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STUDY OF ANTIOXIDANT AND TOTAL PHENOLIC CONTENT FLAVONOID, ANTIMICROBIAL CONTENT OF WHOLE PLANT EXTRACT OF GRANGEA MADERASPATANA

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ABSTRACT

The current research on Grangea maderaspatana (L.) Poir, commonly known as madras carpet, mainly focuses on exploring its antioxidant and antimicrobial activities using different parts of the plant. The study employs characterization techniques such as XRD, TEM, SAED, and UV Spectroscopy to gain insights into the plant's properties. Grangea maderaspatana has been found to exhibit a broad spectrum of activity on various elements, and its different parts have been studied for potential medicinal uses, including antimicrobial, antifertility, analgesic, anti-inflammatory, antiarthritic, flavonoid, and hepatoprotective properties. The plant contains flavonoids, diterepenes, sesquiterpenoids, steroids, and essential oil. While the pharmacological studies confirm its therapeutic value, there is limited information on preclinical, clinical, toxicity, and phyto-analytical studies. Further research, such as clinical evaluation, phyto-analytical studies, and toxicity evaluation, is needed to validate its claims and potential use in treating various ailments. With rigorous scientific and clinical evaluation, Grangea maderaspatana has the potential to offer effective remedies and benefit humanity.

KEYWORDS

1. Grangea maderaspatana
2. Madras carpet
3. Antioxidant activities
4. Antimicrobial activities
5. XRD (X-ray diffraction)
6. TEM (Transmission Electron Microscopy)
7. SAED (Selected Area Electron Diffraction)
8. UV Spectroscopy
9. Medicinal uses
10. Antimicrobial properties

1.1 INTRODUCTION

Medicinal plants throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances, and medicines (Cragg and Newman, 2005). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use.

The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2 600 BC (Heinrich et al., 2004). Among the substances that were used are oils of Cedrus species (cedar) and Cupressus sempervirens (cypress), Glycyrrhiza glabra (licorice), Commiphora species (myrrh) and Papaver somniferum (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. In ancient Egypt, bishop's weed (Ammi majus) was reported to be used to

treat vitiligo, a skin condition characterized by a loss of pigmentation. More recently, a drug (β-methoxypsoralen) has been produced from this plant to treat psoriasis and other skin disorders, as well as T-cell lymphoma.

The interest in nature as a source of potential chemotherapeutic agents continues. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today. Higher plants contribute no less than 25% of the total. In the last 40 years, many potent drugs have been derived from flowering plants; including for example Dioscorea species (diosgenin), from which all anovulatory contraceptive agents have been derived; reserpine and other antihypertensive and tranquilizing alkaloids from Rauwolfia species; pilocarpine to treat glaucoma and 'dry mouth', derived from a group of South American trees 4 (Pilocarpus spp.) in the Citrus family; two powerful anti- cancer agents from the Rosy Periwinkle (Catharanthus roseus); laxative agents from Cassia sp. and a cardiotonic agent to treat heart failure from Digitalis species. Approximately half (125 000) of the world's flowering plant species are found in the tropical forests.

Tropical rain forests continue to support a vast reservoir of potential drug species. They continue to provide natural product chemists with invaluable compounds as starting points for the development of new drugs. The potential for finding more compounds is enormous as to date only about 1% of tropical species have been studied for their pharmaceutical potential (Cragg and Newman, 2005). This proportion is even lower for species confined to the tropical rain forests. To date about 50 drugs have come from tropical plants. The probable undiscovered pharmaceuticals for modern medicine has often been cited as one of the most important reasons for protecting tropical forests.

History of medicinal plants

The earliest records of the use of medicinal plants are that of Chaulmoogra oil from Hydnocarpusgaertn, which was identified to be effective in the treatment of leprosy. Such a use of medicinal plants for the treatment of leprosy was written in the pharmacopoeia of the Emperor of China between 2730 and 3000 B.C. In the same way, the castor seeds and seeds of opium were found from ancient Egyptian tombs, which confirm their use in that part of Africa as for back as 1500 B.C. The written records existing in "Ebers papyrus" also show the use of medicinal plants at that time in Egypt.[2] According to the history of medicinal plants, the Materia Medica of Hippocrates, who is currently known as the father of medicine, composed of herbal formulations, nearly 400 simple formulations having been compiled and explain by him. Plin was a Theophrastus of Ethan's (370- 287B.C) a well-known botanist who wrote a number of manuscripts including the famous Historia planetarium. Just about 500 plants, mostly cultivated, were marked out in this manuscript.[3] However, the significant pharmacological collection of the Greeks was the authoritative text of Dioscoridies. Later than him Pliny the elder (23-79 AD) wrote "Natural History" in 37 volumes. Galen compiled some 30 books on pharmacology beside "Galanicals" his medical formulae.[4] Chinese medicine, with its use of pharmaceutical preparation Known as fangs, also utilized a variety of plants. The written document of Chinese traditional medicine can be marked out toShen Nong Ben Cao Jin (22-250 AD). Later Li Shizhens, a great physician and naturalist, wrote "Ben Cao Gang Mu" published in 1596 that has been regarded as complete pharmacopoeia having a total of 1894 entries. Rig Vedas and Yajurveda's are the main source of Indian medicines. They are mainly based on the use of drugs of plant origin. The Ayurvedic system of medicine is mainly credited to Charaka and Sushruta, who described about 700 medicinal plants. The Muslim rulers introduced their traditional system of medicine in India and included in the native Ayurvedic medicine.

In the last five decades the development and introduction of immuno- stimulants, antibiotics and antitumor agents isolated from plants have led a dramatic success in control of many diseases. During the last decade the use of traditional medicines has expended globally and has gained attractiveness. These are used not only for primary health care of poor people in the developing countries, but are also used in the countries where conventional medicine is predominant in the National Health Care System.[7] According to WHO herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries.

More than 50% of all the medicines in clinical use have a natural product origin. Of the world's 25 bestselling pharmaceutical agent, 12 are natural products derived. More than 600 botanical items have been recognized in various edition of the United State Pharmacopoeia. The most important factors for the continued use of the traditional medicines are its ready accessibility, cheapness and socio-cultural reasons. A long tradition of the use of herbal remedies exists in some countries and the people especially of the rural areas have more faith in the traditional medicines. The fact that most of the medicinal plants have been used over the ages for treatment of diseases is believable evidence that many of the medicinal plants prescription are realistically safe but scientific toxicological trials are still necessary.

1.2 Drug discovery from medicinal plants

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plant(s) of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (i.e. traditionally used herbal remedies) or may involve taxa collected randomly for a large screening

program. It is necessary to respect the intellectualproperty rights of a given country where plant(s) of interest are collected. Phyto chemists (natural product chemists) prepare extracts from the plant material, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets.

1.3 Anti Oxidants

Antioxidants are a class of chemical substances naturally found in our food which can prevent or reduce the oxidative stress of the physiological system. The body is constantly producing free radicals due to regular use of oxygen. These free radicals are responsible for the cell damage in the body and contribute to various kinds of health problems, such as heart disease, diabetes, macular degeneration, and cancer. Antioxidants being fantastic free radical scavengers help in preventing and repairing the cell damage caused by these radicals. Plants and animals are the abundant source of naturally producing antioxidants. Alternately, antioxidants can also be synthesized by chemical process as well as from the different kinds of agro-related wastes using biological process. Based on their solubility, antioxidants are broadly categorized into two groups: water soluble and lipid soluble. In general, water-soluble antioxidants, such as ascorbic acid, glutathione, and uric acid, have functions in the cell cytosol and the blood plasma Ascorbic acid is a redox catalyst which reduces and neutralizes there active oxygen species (ROS), glutathione has antioxidant properties as reducing agent and can be reversibly oxidized and reduced, while α-tocopherol, carotenoid, and ubiquinol are the examples of lipid-soluble antioxidants and protect the cell membranes from lipid peroxidation. Another commonly used classification is on the basis of their mechanism of action, i.e., primary or chain-breaking antioxidants and secondary or preventive antioxidants. Antioxidants can also act as prooxidants when these are not present at the right place at the right concentration at the right time. The relative importance of the antioxidant and prooxidant activities of an antioxidants an area of current research. This chapter discusses the types, sources, synthesis, uses, and protective efficacy of various antioxidants.

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves. Though oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases (Hamid et al. 2010). Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging. Besides, there are some antioxidantsin the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet.

Antioxidants can also act as prooxidants when these are not present at theright place at the right concentration at the right time. The relative importance of the antioxidant and prooxidant activities is not yet explored fully and needs further research.

