9.	TEST FOR CARBOHYDRATE	Molisch's test	Reddish violet zone at the junction of two liquids	+
		Benedict's test	Orange red precipitate	
		Fehling's test	Red precipitate	
10.	TEST FOR PHENOL	Ferric chloride test	Greenish precipitate	+

TABLE 3: DETECTION OF PHYTOCHEMICAL - *Ruta graveolens L.*

SL NO.	CHEMICAL CONSTITUENTS	TEST	OBSERVATION	RESULT
1.	TEST FOR ALKALOID	Wagner's test	Formation of orange- red, green and brown colour precipitate	+
		Dragendroff's reagent		
		Mayer's reagent		
2.	TEST FOR SAPONIN	Foam test	No formation of froth	-
3.	TEST FOR FLAVONOID	Alkaline reagent test	Yellow color obtained showed discoloration on treatment with acid	+
4.	TEST FOR TANNIN	Ferric chloride test	No greenish black colour	-
5.	TEST FOR STEROID	Salkowski test.	Red coloration in lower layer of the solution	+
6.	TEST FOR BITTER	Bitter test	Green colour is formed	+
7.	TEST FOR RESIN	Nitric acid test	Brown colour was observed	+
8.	TEST FOR PROTEIN	Xanthoprotein test	Yellow colour obtained changes to bright orange on addition of 40% NaOH	+
9.	TEST FOR CARBOHYDRATE	Molisch's test	Reddish violet zone at the junction of two liquids	+
		Benedict's test	Orange red precipitate	
		Fehling's test	Red precipitate	
10.	TEST FOR PHENOL	Ferric chloride test	Greenish precipitate	+

TABLE 4

ESTIMATION OF PHYTOCHEMICAL COMPOUND- *Aegle marmelos* Linn correa.

SL NO.	PHYTOCHEMICAL	RESULT
1.	ESTIMATION OF TANNIN	46%
2.	ESTIMATION OF PHENOLIC COMPOUND	2.75 µg/l
3.	ESTIMATION OF CARBOHYDRATE	0.80 mg/g
4.	ESTIMATION OF FLAVONOID	4 mg/l
5.	ESTIMATION OF CHLOROPHYLL CONTENT	3.62 mg/g

TABLE 5:

ESTIMATION OF PHYTOCHEMICAL COMPOUND- *Murraya exotica* L.

SL NO.	PHYTOCHEMICAL	RESULT
1.	ESTIMATION OF TANNIN	39%
2.	ESTIMATION OF PHENOLIC COMPOUND	2.50 µg/l
3.	ESTIMATION OF CARBOHYDRATE	1.30 mg/g
4.	ESTIMATION OF CHLOROPHYLL CONTENT	5.50 mg/g

TABLE 6

ESTIMATION OF PHYTOCHEMICAL COMPOUND - *Ruta graveolens* L.

SL NO.	PHYTOCHEMICAL	RESULT
1.	**ESTIMATION OF PHENOLIC COMPOUND**	2.92 µg/l
2.	**ESTIMATION OF CARBOHYDRATE**	1.45 mg/g
3.	**ESTIMATION OF FLAVONOID**	4.37 mg/l
4.	**ESTIMATION OF CHLOROPHYLL CONTENT**	5.8 mg/g

TABLE 7

ORGANOLEPTIC EVALUATION OF POWDER- *Aegle marmelos* Linn correa.

SL NO.	ORGANOLEPTIC CHARACTERS	INFERENCE
1.	**COLOUR**	DARK GREEN
2.	**ODOUR**	CHARACTERISTIC
3.	**TASTE**	BITTER
4.	**TEXTURE**	GLABROUS

TABLE 8

ORGANOLEPTIC EVALUATION OF POWDER - *Murraya exotica* L.

SL NO.	ORGANOLEPTIC CHARACTERS	INFERENCE
1.	**COLOUR**	BROWNISH GREEN
2.	**ODOUR**	CITRUSY AROMA
3.	**TASTE**	BITTER
4.	**TEXTURE**	GLOSSY

TABLE 9

ORGANOLEPTIC EVALUATION OF POWDER - *Ruta graveolens* L.

SL NO.	ORGANOLEPTIC CHARACTERS	INFERENCE
1.	**COLOUR**	YELLOWISH BROWN
2.	**ODOUR**	UNPLEASANT
3.	**TASTE**	BITTER
4.	**TEXTURE**	SOFT AND GLAUCOUS

TABLE 10

DETERMINATION OF STOMATAL TYPE AND STOMATAL INDEX

SL NO.	NAME OF THE PLANT	STOMATAL TYPE	STOMATAL INDEX
1.	***Aegle marmelos* Linn correa.**	PARACYTIC	16.36
2.	***Murraya exotica* L.**	ANISOCYTIC	26.08
3.	***Ruta graveolens* L.**	ANOMOCYTIC	27.90

TABLE 11

DETERMINATION OF PALISADE RATIO

SL NO.	NAME OF THE PLANT	PALISADE RATIO
1.	***Aegle marmelos* Linn correa.**	5.6
2.	***Murraya exotica* L.**	6.98
3.	***Ruta graveolens* L.**	3.48

TABLE 12

DETERMINATION OF VEIN ISLET AND VEIN TERMINATION NUMBER

SL NO.	NAME OF THE PLANT	VEIN ISLET NUMBER	VEIN TERMINATION NUMBER
1.	***Aegle marmelos* Linn correa.**	6	8
2.	***Murraya exotica* L.**	8	12
3.	***Ruta graveolens* L.**	7	10

TABLE 13

HISTOCHEMICAL STAINING - *Aegle marmelos* Linn correa.

SL NO.	PRINCIPLE	TEST	RESULT
1.	**STARCH**	**Iodine- potassium iodide reaction**	POSITIVE
2.	**POLYPHENOL**	**Toludine blue O Method**	POSITIVE
3.	**LIPIDS**	**Sudan Dye Method**	NEGATIVE

TABLE 14

HISTOCHEMICAL STAINING - *Murraya exotica* L.