Classification of Antioxidants

Antioxidants can be classified into two major types based on their source, i.e., natural and synthetic antioxidants (schematic representation of the classification of anti-oxidants is shown in Fig below.

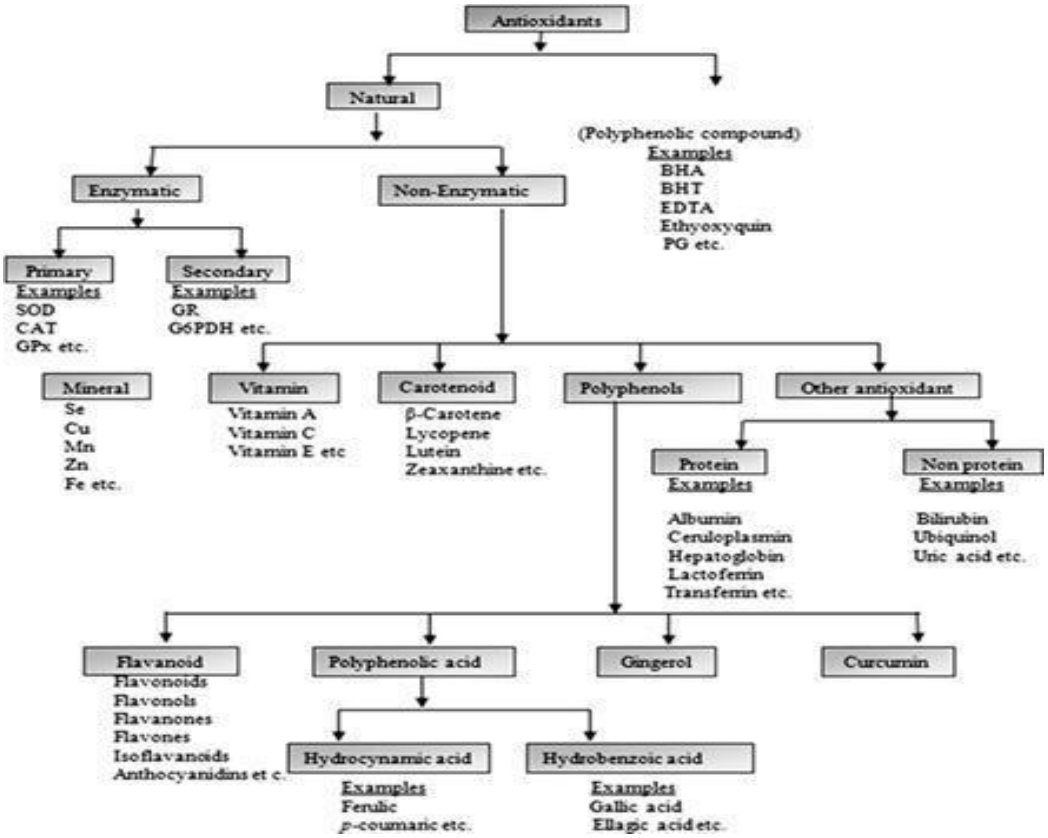


Fig. 1: Classification of antioxidantBenefits of Antioxidants.

- **Protect Against Heart Disease**
- **Protect Against Cancer**
- **Boost Immunity**

Fight Aging

Role of Antioxidants in plants

In order to protect the cells and organs of the plants against free radicals (ROS), a highly combined and complicated system has been evolved in human body, which involve a diversity of components, originated both endogenously and exogenously that function interactively and synergistically to reduce the effect of free radicals.

These are

1. Antioxidants from nutrients such as carotenoids, ascorbic acid (vitamin C), tocopherols and tocotrienols (Vitamin E), and other low molecular weight compounds including glutathione and lipoid acid.
2. Enzymatic antioxidants, which catalyze free radical quenching reactions including glutathione peroxidase, superoxide dismutase, and glutathione reductase.
3. Proteins that bind metals that seizes free iron and copper ions that are capable of catalyzing oxidative reactions, such as lactoferrin, ferritin, albumin, and ceruloplasmin.
4. A number of other antioxidant phytonutrients present in a wide variety of plant foods.

Antioxidants in plants

The use of synthetic and natural food antioxidants regularly in medicine and foods particularly those having fats and oils to shield the food fromoxidation. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are the synthetic and natural food antioxidants which have been used extensively in cosmetic, food and therapeutic industries. But, owing to their instability at high temperatures, high volatility, synthetic antioxidant's carcinogenic behaviour, users inclinations led to shift in the consideration of producers or manufacturers from man-made to natural antioxidants (Papas, 1999).

In consideration of growing risk issues of humans to various lethaldiseases, there has been a universal trend in the direction of the use of natural substances present in dietary and medicinal plants as curative antioxidants. A variety of medicinal plants have been reported to reveal antioxidant activity, including Allium sativum, Zingiber officinale, Crocus sativus, Dodonaea viscose, Barleria noctiflora, Anacardium occidentale, Datura fastuosa, Caesalpinia bonducella and many more as in table below. Numerous antioxidants identified as active oxygen scavengers or free radicals, obtained naturally from the plant sources are used in food, cosmetic and remedial purposes proved to be brilliant alternatives for man-made antioxidants because of their inexpensiveness, and have no any harmful effect on human body. In order to defy the detrimental effects of reactive oxygen species, plants have a powerfully built enzymatic and non-enzymatic scavenging pathway.

Enzymes included are catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione S- transferase (GST), de-hydro ascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), peroxidases (POX) and glutathione peroxidase (GPX). Non enzymatic compounds include glutathione (GSH), carotenoids tocopherols and ascorbate (ASA). There are unambiguous, well synchronized ROS generating and scavenging systems present in different organelles of the plant cells. Lesser levels of ROS comparatively act as signalling essences that arouses abiotic stress tolerance by altering the expression of resistant genes. In plants, elevated levels of antioxidants have been accounted to demonstrate better resistance to different types of environmental stresses.

1.4 Flavonoids

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure,widely found in fruits, vegetables and certain beverages. They have miscellaneous favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc.(,1–3). Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase.[4-6]

Flavonoids play a variety of biological activities in plants, animals and bacteria. In plants, flavonoids have long been known to be synthesised in particular sites and are responsible for the colour and aroma of flowers, and in fruits to attract pollinators and consequently fruit dispersion to help in seed andspore germination, and the growth and development of seedlings. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV filters function as signal molecules, allopathic compounds, phytoalexins, detoxifying agents and antimicrobial defensive compounds. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimatisation and freezing tolerance.

Classification of flavonoids

Flavonoids can be subdivided into different subgroups depending on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring. Flavonoids in which the B ring is linked in position 3 of the C ring are called isoflavones. Those in which the B ring is linked in position 4 are called neoflavonoids while those in which the B ring is linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring.

These subgroups are:Flavones Flavonols Flavanones Flavanonols Flavanols or catechinsAnthocyanins Chalcones.

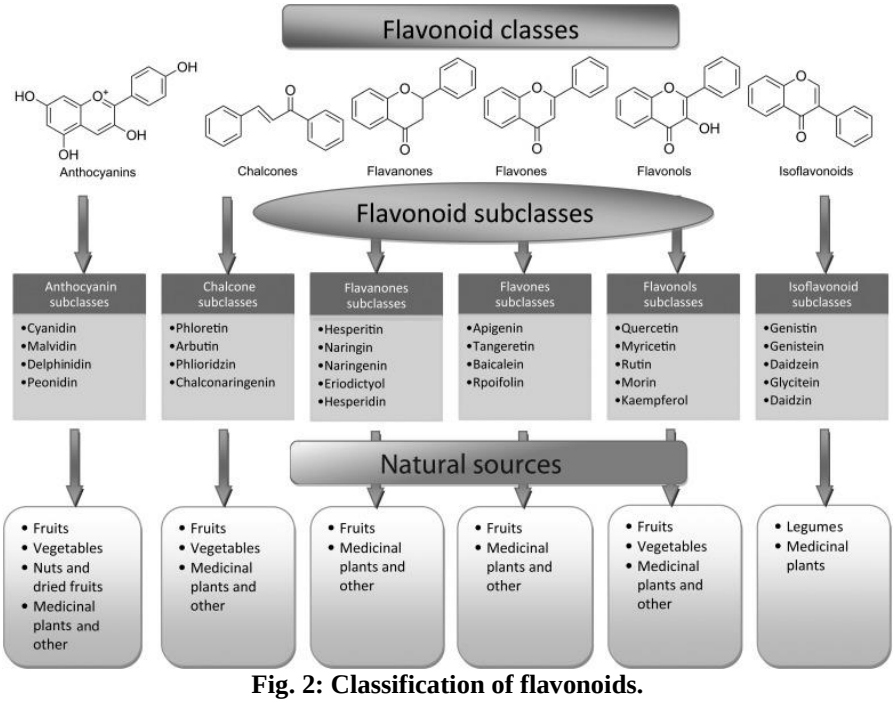


Fig. 2: Classification of flavonoids.

Functions and applications of flavonoids in plants

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These

substances, traditionally referred to as secondary metabolites (flavonoids), often are differentially distributed among limited taxonomic groups within the plant kingdom. The flavonoids are categorised in different classes as alkaloids, terpenoids and phenolics. Flavonoids carry out a number of protective functions in the human body. Many flavonoids have evolved as bioactive compounds that interfere with nucleic acid or proteins and show antimicrobial or insecticidal and pharmacological properties. Flavonoids are therefore of interest in medicine as therapeutics and at the same instance in agriculture as pesticides. In vitro technology has given new insight to explore the potency of plant cell tissue culture to produce the same valuable chemical compounds as those of the parent plant. The advancement in plant tissue culture methods for flavonoid production has bloomed beyond expectations. Plant tissue culture is an aseptic technique whereby proper manipulation of the nutrients, culture conditions, and phyto-hormone supply, one may be able to produce the desired quality and quantity of plants as well as metabolites. With the culture of differentiated cells it is possible to obtain production of the desired compounds in levels comparable with that of the plant. Flavonoids are associated with a broad spectrum of health-promoting effects. They are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. Flavonoids act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including anti-allergenic, antiviral, anti-inflammatory and vasodilating actions.

1.5 Phenolics

Phenolics are a type of secondary metabolite that can be found almost all over in plants. They are an aromatic molecule with a benzene ring (C6) and one or more hydroxyl groups that belong to a broad and diversified group. In general, phenolics are classified according to the number of carbon atoms in themolecule.

Three different biosynthetic pathways produce phenolics:

1. The shikimate/chorizmate or succinylbenzoate pathway, which produces phenylpropanoid derivatives (C6–C3)
2. The acetate/malonate or polyketide pathway, which produces side-chain- elongated phenylpropanoids, including the large group of flavonoids (C6–C3–C6) and some quinones
3. The acetate/mevalonate pathway, which produces the aromatic terpenoids, mostly monoterpenes, by dehydrogenation reactions.

Phenolic in plants

Phenolics perform a dual function in the plant's environment, repelling and attracting various organisms. They act as inhibitors, natural animal toxicants, and pesticides against invading organisms, such as herbivores, phytophagous insects, nematodes, fungal and bacterial pathogens. On the plantsurface, simple phenolic acids, complex tannins, and phenolic resins deter birdsby interfering with the gut microflora and impairing their digestive ability. Low-molecular-weight phenylpropanol derivatives attract symbiotic microbes, pollinators, and animals that disperse fruit.

Thus, phenolic compounds have been proposed as useful alternatives to chemical control of agricultural crop pathogens for some time. The majority of polyphenols have been shown to have a negative effect on microbes. Plants accumulate phytoalexins in response to pathogen attacks, such as hydroxycoumarins and hydroxycinnamate conjugates. The synthesis, release, and accumulation of phenolic compounds—in particular, salicylic acid are critical for a variety of plant defence strategies against microbial invaders. Phenolics are synthesised when plant pattern recognition receptors recognise potential pathogens via conserved pathogen-associated molecular patterns (PAMPs), resulting in PAMP-triggered immunity. As a result, the pathogen's progress is slowed significantly before it takes complete control of the plant.

Classification of phenolic compounds

Polyphenols are classified according to the number of phenol rings they contain and the structural elements that connect these rings.

The major classes of polyphenols arePhenolic acids
FlavonoidStilbenes
Lignans

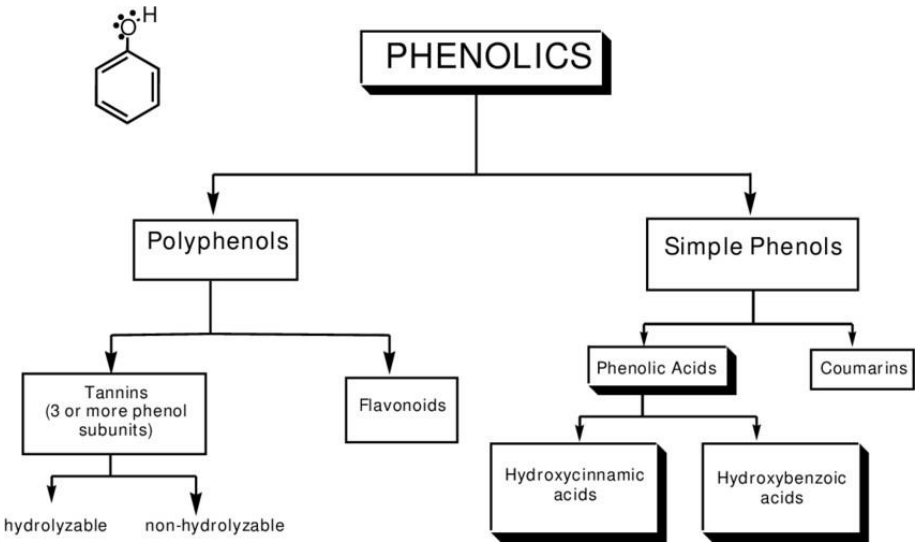


Fig. 3: Classification of phenolics.

Applications of phenolic acids in plants

Phenolic acids as signalling molecules. Phenolic acids in food.

Phenolic acids as antioxidants. Phenolic acids as antidiabetic agent. Phenolic acids as antimicrobial agent.

Phenolic acids in cancer cure and treatment. Phenolic acids as antimicrobial agent

1.6 Antimicrobial Activity

Antibiotic resistance has become one of the major problems of humanity since late 20th century. The need for new antimicrobials, which could effectively fight against resistant microbes, has tremendously increased. Traditional approaches to find new antimicrobial drugs are not sufficiently successful anymore due to the rapid resistance development against them. Consequently, it is very important to find new approaches of antimicrobial compounds discovery. Plant materials are demonstrated to be one of the most promising sources. Plant-derived antimicrobials are also considered to be safer compared with synthetic compounds because of their natural origin. It is well known that about quarter part of current medications is derived from compounds of plant origin. Plant-derived compounds could have other target sites than traditional antimicrobials and subsequently having different mechanisms of action against microbes. Plant secondary metabolites are mostly responsible for their antimicrobial activity. Major groups of phytochemicals which possess antimicrobial properties are phenolics and polyphenols (flavonoids, quinones, tannins, coumarins), terpenoids, alkaloids, lectins and polypeptides. There are several mechanisms that underlie antimicrobial action of plant-derived compounds. Phytochemicals can act by disrupting microbial membranes (carvacrol, thymol, eugenol, etc.) or impairing cellular metabolism (cinnamaldehyde). They can also control biofilm formation (trans- cinnamaldehyde, carvacrol, thymol, geraniol, etc.). Plant antimicrobials can inhibit bacterial capsule production (salicylic acid and its derivatives). Some plant compounds can attenuate bacterial virulence by controlling quorum- sensing. Another mechanism of plant metabolites' antimicrobial action is the reduction of microbial toxin production (dihydro isosteviol, RG-tannin, etc.). Plant metabolites can also act as resistance-modifying agents (RMAs). Nowadays RMAs are considered as one of the most prospective ways to combat bacterial resistance. Some studies already showed that plant-derived compounds can enhance therapeutic effect of antibiotics acting as RMAs (nerolidol, bisabolol, apritone, etc.).

Plant species are estimated to be around 250–500 thousands. However, only a small part of them were investigated for antimicrobial activity. People started to use plant materials to treat infectious diseases since ancient times even without any knowledge on their causative agents. Nowadays, herbs continue to be used in traditional medicine to heal various infectious conditions in many countries, including Armenia. Moreover, in the last decades this tendency has increased.

2.1 Biological Review

Grangea is a genus of suberect or prostate annual herbs: Fourteen species of Granges are fund in tropical and sub-tropical Asia and Africa Granges maderaspataria (synonym Artemisia maderaspatana) is a common weed usually occurring in sandy lands and waste placest Vernacular name of this plant is Mukhari and Machi pathree. The leaves of G maderaspatana are used as sedative, analgesic, carminative, stomachic and in cases of obstructed menses 2 Steroidal constituents3, hardwickiic acid, the corresponding 1.2-dehydro-derivative, acetylenic compounds 4, eight new clerodane diterpenes including nor-clerodane, a seco-clerodane and nor-secoclerodane derivatives along with auranamides, grangolide and cudesmano-lides6 have been reported from various extracts of this species, Preliminary chemical screening of essential oil of G maderaspatana has been done earlier7,8 Objective of the present study was to investigate chemical composition, in vitro antioxidant and antimicrobial activity of the essential oil of G. madrascarpet Herbal medicines play an important

role in preventing and treating diseases.