SL NO.	PRINCIPLE	TEST	RESULT
1.	**STARCH**	**Iodine- potassium iodide reaction**	POSITIVE
2.	**POLYPHENOL**	**Toludine blue O Method**	POSITIVE
3.	**LIPIDS**	**Sudan Dye Method**	NEGATIVE

TABLE 15

HISTOCHEMICAL STAINING - *Ruta graveolens* L.

SL NO.	PRINCIPLE	TEST	RESULT
1.	**STARCH**	**Iodine- potassium iodide reaction**	POSITIVE
2.	**POLYPHENOL**	**Toludine blue O Method**	POSITIVE
3.	**LIPIDS**	**Sudan Dye Method**	NEGATIVE

ALKALOID DETECTION

Figure 1 ***Aegle marmelos*** **Linn correa.**

Figure 2 ***Murraya exotica*** **L.**

Figure 3 ***Ruta graveolens*** **L.**

DETECTION OF SAPONIN

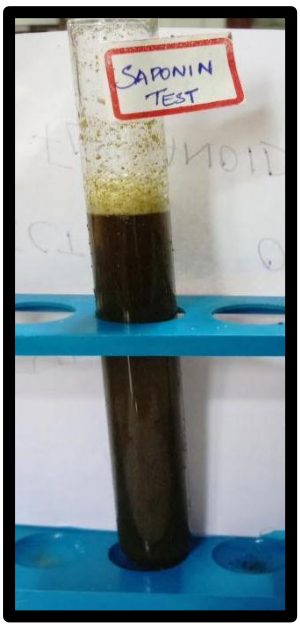

Figure 4 ***Aegle marmelos*** **Linn correa.**

Figure 5 ***Murraya exotica*** **L.**

DETECTION OF FLAVONOID

Figure 6 ***Aegle marmelos*** **Linn correa.**

Figure 7 ***Ruta graveolens*** **L.**

DETECTION OF TANNIN

Figure 8 ***Aegle marmelos*** **Linn correa.**

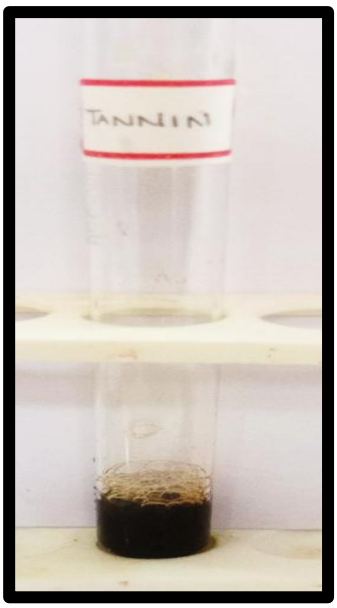

Figure 9 ***Murraya exotica*** **L.**

DETECTION OF STEROID

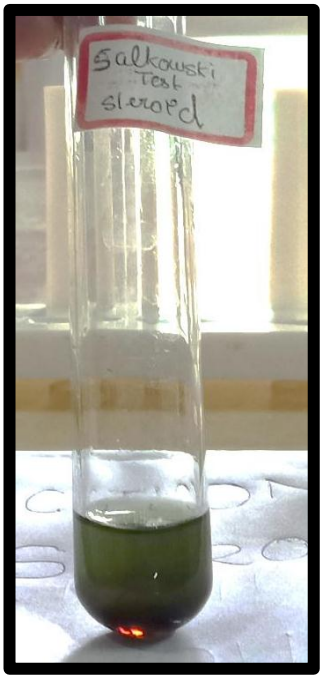

Figure 10 ***Aegle marmelos*** **Linn correa.**

Figure 11 ***Murraya exotica*** **L.**

Figure 12 ***Ruta graveolens*** **L**

DETECTION OF BITTER

Figure 13 ***Aegle marmelos*** **Linn correa.**

Figure 14 ***Murraya exotica*** **L.**

Figure 15 ***Ruta graveolens*** **L.**

DETECTION OF RESIN

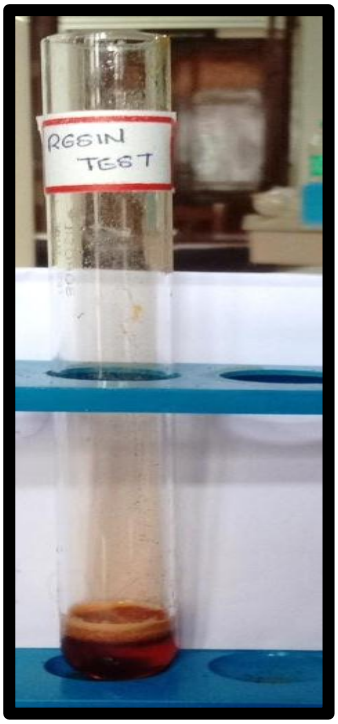

Figure 16 ***Aegle marmelos*** **Linn correa.**

Figure 17 ***Murraya exotica*** **L.**

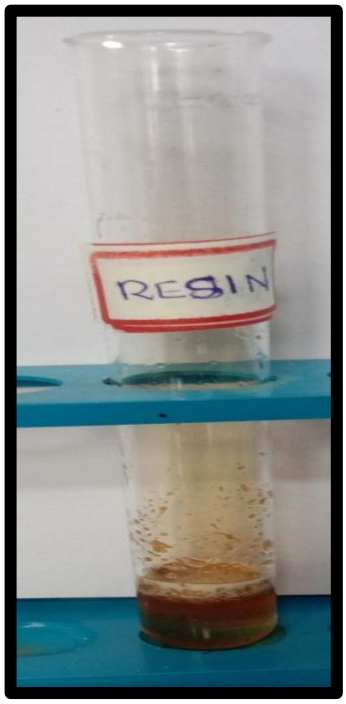

Figure 18 ***Ruta graveolens*** **L.**

DETECTION OF PROTEIN

Figure 19 ***Aegle marmelos*** **Linn correa.**

Figure 20 ***Murraya exotica*** **L.**

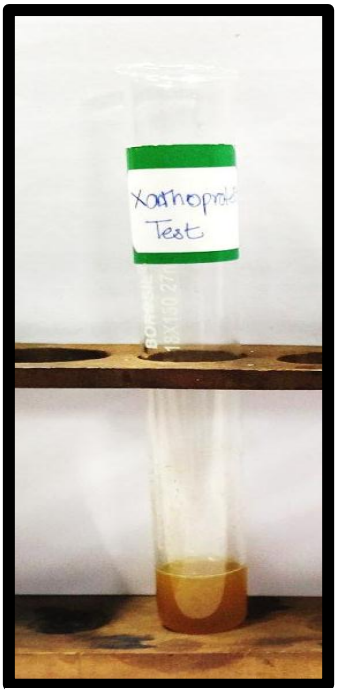

Figure 21 ***Ruta graveolens*** **L.**

DETECTION OF CARBOHYDRATE

Figure 22 ***Aegle marmelos*** **Linn correa.**

Figure 23 ***Murraya exotica*** **L.**

Figure 24 ***Ruta graveolens*** **L.**

DETECTION OF PHENOL

Figure 25 ***Aegle marmelos*** **Linn correa.**

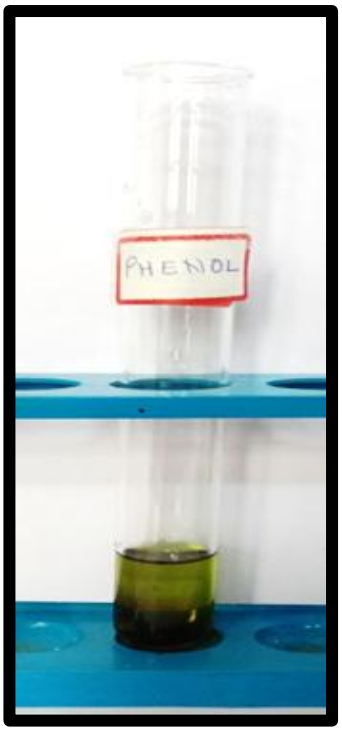

Figure 26 ***Murraya exotica*** **L.**

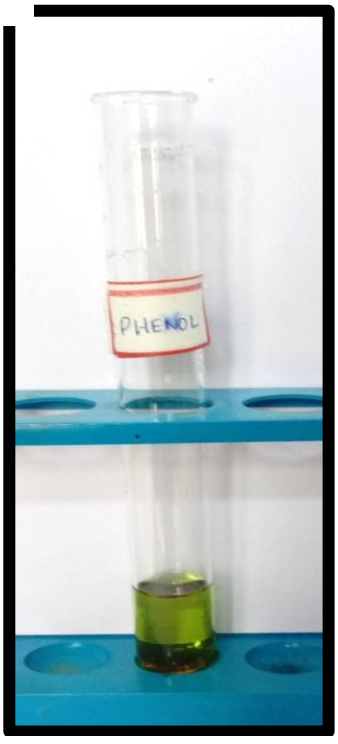

Figure 27 ***Ruta graveolens*** **L.**

PHARMACOGNOSTIC ANALYSIS

MORPHOLOGICAL EVALUATION

Figure 28 *Aegle marmelos* Linn correa.

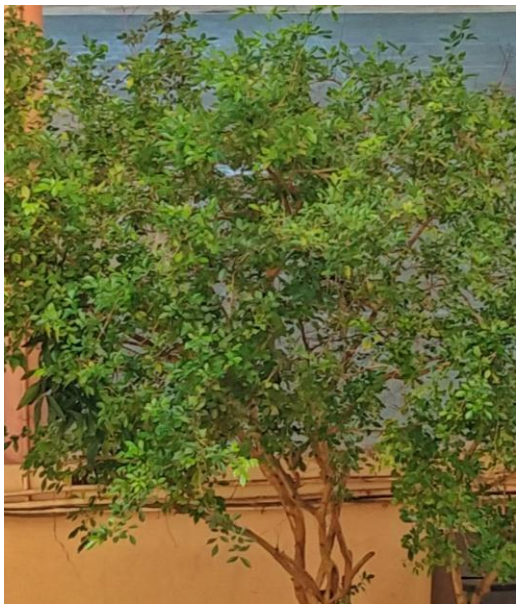

Figure 29 *Murraya exotica* L.

Figure 30 *Ruta graveolens* L.