In recent years there has been a resurgence of interest rediscovermedicinal plants as a source of potential drug candidate. The asteraceae family is one of the largest plant families more than one thousand genres and twentythousand species. Many of the plants of this are used in traditional medicine. Since ancient times, plants have been the used in traditional medicine systemssuch as Ayurvedic. Chinese and African traditional medicines. interest of modern medicine for medicinal plants significantly increased due to the recently enormous therapeutic potential of these plants. Thus, many plants of the Asteraceae are used for symptomatic relief of several neuropsychiatric disorders. More than that, and cellular modelling experiments were performed to present scientific proof for their correlations between traditional use and scientific research on Asteraceae family plants for treatments ofneuropsychiatric disorders such as AD. PD, schizophrenia, autism, depression. In this way, this review aims to assemble traditional and scientific knowledge of Asteraceae in neuropsychiatric disorders therapy. These plants are widely used in alternative medicine due to their effects on the nervous system,being studied both on cellular and animal models. In this way, medicinal plants are preferred in drug therapy research due to the wide population interest. The plants phytochemical composition is mainly consisted in cholinergic dopaminergic or serotonergic molecules which are involved in the pathophysiology of the neuropsychiatric disorders like schizophrenia, autism, anxiety or depression. Therefore, this review aims to comprise all the available information regarding this family plants in order to find further correlations between or to explain their neuropsychiatric use Thus, many plants of this family are used for symptomatic relief of several neuropsychiatric disorders More than that, animal and cellular modelling experiments were performed to present scientific proof for their properties Correlations between traditional use and scientific research on family plants for treatments of neuropsychiatric disorders such as AD, PD, schizophrenia, autism, depression and anxiety are necessary for neuropsychiatric potential molecules discovery. Unlike the potentially addictive and forceful action of widely used psychostimulants, chronic and moderate administration of g.maderaspatana, appears to nourish rather than deplete neurons.

In this way, this review aims to assemble traditional and Asteraceae family plants.

Fig. 4: Grangea maderaspatana.

G.maderaspatana -Plant profile Kingdom: Taxonomic Classification

Domain	: Eukaryota
Kingdom	: Plantae
Sub- Kingdom	: Viridaeplantae
Phylum	: Tracheophyta
Sub- Kingdom	: Euphyllophytina
Class	: Magnoliopsida
Sub- Class	: Asteridae
Superorder	: Asteranae
Order	: Asterales
Family	: Asteraceae
Genus	: Grangea
Species	: Maderaspatana

Fig. 5: Flowers of grangea maderaspatana.

Fig. 6: Fruit of Grangea maderaspatana.

Fig. 7: Roots of grangea maderaspatana.

Leaves and flowers of the plant

Vernacular name

Gujarati	: Jhinkimundi, Nahanigora,
KhamundiHindi	: Mukhatari, Mustaru
Malayalam	: Nelampala;
Marathi	: Mashipatri
Tamil	: Mashipatri
Telugu	: Machi-Patri
Urdu	: Afsantin

Descreption grangea maderaspatana (l.) Poir

Nomenclature of madras carpet

Botanical name Grangea maderaspatana.

Synonyms Artemisia maderaspatana, Perdicium. Family Tomentosum.

Phytography of Grangea maderaspatana (L.) Poir. (Macroscopic Characters)

Madras Carpet is a herb commonly seen in flat bunches in harvested fields, dry river and pond beds. It is a common weed usually grown in sandy soil and waste places. This hairy, branched herb spreads from the roots and grows up to 70 cm in height. It is an annual herb, stems many; prostrate, spreading form the centre, 10-30 cm long, hairy with soft white hairs.

Leaves numerous, alternate, sessile, 2.5-6.3 cm. Long, sinuately pinnatifid with 2-4 pairs of opposite or subopposite lobes smaller towards the base, the terminal lobe the largest, all coarsely serrate-dentate, pubescent on both surfaces, oblongor oblenceolate. Heads globose, 6-8 mm diameter solitary or bipinate, on short leaf opposed peduncles; flowers yellow.

Involucral bracts elliptic, obtuse, rigid densely pubescent. Pappus a shorttube with fimbriate mouth.

Fruits, Achenes glandular, 2.5 cm long including the pappus tube. The odour of leaves resembles that of wormwood; some of the vernacular names of the plant are probably those of Artemisia spp.

Flowers The inflorescence is terminal, truncate spherical head, 6-10 mm in diameter, solitary or 2-3 together, yellow and many flowered. The peduncle is 1-4 cm long.

The involucral bracts are 2-3 seriate where the outer ones are oblong and acute while the inner ones are elliptical, yellow, involucral bracts elliptic, obtuse, rigid, densely pubescent, Pappus a short tube with fimbriate mouth. Achenes glandular, 2.5 cm long including the pappus-tube.

Phenology: Flowering and fruiting.

December-April. Distribution throughout India, Pakistan and Bangladesh, Africa, Indochina and Java, Baluchistan. Ecology and Cultivation Common weed of rice-fields, wastelands and meadows, forming patches 15-30 cm wide; also on the bank of rivers and nullahs; wild.

2.2 Pharmacological review

Antioxidant activity

Patel et al., 2009 evaluated the antioxidant activity of the methanolextract of Grangea maderaspatana using five in vitro assays and was compared to standard antioxidant ascorbic acid. The extract exhibited significant ($p<0.05$) reducing power ability, 1,1-diphenyl- 2-picrylhydrazyl (DPPH) radical scavenging activity, nitric oxide radical scavenging activity, hydrogen peroxide (H_2O_2) scavenging activity and inhibition of β-carotene bleaching. The activity depends on concentration and increased with increasing amount of the extract. The free radical scavenging and antioxidant activities may be attributed to thepresence of phenolic and flavonoid compounds present in the extract.

Singh et al., 2013 also assessed in vitro antioxidant potential of the oil obtained by steam distillation of extract of aerial parts of Grangea maderaspatana (L.) Poir., using, DPPH radical scavenging, metal chelating and reducing power assays. The oil showed antioxidant potential with significant reducing power (ASE/mL 2.01 ± 0.00), chelating activity (IC50 1.80± 0.15) and DPPH radical scavenging activity (IC50 2.90 ± 0.96).

Seifried and Pilch, 2013 evaluated antioxidant or oxidation inhibitor is any compound that inhibits the production of free radicals. It prevents the damage of cellular components that arises due to chemical reactions, which yield free radicals.

Lee et al., 2007 these reactive oxygen species are the ones that cause wide range of diseases. Natural antioxidants have been shown to possess natural bioactive compounds that prevent these diseases. Natural antioxidants such as; ascorbic acid, carotenoids, and phenolic compounds are known to inhibit lipid peroxidation. Free radicals and active oxygen species reacts through a reaction cycle and chelate heavy metal ions.

Tlili et al., 2013 the method of choice for determining antioxidant activity in medicinal plants is DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay since it is reliable, it gives accurate results, does not require special reactions and device which is illustrated below. Other methods may be limited with those compounds that are soluble in selected solvents. The advantage of DPPH is that it reacts with whole sample and it is stable in methanol.

Flavonoid activity

Jain et al., 1993 assessed a mixture of flavonoids extracted from the Grangea maderaspatana plant for oestrogenicity and antiimplantational activities, in the mouse. In the 3 day uterotrophic bioassay, administration of the drug at a dose of 20 mg/kg body weight per day, intramuscularly to ovariectomized females, resulted in a highly significant ($p<0.001$) increase in the wet uterine and vaginal weights. However, in comparison with

conjugated oestrogen, the extract proved to be mildly oestrogenic. Flavonoids, administered orally at the same dose level effectively interfered with all stages of pregnancy.Maximum interceptory efficacy was recorded when the drug was administered from days 4-6 post coitum. However, there was a reduction in anti flavonoid activity only if the drug was administered from days 1-3 and 7-9 post coitum.

Patel et al., 2014 aluminum chloride colorimetric method was used with some modifications to determine the amount of flavonoids content.