STOMATAL TYPE AND STOMATAL INDEX

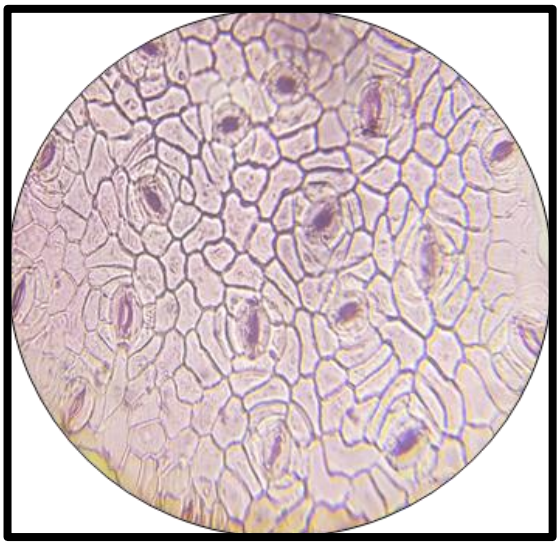

Figure 31 ***Aegle marmelos*** **Linn correa. - PARACYTIC STOMATA**

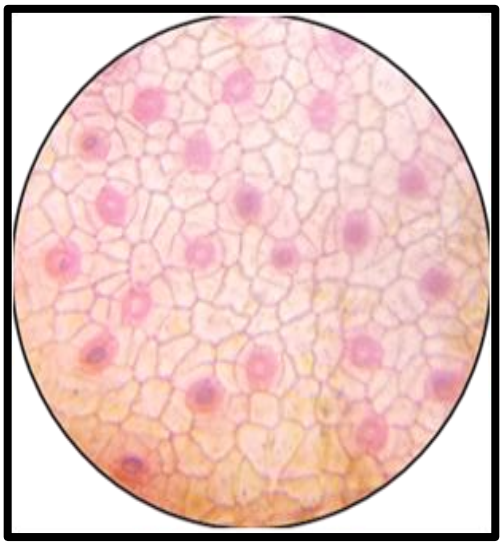

Figure 32 ***Murraya exotica*** **L. - ANISOCYTIC STOMATA**

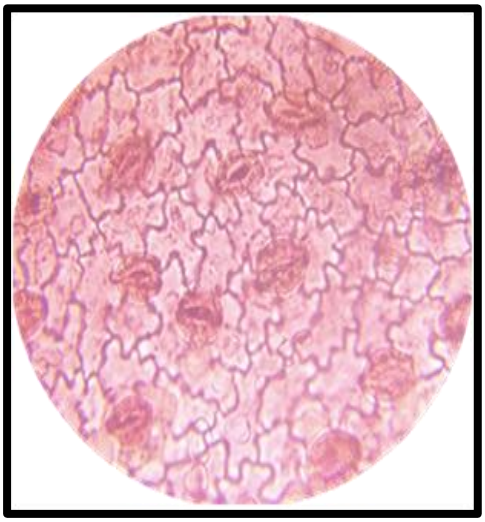

Figure 33 ***Ruta graveolens*** **L. - ANOMOCYTIC STOMATA**

VEIN ISLET AND VEIN TERMINATION NUMBER

Figure 34 ***Aegle marmelos*** **Linn correa.**

Figure 35 ***Murraya exotica*** **L.**

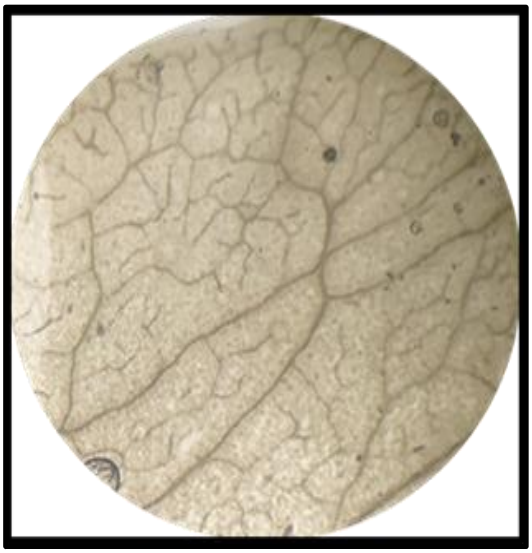

Figure 36 ***Ruta graveolens*** **L.**

LEAF ANATOMY

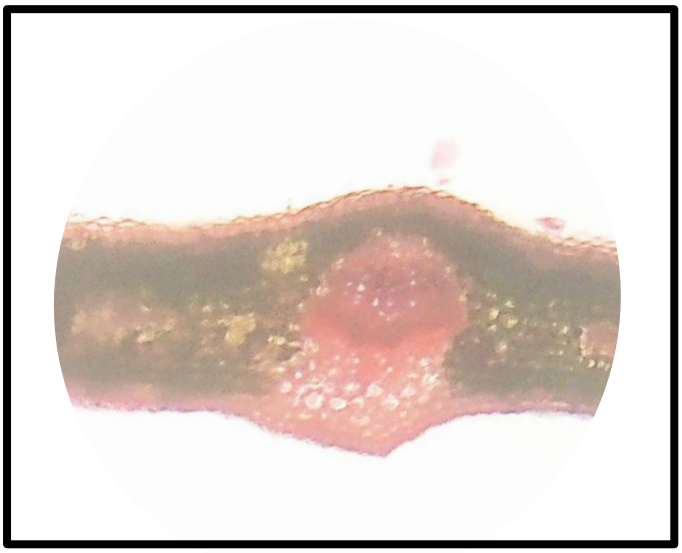

Figure 37 *Aegle marmelos* Linn correa.

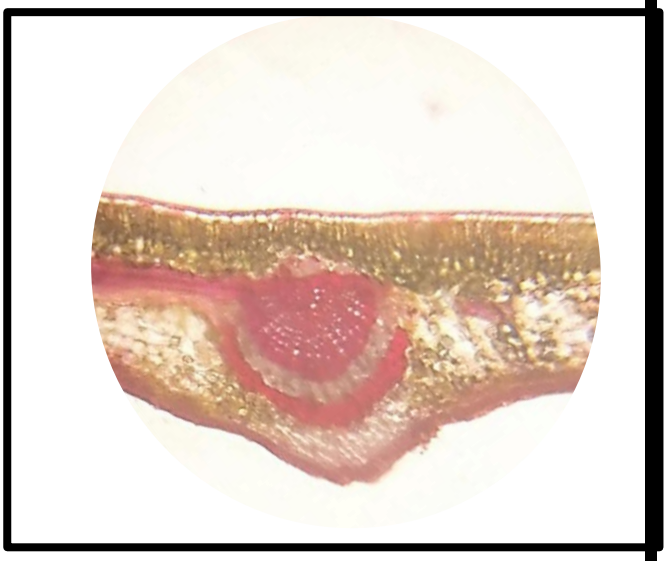

Figure 38- *Murraya exotica* L.

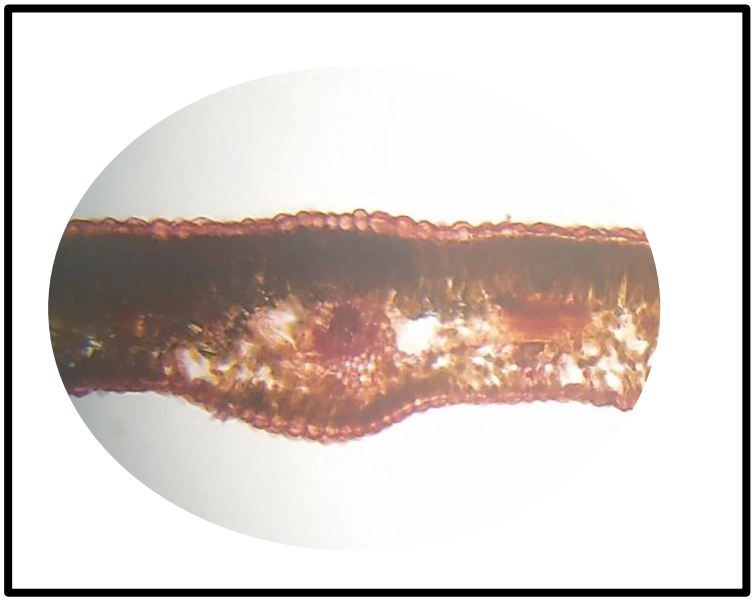

Figure 39 *Ruta graveolens* L.

HISTOCHEMICAL STAINING

DETECTION OF STARCH

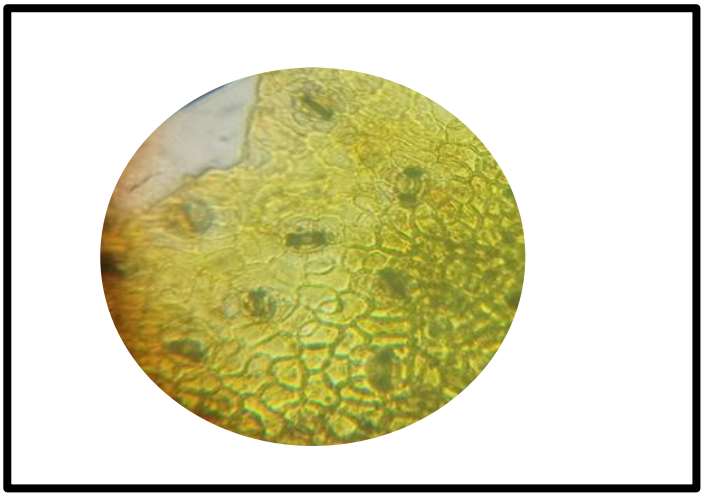

Figure 40 *Aegle marmelos* Linn correa.

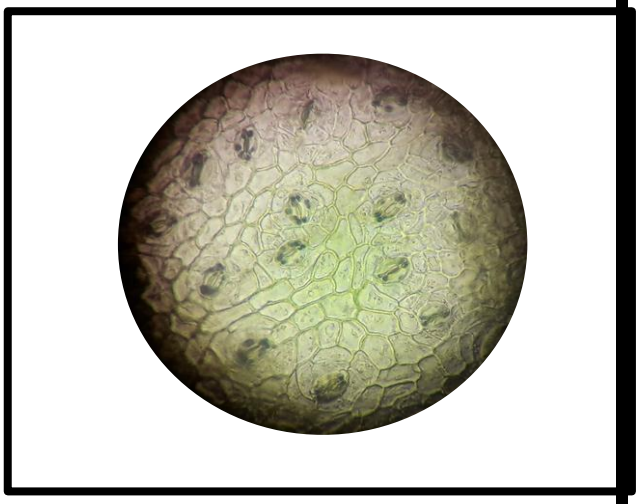

Figure 41- *Murraya exotica* L.

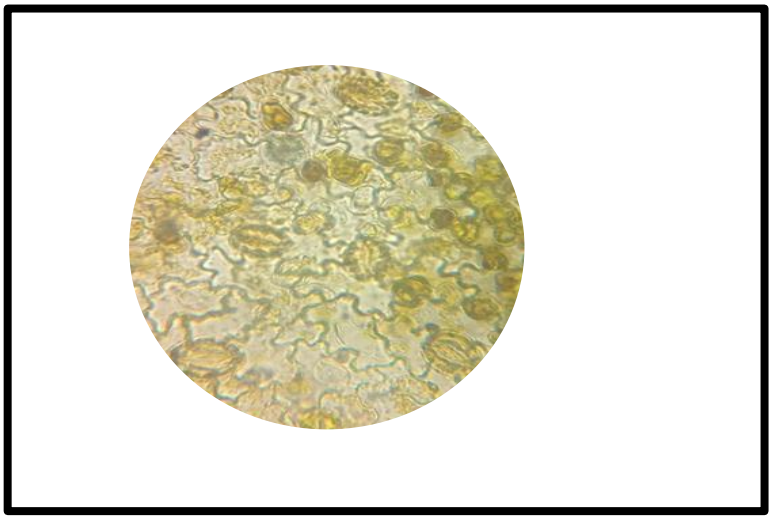

Figure 42 *Ruta graveolens* L.