Patel et al., 2014 Analysed of grangea maderaspatana showed the presence of flavanols such as Quercetin, 3'-Methoxyquercetin and 3',4'- Dimethoxy quercetin. Phenolic acids like vanillic, syringic acid, mela lotic acid, p – coumaric, caffeic acids and ferulic are also present. Anthocyanidins like pelargonidin and cyanidin and are present. Iridoids and alkaloids are also found. Total flavonoid content has been discussed by several authors The measurementof an extract's flavonoid concentration is based on the method described by **Moreno et al. (2000)** with a slight modification, and the results are expressed as quercetin equivalents. An aliquot of 1ml of a methanol solution containing 1mg of extract is added to test tubes containing 0.1ml of 10% aluminium nitrate, 0.1ml of a 1 M potassium acetate solution and 3.8ml of methanol. After 40min at room temperature, the absorbance is measured at 415 nm. Quercetin is used as a standard.

Total phenolic content

Singh et al., 2013 demonstrated an phenolic activity of the oil obtained by steam distillation of aerial parts of Grangea maderaspatana (L.) Poir. against gram positive bacteria, gram negative bacteria and fungi using agar well diffusion method. The zone of inhibition (ZOI) values of the oil was in the rangeof 2.67 ± 0.58 to 11.00 ± 0.00 mm and minimum inhibitory concentration (MIC)of the oil was ranged from 5 to 30 µL/ mL for tested microorganisms. The activity was more pronounced against Candida albicans (ZOI = 11.00 ± 0.00 mm, MIC = 5 µL/mL) followed by Streptomyces candidus (ZOI = 9.33 ± 0.58 mm,MIC = 5 µL/mL), while the oil was least effective against Aeromonas hydrophila and Klebsiella pneumoniae.

Arunachalam et al., 2002 Methanol extract of dried leaves of grangea maderaspatana and its fractions were investigated for its anti inflammatory activity in carrageenan-induced rat paw oedema.

Delaporte et al., 2002 phenolic content of an ethanolic extract from grangea maderaspatana leaves has been demonstrated.

Parthasarathy, 2010 The methanol extract of grangea maderaspatana whole plant was investigated for its phenolic content in caragenan

Sutha et al.,2011 The ethanol extract of grangea maderaspatana leaves was investigated for its phenolic content in carrageenam.

Kalpanadevi et al., 2012 The ethanol extract of grangea maderaspatana seed was investigated for phenolic activity.

lodun et al. 2006 reported the occurrence of flavonoids, phenolic contentand phorbol estersin in the aqueous and methanol extracts of grangea maderaspatana.

Raghavendra et al. 2006 examined the powdered leaf material of different solvent of grangea maderaspatana and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins.

Antimicrobial activity

Ray and Majumdar 2005 studied the antimicrobial activity of different plant parts of 105 Indian species. Only 30 species showed antibacterial activity (of which 20 also had antifungal activity). These include roots of Grangea maderaspatana.

Jaffer et al. 2009 studied the antimicrobial activity of Grangea maderaspatana extract against different gram positive, gram negative and candida species and no antimicrobial activity against gram negative bacteria was observed. How ever leaf chloroform leaf methanolic and stem chloroform extract displayed most significant antibacterial activity against gram positive bacteria.

Singh et al., 2013 demonstrated an Antimicrobial activity of the oil obtained by steam distillation of aerial parts of Grangea maderaspatana (L.).

Poir. against gram positive bacteria, gram negative bacteria and fungi using agarwell diffusion method. The zone of inhibition (ZOI) values of the oil was in therange of 2.67 ± 0.58 to 11.00 ± 0.00 mm and minimum inhibitory concentration(MIC) of the oil was ranged from 5 to 30 µL/ mL for tested microorganisms. The activity was more pronounced against Candida albicans (ZOI = 11.00 ± 0.00 mm, MIC = 5 µL/mL) followed by Streptomyces candidus (ZOI = 9.33 ± 0.58 mm, MIC = 5 µL/mL), while the oil was least effective against Aeromonashydrophila and Klebsiella pneumoniae.

2.3Chemical review

The chemical constituents reported in different parts of G. maderaspatana.

L. are mentioned below

Various parts of the plant have been reported to contain steroidal constituents like hardwickiic acid, the corresponding 1, 2- dehydro derivative and acetylenic compounds.

Eight new clerodane diterpenes including five clerodane, a nor clerodane, a secoclerodane and a norseco clerodane

derivatives along with auranamide werealso isolated.

A clerodane derivative, 15-hydroxy-16- oxo-15,16H-hardwickiic acid has beenisolated from the aerial parts of G. maderaspatana.

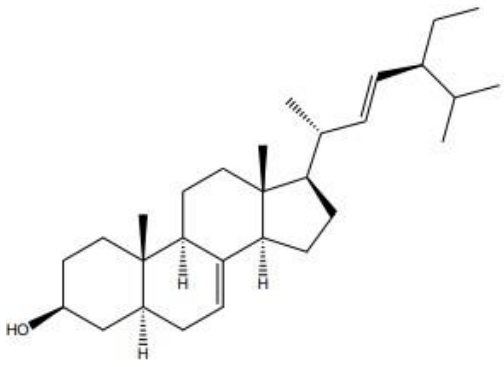
Three components viz., eudesmanolide, (-) frullanolide,(-) -7- alphahydroxyfrullanolide and a new eudesmanolide (+) -4 alpha, 13-dihydroxyfrullanolide have been isolated from the whole plant of G. maderaspatana. A new eudesmanolide was named (+) – Grangolide.

Penta and hexamethoxy flavones have been isolated as 3 ‟5- dihydroxy- 3,4‟,5 ‟,6,7-pentamethoxy flavone, 4‟,5-dihydroxy-3,3 ‟,5 ‟,6,7-pentamethoxy flavone (murrayanol) and 5-hy droxy-3,3 ‟,4 ‟,5 ‟6,7-hexamethoxy flavone in addition to previously reported clerodane ditepenes from the Diethyl ether – Petrol – Methanol (1:1:1) extract of the aerial parts of Grangea maderaspatana. Two new 5-deoxyflavones, 6-hydroxy-2′,4′,5′-trimethoxyflavone, 6-hydroxy- 3′,4′,5′- tri -methoxy flavone and a known flavone, 7,2′,4′-trimethoxyflavone have been isolated from the whole plant of Grangea maderaspatana.

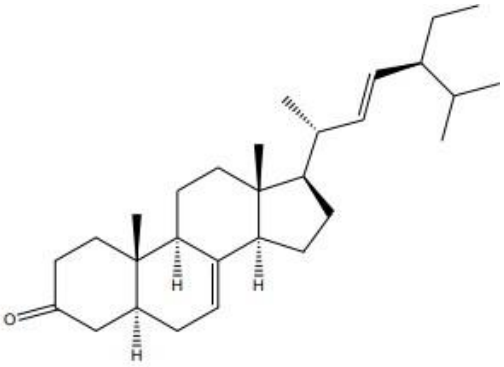
The plant contains diterpenoid compounds of labdane and clerodatetrean type, 15, 16- epoxy-7-hydroxy-3, 13, 14-clerodatrien-18-oic acid; steroids, chondrillasterone and chondril -lasterol; diterpene, strictic acid, a phenylalanine derivative, auranamide and the allergenic compounds, eudesmanolides, (-)- frullanolide, (-)-hydroxyfrullanolide and (+)-grangolide.

A new diterpenoid has been isolated as 8-hydroxy- 13 E -labdane-15yl-acetate from the acetone extract of Grangea maderaspatana.

The aerial parts of Grangea maderaspatana (L.) Poir contain 91.5% of oil constituting 21 different constituents. It was characterized by the dominant presence of sesquiterpenoids (sesquiterpenoid hydrocarbons 36.1 % and oxygenated sesquiterpenoids 28.4 %). Most abundant compounds are γ- gurjunene (26.5%), terpinyl acetate (20.8%) and hinesol (11.7%).