DETECTION OF POLYPHENOL

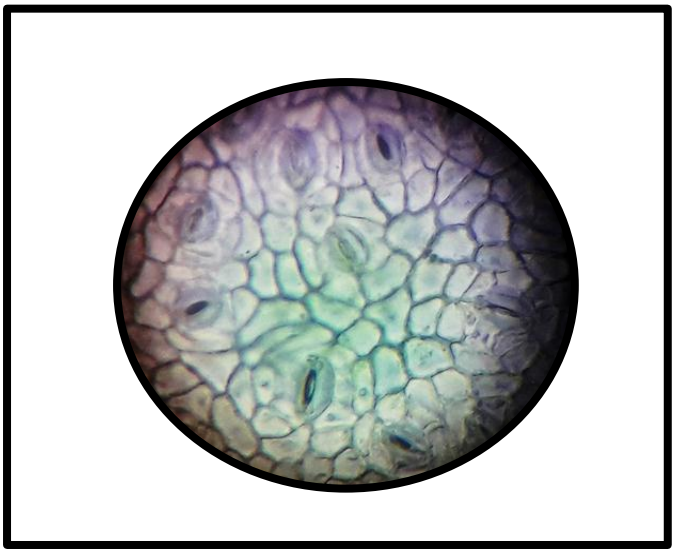

Figure 43 *Aegle marmelos* Linn correa.

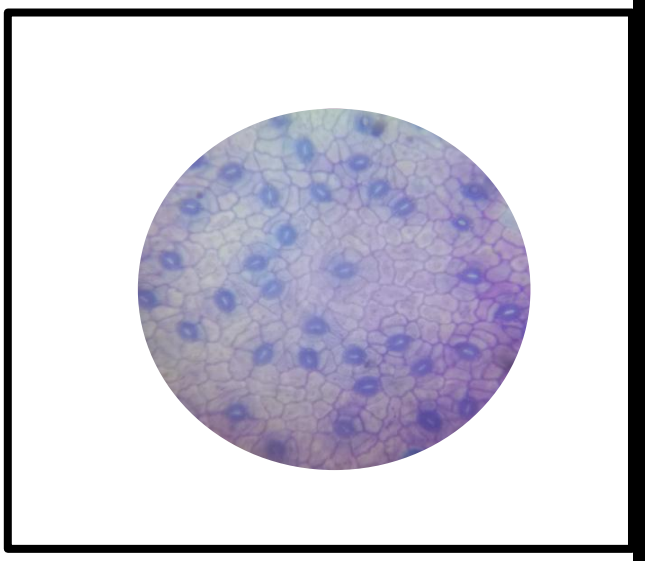

Figure 44- *Murraya exotica* L.

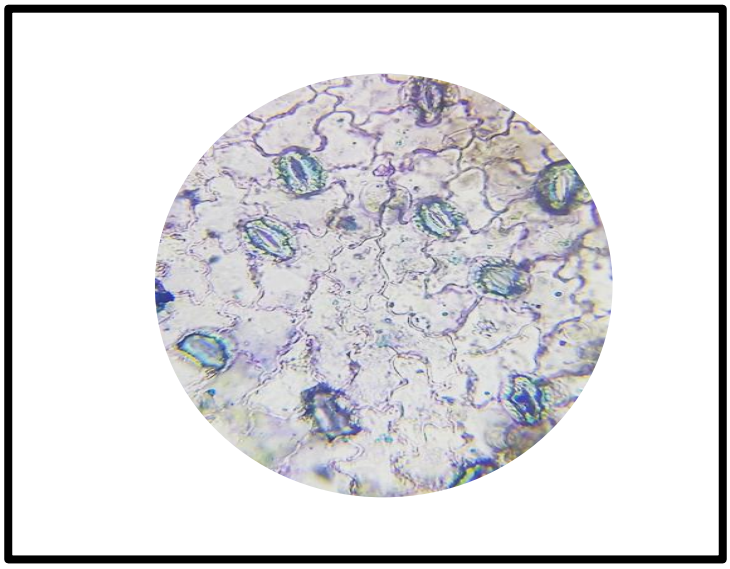

Figure 45 *Ruta graveolens* L.

DISCUSSION

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing demand for medicinal plants. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional herbal medicines, as the drugs derived from the plants are easily available, less expensive, safe, and efficient and rarely have side effects.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (Yadav et. al., 2011).

The present study is aimed to analyze the pharmacognostic, phytochemical, and histochemical features of the three medicinal plants selected from rutaceae family.

Rutaceous members are the relevant medicinal family and broadly used in Ayurvedic, Siddha, Naturopathy, Rasayana and medicinal system. Majority of these family members consist of a number of phytoconstituents which are the fundamental elements in the therapeutic value of these plants. Almost all parts of each plants starting from root to stem to leaves, are having the crucial role in curing disease (Monalisa Panda et. al., 2019). The present study shows that the three medicinal plants of rutaceae family - *Aegle marmelos* Linn Correa., *Murraya exotica* L., *Ruta graveolens* L., showed the presence of phytochemicals like alkaloids, steroids, phenols, protein, carbohydrate, resin etc., thus imparts anti-inflammatory, antioxidant, antimicrobial, cytotoxicity properties.

The methanolic extract of *Ruta graveolens* L., markedly reduces cell influx, edema, release of mediators and oxidative stress associated with arthritic condition, and therefore has the

potential to be used as an anti-arthritic agent. These effects may be due to the presence of the broad range of flavonoids present in the plant extract especially rutin and quercetin (Ratheesh et. al., 2009). Its extract and the major isolated flavonoids, quercetin and rutin, had potent activity on guinea pig liver XO. Rutin exhibits multiple pharmacological activities including antibacterial, antitumor, anti-inflammatory, antidiarrheal, antiulcer, antimutagenic, myocardial protecting, vasodilator, immunomodulators and hepatoprotective activities. It can be useful in the development of new drugs to treat various diseases and also helpful for preventing cardiovascular disease and cancer (Jinous Asgarpanah *et. al.*, 2012). The result of the present study showed that the plant extract of *R. graveolens* L. has the presence of flavonoids.