(A)



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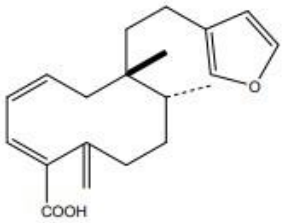

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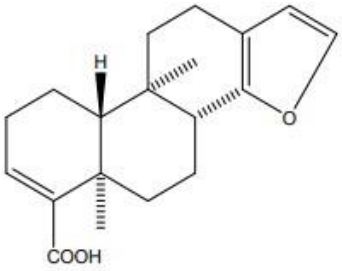


(D)

Phytochemicals isolated & reported from the Grangea maderaspatana. (L.) Poir.[Chemical Structures]

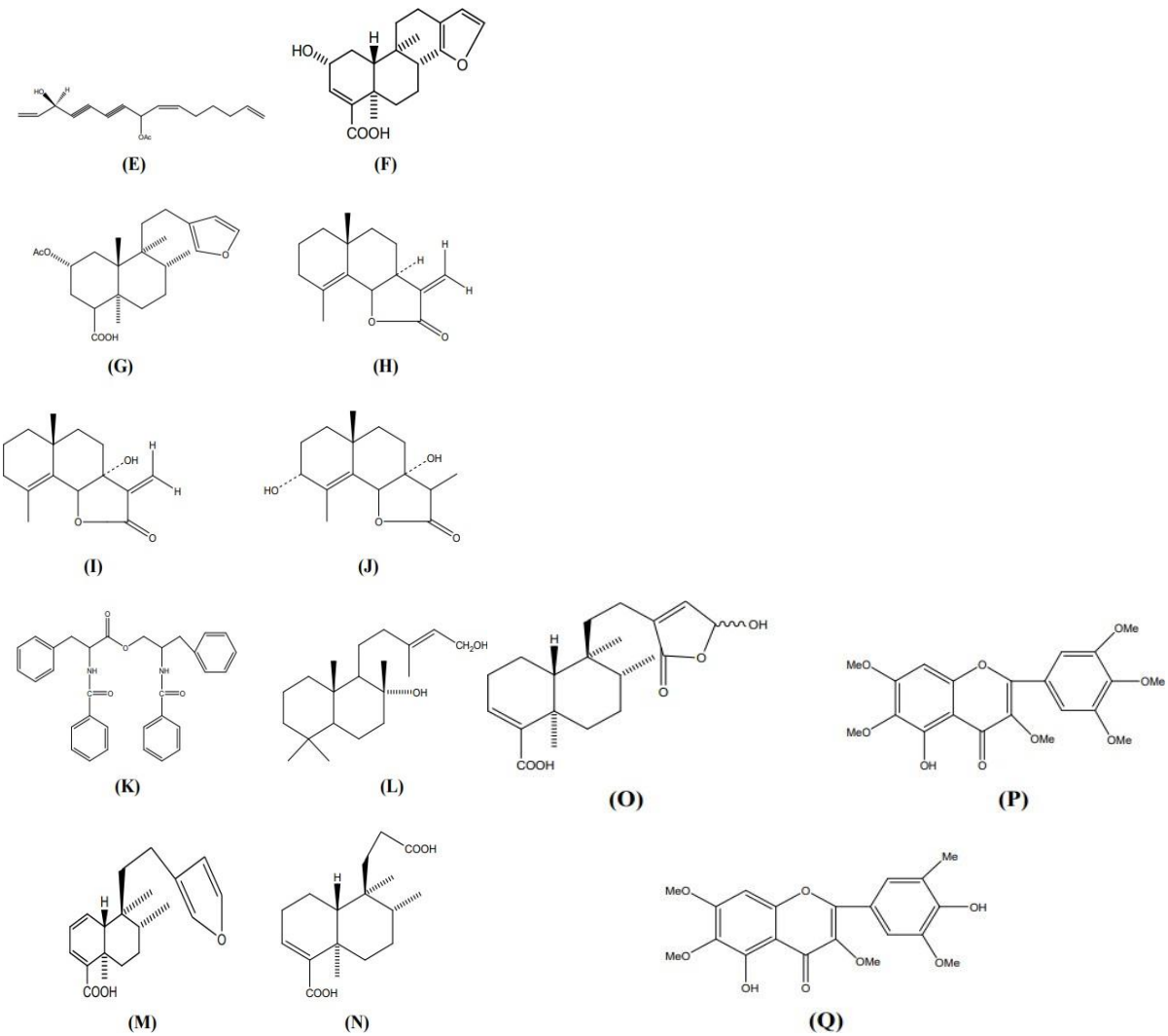


Fig. 8: Chemical constituents of Grangea maderaspatana.

Sr.No	Compound name	Chemical class	Plant part (s)
A	Chondrillasterol	Sterol	*Entire plant*
B	Chondrillasterone	Sterol	*Entire plant*
C	Strictic acid	Terpenoid	*Entire plant*
D	Hardwickiic acid	Furanodi-terpenes	*Entire plant*
E	Acetylenic alcohol	Alcohol	*Entire plant*
F	2α hydroxy Hardwickiic acid	Furanodi-terpenes	*Aerial Part*
G	2αAcetoxy-hardwickiic acid	Furanodi-terpenes	*Aerial Part*
H	Eudesmanolide (-)- frullanolide	Sesqui-terpene lactone	*Entire plant*
I	(-)- 7α hydroxyl-frullanolide	Sesqui-terpene lactone	*Entire plant*
J	Grangolide	Sesqui-terpene lactone	*Entire plant*
K	Auranamide	Phenyl-alanine	*Aerial Part*
L	8-hydroxy-13E-Labdane	Diterpenoid	*Aerial Part*
M	10-epi-nidoresedic acid	Clerodane diterpenes	*Aerial Part*
N	nor hardwickiic acid	Clerodane diterpenes	*Aerial Part*
O	15-hydroxy-16-oxo-15,16H-hardwickiic acid, 15-hydroxycleroda-3,13-dien-15,16-olid-18-oic acid,	TRANS-Clerodane Diterpenes	*Aerial Part*
P	5-hydroxy-3,3',4',5',6,7-hexamethoxy flavone	Flavonols	*Aerial Part*
Q	Murrayanol	Flavonols	*Aerial Part*

MATERIALS AND METHODS

4.1 Collection of plant materials

The plant of grangea madrespatana was collected from the nearby surroundings of lepakshi area, Ananthapur District. In the month of Feburuary2023.

4.2 Extraction of drug

The whole plant material was dried in shade and was ground to get a coarse powder. The powdered plant material of Grangea maderaspatana was extracted with methanol using soxhlet apparatus. The crude extract was filtered, concentrated, and dried. Freshly prepared aqueous solution of dried methanolic extract of Grangea maderaspatana was used in the experimental study.

Continuous extractionSoxhlet extraction

Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor several times. The process involves extraction followed by evaporation of the solvent. The vapours of the solvent are taken to a condenser and the condensed liquid is returned to the drugfor continuous extraction.

Fig 9 Soxhlet apparatus

Extraction assembly is set up by fixing a condenser and a distillation flask. Initially for the setting of the powder, the solvent is allowed to pass through siphon once before heating Fresh activated porcelain pieces are added to flask to avoid bumping of the solvent the vapours pass through the side tube and the condensed liquid gradually increases the level of liquid in the extractor and in the siphon tube. A siphon is set up as the liquid reaches the point of return and the content of the extraction chamber are transferred to the flask. The cycle of solvent evaporation and siphoning back can be continued as many times as possible without changing the solvent, so as to get efficient. This method although a continuous extraction process, is nothing but a series of short macerations.

Similar methodology can be adopted in large scale production in which the operation principles may resemble the laboratory equipment. Soxhlet extraction is advantageous in a way that less solvent is needed for yielding more concentrated products. The extraction can be continued until complete exhaustion of the drug. The main disadvantage is that this process ted to pure boiling solvents or to azeotrops.

4.3 Preliminary phytochemical screening

Preliminary phyto chemical screening for carbohydrates

Walsh's Test General test: To 2-3ml. aqueous extract few drops of alpha napthol solution in alcohol, shake and add concentrate H2SO, from sides of testtube, violet ring, violet ring is formed at the junction of two liquids

TESTS FOR REDUCING SUGARS

Fehling's test: mix 1ml fehling's A and 1ml B solution heat for 1 min. Add equal volume solution. Heat in boiling water bath for 5-10 minute. First a yellow or red ppt is observed.

Benedict's Test: Mix equal volume of benedict's reagent. Heat in boilingwater bath for 5 minutes. Solution appears green, yellow or red depending on reducing sugar present in solution.

TEST FOR MONOSACCHARIDES

Barfoed's test: mix equal volume of barfoed's reagent and test solution.

Heat for 1-2 min boiling water bath and cool. Red ppt is observed.

TEST FOR PENTOSE SUGARS

Pentose are components of certain gums.

BIAL'S ORCINOL TEST: To boiling bial's reagent add few drops test solution. Green purple coloration appears.

ANILINE ACETATE TEST: Boil test solution in test tube. Hold filter paper soaked aniline acetate in the vapour. Filter paper turns pink.

Mix equal amount of test solution and Hel Heat. Add a crystal of pholoroglycinol. Red colour appears.

TEST FOR HEXOSE SUGARS
SELWINOFF'S TEST (FOR KETONE HEXOSE LIKE
FRUCTOSE): Heat 3ml of selwinoff's reagent and 1ml test solution in bearing water bath for 1-2min red colour is formed.

TEST FOR NON-REDUCING SUGARS
Test solution does not give response to fehlings and benedes test.
Hydrolyse test solution fehling's and benedic's tests are positive.

FOR NON-REDUCING POLYSACCHARIDES STARCH
IODINE TEST Mix 3m of test solution and few drops of dilute iodine solution blue colour appears it disappears on boiling and reappear on cooling.

TANNIC ACID TEST FOR STARCH: With 20% tannic acid, test solution give ppt(0) TEST FOR GUMS Hydrolyse test solution using dilute HCL. Perform fehling's or benedict's test. Red colour is developed

TEST FOR MUCILIGE
Powder drug swells in water or aqueous KOH.

TEST FOR PROTIENS
Biuret test (general test): To 3 ml TS add 4% NaOH and few drops of Cu50 solution violet or pink colour appears.

Million's test (for protein): Mix 3 ml TS with 5 ml Millions reagent. White ppt. warm. Ppt turns brick red or deep ppt dissolves giving red coloured solution.

Xanthoprotein test (for protein containing tyrosine or tryptophan): Mix 3 ml TS with 1ml Conc. H2SO. White ppt is formed. Boil. Precipitate turns yellow Add NH.OH.ppt turns orange.

Test for proteins containing sulphur
Mix 5 TS with 2 ml. 40% NaOH and 2 drops 10% lead acetate solution. Boil Solution turns black or brownish due to PS formation.

Precipitation test: The test solution gives white colloidal ppt with following reagents (a) absolute alcohol, (b) 5% HgCL solution.(c) 5% CuSO, solution, (d) 5% lead acetate. (e) 5% aluminium sulphate.

Ninhydrin test (General test): Heat 3 ml TS and 3 drops % ninhydrin solution in boiling water bath 10 min Purple or bluish colour appears.

Test for tyrosine: Heat 3 ml TS and 3 drops Million's reagent Solutionshows dark colour.

Test for tryptophan: To 3 ml TS and few drops glyoxalic acid and come H2S04 Reddish coloured ring appears at junction of two layers.

Test for cysteine: To 5 ml T.S. add few drops of 40% NaOH and 10% lead acetate solution Boil Black ppt of lead sulphate is formed.

TESTS FOR FATS AND OILS
1. Place a thick section of drug on glass slide. Add a drop of Sudan Red reagent After 2 min. wash with 50% alcohol. Mount in glycerine. Observe under microscope Oil globules appear red
2. To thin section add a drop of 1% osmic acid. After a one minute, observe under microscope. Oil drops appear black
3. Solubility test. Oils are soluble in ether benzene and chloroform, but insoluble in 90% ethanol and in water (Exception-Castor oil soluble in alcohol)
4. Filter paper gets permanently stained with oils
5. Extract gives red colour with 2-3 drops of tincture alkane
6. Saponification test: Evaporate extract to get 10 ml oil. Too oil add 25 ml. 10% NaOH. Boil in boiling water bath for 30 min. Cool Add excess Na.SO solution. Soap forms and raise to the top filter too filterate add H.SO, Evaporate. Collect residue, it contains glycerol. Dissolve residue ethanol.

With ethanolic solution, perform following tests:
1. To ethanolic solution, add few crystals of KHSO, Heat vigorously Pungent odour acrylic aldehyde is produced.
2. To ethanolic solution, add few drops of CuSO, and NaOH solutions Clear blue solution is observed.

TESTS FOR STEROID
1. **Salkowski reaction:** To 2 ml. of extract, add 2 ml chloroform and 2ml conc. H2SO Shake well Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
2. **Libbermann-Burchard reaction:** Mix 2 ml extract with chloroform. Add 1-2 ml, acetic anhydride and 2 drops conc. H.SO, from the side of test tube,red, then blue and finally green colour appears.
3. **Libbermann's reaction:** Mix 3 ml. extract with 3 ml, acetic anhydride. Heat and cool. Add few drops conc. H_2SO, Blue colour appears.

TESTS FOR VOLATILE OILS
Hydro disilicate material. Separate volatile oil from

distillate and perform thefollowing
1. Volatile oils have characteristic odour.
2. Filter paper is not permanently stained with volatile oil.
3. Solubility test; Volatile oils are soluble in 90% alcohol

TEST FOR GLYCOSIDES
Determine free sugar content of the extract. Hydrolyse the extract with mineral acid (dil HCI dil H2SO4) Agar determine the total sugar content of the hydrolyse the extract. Increase in sugar content indicate presence of in the extract Glycoside Aglycon(genin)+ Glycon(sugar)

TEST FOR CARDIAC GIYCOSIDES
(a) Baljet's test: A thick section shows yellow to orange colour withsodium picrate.
(b) Legal's test (test for cardenoloids): To aqueous or alcoholic extract, add 1 ml. pyridine and 1ml sodium nitro prusside. Pink to red colour appears. **Libermann's test (test for bufadenoloids):** see tests for steroids

TESTS FOR ANTHRAQUINONE GLYCOSIDES
(a) Borntrager's test for anthraquinone glycosides: To 3 ml extract, add dil.H.SO. Boil and filterate, cold filtrate, add equal volume of benzene or chloroform Shake well. Separate the organic solvent. Add ammonium ammonical layer turns pink to red.
(b) Modified Borntrager's test for e- glycosides: To 5 ml. extract add 5ml. 5% FeCl, and 5 ml. Diluted HCL. Heat for 5 min in boiling water bath cool and add benzene or any organic solvent. Shake well. Separate layers add equal volume of dil. ammonia ammonical layer shows pinkish red colour.

TESTS FOR SAPONIN GIYCOSIDES
(a) Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.
(b) Haemolytic test: Add drug extract or dry powder to one drop of bloodplaced on glass slide haemolytic zone appears.

TESTS FOR CYANOGENETIC GLYCOSIDES
(a) Guignard reaction or sodium picrate test: Soak a filter paper strip first in 10% picric acid, then 10% sodium carbonate. Dry In a conical flask place moistened powdered drug. Cork it, place the above filter paper strip in the slit in cork. The filter paper turns brick red or maroon.
(b) To dry drug powder or extract, add 3% aqueous mercurous nitrate solution.
Metallic mercury forms
(c) Dip a piece of filter paper in guaiacum resin and moist it with dil. Copper sulphur solution. Expose to freshly cut surface of drug, blue strain is produced.

TESTS FOR COUMARIN GLYCOSIDES:
(a) Coumarin glycosides have aromatic odour
(b) Alcoholic extract When made alkaline Shows blue or green fluroscence.
(c) he moistened dry powder in test tube. Cover test tube with filter paper soaked in dilute NOH Keep in water bath. After sometime expose filter paper to U.V light. It shows yellowish-green flouroscence.

TESTS FOR FLAVONOIDS
(a) Shinoda test: To dry powder or extract, add 5 ml 95% ethanol, few drops HCI and 0.5 g magnesium turnings pink colour is observed.
(b) To small quantity of residue, add lead acetate solution. Yellow coloured pptis formed
(c) Addition of increasing amount of sodium hydroxide to the residue shows yellow colouration, which decolourises after addition of acid

TESTS FOR ALKALOIDS
Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add di HCI Shake well and filter With filtrate, perform following tests:
(a) Dragendorff's test: To 2-3 ml filtrate, add few drops Dragendorff's reagent orange brown ppt is formed.
(b) Mayer's test: 2-3 ml filtrate with few drops Mayer's reagent gives ppt
(c) Hager's test: 2-3 mi. filtrate with Hager's reagent gives yellow ppt.
(d) Wagner's test: 2-3 ml filtrate with few drops Wagner's reagent gives reddishbrown ppt
(e) Murexide test for purine alkaloids: To 3-4 ml. test solution, add 3-4 drops of Conc.HNO3. Evaporate to dryness. Cool and add 2 drops of NH4OH Purple colour is observed.The plant G. Madrespatana was collected from the nearby surroundings of lepakshi area, Ananthapur District.

All the solutions were prepared using double distilled water. The whole plant was rinsed with tap water followed by distilled water to remove dust and impurities, the washed plant was dried in dust free conditions and powdered in a conventional grinder to obtain a fine powder. The dried plant powder was then sieved and stored in an amber coloured air tight container for further use in preparation of methanolic extract of g. Maderaspatana.

4.4 PREPARTION OF METHANOLIC EXTRACT OFG.MADRESPATANA
The methanolic extract of madras carpet was prepared by adding the powder of the dried plant of madras carpet were extracted in soxhlet apparatus using methanol as solvent. Thus, obtained methanolic extract of madras carpet was stored in amber coloured air tight bottles under refrigeration.