The alkaloids are secondary plant substances. The main four alkaloids of *Aegle marmelos* Linn Correa., leaves are N-2-[4-(3', 3'- dimethylallyloxy) phenyl] ethylcinnamide, N-2-hydroxy-2- (4-hydroxyphenyl) ethylcinnamide, Marceline and *Angel*ine. Shahidine, an alkaloid having oxazoline core has been isolated as a major constituent from the fresh leaves of *Aegle marmelos* Linn Correa., and it showed activity against a few Gram-positive bacteria (Asha Jhajhria et. al., 2016). In this study the phytochemical analysis of the leaf extract of *Aegle marmelos* Linn Correa., show the presence of alkaloid.

The ethyl acetate fraction of *Murraya exotica* L., leaves were found to have high total phenolic content which might be responsible for the maximum antioxidant activity (Davinder Kaur et. al., 2016). In the present study the *Murraya exotica* L., show the presence of phenol.

Investigation using GC-MS analysis of the methanolic extract of the leaves of *Aegle marmelos* Linn Correa., a novel neuro-steroid -3- hydroxy-pregnan-20-1 has been reported. It is a progesterone derivative. Pregnanolone has sedative, anxiolytic, anesthetic, and anticonvulsant effects. The compound is potentially used against anxiety disorder, seizure and depression disorder (Santi Ranjan Dey et. al., 2020). The present study shows that the leaf extract of *Aegle marmelos* Linn Correa., showed the presence of steroid.

The carbohydrates or saccharides are mostly sweet compounds are found abundantly in higher terrestrial plants, fungi, and seaweed and consist of compounds such as sugars, starch, and cellulose. Polysaccharides of higher plants possess immunostimulatory, anti-complementary, anti-inflammatory, hypoglycemic, and anti-viral activities (Saranraj et. al., 2016). In this study qualitative analysis of phytochemical constituents showed the presence of

carbohydrate in the plants *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L.

Extensive investigations of different parts of *Aegle marmelos* Linn Correa., revealed varied classes of compounds Coumarins (Marmelosin, marmesin,), alkaloids (Aeglin, aegelenine), Tannins (skimmianine), Carotenoids , phenols, steroids, resins,. So, it has been used in ethnomedicine to exploit its medicinal properties including antidiabetic, antiulcer, antioxidant, antimalarial, anti-inflammatory, anticancer, radioprotective, antifungal, antibacterial and antiviral activities (Sandeep Dhankhar et. al., 2010). In this study the phytochemical analysis of the leaf of *Aegle marmelos* Linn Correa., show the presence of alkaloids, saponin, phenol, flavonoids, bitter, resin, protein, steroid, tannin and carbohydrate.

Saponins are plant glycosides, which have the property of forming a soapy lather when shaken with water. Saponins exhibit divergent antimicrobial, anti-inflammatory, antibiotic, hemolytic analgesic, hypoglycemic, anthelmintic and cytotoxic activities (Saranraj et. al., 2016). In the present study the *Aegle marmelos* Linn Correa., and *Murraya exotica* L., showed the presence of saponins.

Chlorophyll is the green pigment present in plant plays vital role in photosynthesis which absorbs light from sun and uses its energy to synthesize carbohydrates from CO2 and water. Leaves of *Aegle marmelos* Linn Correa., generally accumulated highest amount of chlorophyll a (2.60 mg/g fresh wt.), chlorophyll b (1.73 mg/g fresh wt.) and carotenoid (1.51 mg/g fresh wt.) in summer season compare to monsoon and winter (Khairnar, 2017). In the present study the total chlorophyll content of the *Aegle marmelos* Linn Correa., was found to be 3.62mg/g.

The *Murraya exotica* Linn., was traditionally used as medicinal plant. The study revealed the presence of reducing sugars, tannins, saponins and alkaloids and thus was evaluated for possible cytotoxic, thrombolytic and antioxidant activities (Amina Khatun et. al., 2014). In the present study the plant *Murraya exotica* Linn., show the presence of alkaloids, saponin, phenols, tannin, steroid, resin, bitter, protein and carbohydrate.

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acids and minerals, etc. The tannin-containing plant extracts has anti-inflammatory, antiseptic, antioxidant properties (Mamta Saxena et. al., 2013). In the present

study the *Aegle marmelos* Linn Correa., and *Murraya exotica* L., showed the presence of tannins.

Resins are complex amorphous secondary products, produced in special schizogenous ducts or cavities or in special cells or glands and are often stored or sometimes secreted out. Their functions are helping against dehydration, mechanical damage and bacterial and insect attack (Sabins and Daniel, 1990). The present study shows that the three plants showed the presence of resin.

Phytochemical analysis of the *Ruta graveolens* L., revealed the presence of various secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins and carotenoid. GC-MS analysis of methanolic leaf extract revealed the presence of approximately 26 phytochemical constituents (Azalework et. al., 2017). In the present study the presence of alkaloids, phenol, flavonoid, steroid, bitter, protein, resin and carbohydrate was observed in the leaf extract of *Ruta graveolens* L.

Foliar epidermal evaluation solves the problem by differentiating the genuine material from the adulterants, substitutes and spurious drugs. Therefore, the characterization of plants with such study is an ideal approach for the identification of medicinal plant species and populations of the same species. The study of the epidermal leaf features like type of stomata, stomatal index etc. of medicinal plant species are highly significant and taxonomically important tools in plant (shilpi singh et al 2021). In this present study the type of stomata of *Aegle marmelos* Linn Correa., was found to be paracytic with stomatal index 16.36. For *Murraya exotica L.*, type of stomata was identified as anisocytic with stomatal index 26.08 and for *Ruta graveolens L.*, type of stomata was found to be anomocytic with stomatal index 27.90.

Morphological measurement of stem, leaf and root give an overall appearance of the plant. They are used to identify the plant externally for the characterization and identification of the plant (Maheswari, 1830). *Aegle marmelos* Linn Correa., is a small deciduous thorny tree. Leaves are ovate in shape, reticulate venation; alternate and 3- foliate arrangement. Flowers are greenish white in color and fruits are yellowish green, with small dots. *Murraya exotica L.*, are an evergreen shrub. Leaves alternate, spiral, imparipinnate, exstipulate, leaflets usually 3-5. The flowers are white or cream-coloured with terminal or axillary panicle. The fruit is an oval, glabrous, orange-red berry. *Ruta graveolens L.*, is a perennial, scented and glabrous herb or a sub-shrub. Leaves are dissected pinnately into oblong or spoon shaped segments,

alternate, reticulate arrangement. Flowers are yellow in colour and Fruits are dry, hard, and roundish.

The taste and smell of the environment are of particular relevance for the selection of medicinal versus non medicinal plant species (Marco Leonti et. al., (2002). In the present study the organoleptic characters for the *Aegle marmelos* Linn Correa., was found to be dark green in colour with characteristic odour, glabrous texture and bitter taste. For *Murraya exotica L.,* it shows brownish green in colour with citrusy aroma, glossy texture and bitter taste. For *Ruta graveolens L.,* it was found to be yellowish brown in colour with unpleasant odour, soft and glaucous texture with bitter taste.

The anatomical study, quantitative microscopy methods like epidermal number, stomatal number, stomatal index, vein islet number, vein islet ratio, palisade ratio of the aerial parts of the plant can be a solution to mitigate this drawback for the proper identification and authentication of the plants since both are used in traditional systems of medicines, the adulteration or substitution of drugs can prevent to some extent (Rubeena et. al., 2018). The palisade ratio of the *Aegle marmelos* Linn Correa., was found to be 5.6 with vein islet and vein termination number 6 and 8 and for *Murraya exotica L.,* it showed palisade ratio of 6.98 with vein islet and vein termination number 8and 12. The palisade ratio, vein islet and vein termination number of *Ruta graveolens L.,* was found to be 3.48, 7 and 10.

The study of morphology-anatomical signs of plants of the different age conditions is important at determination of effectiveness of the variety introduction in new ecological conditions and favours the spread of assortment of resistant plants for the use in greenery planting. Anatomical analysis provides the structural details of plant as well as the understanding about functional, biochemical and developmental aspects. (Boyko, 2017) For the present study leaf of the plants was used. Anatomical studies revealed the microscopic characters of the leaves such as nature of the mesophylls cells, vascular bundles etc.

Histochemical analysis provides a best tool for the botanical identification and standardization of crude drugs (Momin 2011). The living cells of all higher plants possess different types of plastids that are mainly concerned with synthesis of starch (Winkler, 1898). Iodine potassium iodide reaction was employed for detection of starch. The present study shows that the three plants showed the presence of starch.

The presence of polyphenols was identified using toludine blue O stain method. The blue colour indicated the presence of polyphenols. The distribution of polyphenol deposits is influenced by different environmental factor (Tempel, 1981). The present study shows that the three plants showed the presence of polyphenol.

This is a small study which emphasis on phytochemical, pharmacognostic and histochemical analysis of selected medicinal plants of rutaceae family. Through this study by determining various phytochemicals constituents, it may be concluded that it can be used as valuable tool for authentication and identification of drug. The present study, act as a primary platform for further phytochemical and pharmacognostic studies. Further studies can be carried out for the analysis and improvement of plant products for their improved economic and therapeutic implementation.

SUMMARY AND CONCLUSION

Plants are the integral part of the nature. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing in demand for medicinal plants. Medicinal plants is any plant which, in one or more of its organs, contain some organic compounds which provide definite physiological action on human body. About 80% of individuals from developed countries use traditional medicines, as drugs derived from the plant are easily available, less expensive, safe, and efficient and rarely have side effects.

The plants selected for the present study are *Aegle marmelos* Linn Correa., *Murraya exotica* L., and *Ruta graveolens* L., *Aegle marmelos* Linn Correa., is noted for its hypoglycemic and antioxidant property, *Murraya exotica* L., was found to exhibit the cytotoxic properties and *Ruta graveolens* L., is noted for its anti-oxidant and anti-inflammatory activities. Phytochemical, pharmacognostic, anatomical and histochemical studies were conducted on these plants.

Phytochemical studies of the plants showed the presence of alkaloid, saponin, flavonoid, tannin, phenol, steroid, bitter, resin, phenol, protein and carbohydrate. Quantitative determination of secondary metabolites (phenol, flavanoid, tannin, carbohydrate, chlorophyll) has been carried out and the conclusion denoted that their qualitative analysis revealed their appearance where as their quantitative analysis give almost approximate idea for their quantity present.

In the pharmacognostic studies, morphology, organoleptic evaluation, and anatomical features were studied. The microscopic characters were studied by determining the type of stomata, stomatal index, palisade ratio, vein islet as well as vein termination number. The three plants possess different types of stomata. The type of stomata of *Aegle marmelos* Linn Correa., was found to be paracytic with stomatal index 16.36. For *Murraya exotica* L., type of stomata was identified as anisocytic with stomatal index 26.08 and for *Ruta graveolens* L., type of stomata was found to be anomocytic with stomatal index 27.90. Anatomical features of leaf were also studied. Anatomical studies revealed the microscopic characters of the leaves such as nature of the mesophylls cells, vascular bundles etc. The results obtained from pharmacognostic studies helps in achieving a trouble-free identification and authenticity of the plant leaf or in powder form in future.

To detect primary metabolites like starch, polyphenols and lipids histochemical analysis was done with specific dyes in epidermal peel of leaf tissue. Through histochemical tests it was possible to demonstrate the sites of production or accumulation of some metabolites of pharmacological use in the leaves the plant.

The results revealed the presence of medicinally important constituents in the plants studied. Several studies confirmed the presence of these phytochemicals contributing to the medicinal as well as physiological properties of these plants.

Therefore, extracts from these plants could be considered as good source for useful drugs. The traditional medicinal practitioners strongly recommend these drugs for the treatment of ailments. Further work may be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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