4.5 METHODS FOR EVALUATION Estimation of total phenolic content
Total phenolics contents (TPC) of the extracts were determined by the method described by Singleton and Rossi using Folin-Ciocalteu reagent. In thismethod, 60 µL of Folin-Ciocalteu reagent (FCR) were added to 60 µL of suitably diluted extract. The mixture was left at room

temperature for eight minutes to allow complete reaction of the RFC on oxidisable substances or phenolates. Then 120 µL of a 7.5% solution of is added to neutralise the residual reagent. Absorbances were recorded at 760 nm with a microplate reader (spectrophotometer MP96, SAFAS) after incubation at 37 °C for 30 minutes. The results, determined from the equation of a calibration curve (y = 21.298x + 0.354; = 0.9956) established from the gallic acid taken as reference, were expressed in mg of Gallic Acid Equivalent per gram of extract (mg GAE/ g). The total phenolic content was determined by the spectrophotometric method[27]. In brief, a 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of a 7% Na2CO3 solution wasadded to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at23°C, after which the absorbance was read at 750 nm. The TPC was determinedfrom extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g ofdried sample.

Estimation of total flavonoid content

Total flavonoid content was determined following a method by Park et al(2008) [28]. In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M) and 0.15 ml of AlCl3.6H2O (0.3 M) were mixed. After 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using rutin standard solution (0 to 100 mg/l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per g of dried fraction.

Total flavonoids contents of the extracts were assessed according to the colorimetric method described by Zhishen et al. [17]. 50 µL of the suitably diluted extracts were mixed with 150 µL of bi-distilled water, followed by 15 µL of 5% (w/v) sodium hydroxide. 5 min later, 15 µL of a 10 % (w/v) aluminium trichloride solution is added. The mixture was allowed to stand at room temperature for 6 min. Then, 50 µL of a 1 N sodium hydroxide solution were added and the absorbance of the pinkish mixture, was measured at 510 nm using a microplate reader (spectrophotometer MP96, SAFAS). A calibration curve was established using quercetin as a reference according to the same procedure as the sample. Flavonoid contents of the extracts, expressed in mg of QuercetinEquivalent per gram of extract, were calculated by relating the absorbances values on the calibration curve.

Estimation of Antioxidant content

There are many ways to estimate antioxidant content in plant using methanolic extraction. Some of them are:

DPPH radical scavenging activity assayHydroxyl radical scavenging assay Hydrogen peroxide scavenging activity.

Antioxidant assays

Each sample was dissolved in 95% methanol to make a concentration of 1 mg/ml and then diluted to prepare the series concentrations for antioxidant assays. Reference chemicals were used for comparison in all assays.

DPPH radical scavenging activity assay

The free radical scavenging activity of the fractions was measured in vitro by 2,2′- diphenyl-1-picrylhydrazyl (DPPH) assay according to the methoddescribed earlier. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (10-500µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the followingequation:

Scavenging effect (%) = [(controlabsorbance−sampleabsorbance)/(controlasbsorbance)]×100

Hydrogen peroxide scavenging activity

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different fractions was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank.[23] The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

Hydrogen peroxide scavenging activity= (1−absorbanceofsample/absorbanceofsample)×100

Hydroxyl radical scavenging assay

The scavenging activity of the extract against hydroxyl radical was measured using the deoxyribose test-tube method[39] with minor changes. All solutions used was freshly prepared; 200 µL of 2.8 mM 2-deoxy-2-ribose, 5 µLof N.laevis leaf extract ,400 µL of 200 mM FeCl3, 1.04 mM EDTA, 200µL

H2O2 (1.0 mM), 200 µL ascorbic acid(1.0 mM) and various concentrations (10– 400 µg/ml) of the plant extract was mixed to form a reaction mixture. The mixture was incubated for 1 hour at 37°C. The extent of deoxyribose degradation was measured by TBA reaction. TCA (1.5 ml of 2.8% TCA) was added and kept for 20 mins. The solution was incubated at 90°C for 15 min to develop the colour. Afterwards, the solution was cooled

and the absorbance measured at 532 nm against an appropriate blank solution Mannitol, a classical •OH scavenger was used as a positive control. The percentage antioxidant activity was calculated using the formula described in equation.

Hydrogen peroxide scavenging activity= (1−absorbanceofsample/absorbanceofsample)×100

Evaluation of antimicrobial activity

The plant extracts were tested for antimicrobial activity by the cup plate /well plate diffusion method 9 using nine bacterial cultures. 0.1 ml of bacterial suspension was thoroughly mixed with 25 ml of sterile molten Nutrient Agar (Hi-media Pvt. Ltd) respectively and poured in presterilized petri plates and set aside. After congealing the seeded agar was punched out (Dug out) with flamed cork borer in order to make 3 cups (10mm diameter) at a spaced out position in the petri plate. All the three cups were filled with 0.1 ml (100 l) of each extract(100mg/ml) with micropipette. Each extract was tested in triplicate. Culture control and DMSO (Dimethyl sulphoxide) as solvent control were also maintained. These agar plates were set aside at room temperature for one hour for diffusion and then incubated at 37℃ for 18-24 hours. After incubation, the zone of inhibition was measured in mm diameter and the mean value of triplicates was recorded.

Assay of Antimicrobial activity using Agar well diffusion method

The 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplate, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher at various concentration (20, 30, 40, 50 and 60) of eachplant extract were added to the wells. The plates were incubated for 24 hours at37C. After incubation the diameter of inhibitory zones formed around each discswere measured in mm and recorded.

Minimum Inhibitory Concentration (MIC) Assay

The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disk diffusion (Kirby–Bauer) method. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of a microorganism is known as MIC10. Selected plant extracts were subjected to a serial dilution (25 mg/ml to 0.37 mg/ml) using sterile nutrient broth medium as a diluent. In a 96-well titre plate 20 µl of an individual microorganism and 20 µl of selected plant extract were loaded and inoculated at 37℃ for 24 h. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract. A control experiment was run in parallel to study the impact of the solvent alone (without plant extracts) on growth of the nine test organisms. Methanol was diluted in a similar pattern with sterile nutrient broth followed by inoculation and incubation.

Anti Bacterial activity

Test organisms

a) Gram (+)ve bacteria
1. Bacillus subtilis
2. Staphylococcus aureus

b) Gram (-)ve bacteria
1. Escherichia coli
2. Pseudomonas aeruginosa

Composition of nutrient agar medium

Peptone	-5gm
Sodium chloride	-5gm
Meat extract	-3gm
Agar	-20gm
Ph	-7.4±02.

Distilled water to make up to 1000ml

Peptone meat extract and sodium chloride were dissolved in distilled water andpH of the medium was adjusted to 7.2. Agar was dissolved and distributed in 40ml quantities into 1000ml conical flask and was sterilized in an autoclave at 12℃ (151lbs/sq.in) for 20min.The medium was inoculated at 1% level with 18 hrs of old cultures of the test mentioned above organism and transferred into sterile 15cm diameter petridishes.

The medium in the plates was allowed to set at room temperature for 30 min. For the preparation of cup agar plates, 6mm diameter holes were made withthe help of a sterile border at the corner of the plate at an equal distance. Solution of test compounds in the concentrations of 100gm/ml and 200mg/ml.

Concentrations were prepared in DMSO, and two drops (0.05ml) of eachconcentration were placed in the cups by means of sterile pipettes. In each plate,one cup was used for control with 2 drops(0.05ml) of DMSO Neomycin sulfate in 10µg/ml concentration was used as standard. The plates thus prepared were left 90min at room temperature for diffusion. After incubation for 24 hrs at 37℃,the plates were examined for inhibition zones. The experiments were run in duplicate and the average diameter of the zones of inhibition was recorded and noted.

RESULTS AND DISCUSSIONS

Collection of plant materials

The plant of grangea madrespatana was collected from the nearby surroundings of lepakshi area, Ananthapur District. In the month of Feburuary2023.

Phytochemical screening of madras carpet

Chemical constituents	Petroleum ether extract	Chloroform extract	Ethylacetate extract	Methanol extract	Water extract
Carbohydrate	-	-	-	+	+
Protein	-	-	-	-	-
Phenolics & Tannins	-	-	-	+	+
Saponins	-	-	-	+	++
Flavanoids	-	-	-	+	+
Terpenes	++	++	+	+	-
Steroids	++	++	-	-	-
Alkaloids	-	-	-	-	-

(+ - Positive test, - - Negative test)

Antioxidant Activity

Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of various methods the total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H2O2, O2•− and OH• Quenchingassays were used for the evaluation of antioxidant activities of extracts. For the measurements of the reductive ability, we observed the transformation of Fe3+ -Fe2+in the presence of oil samples using the method of Apati et al. (2003)13. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Like the antioxidant activity, the reducing power of essential oil increased significantly with concentration.

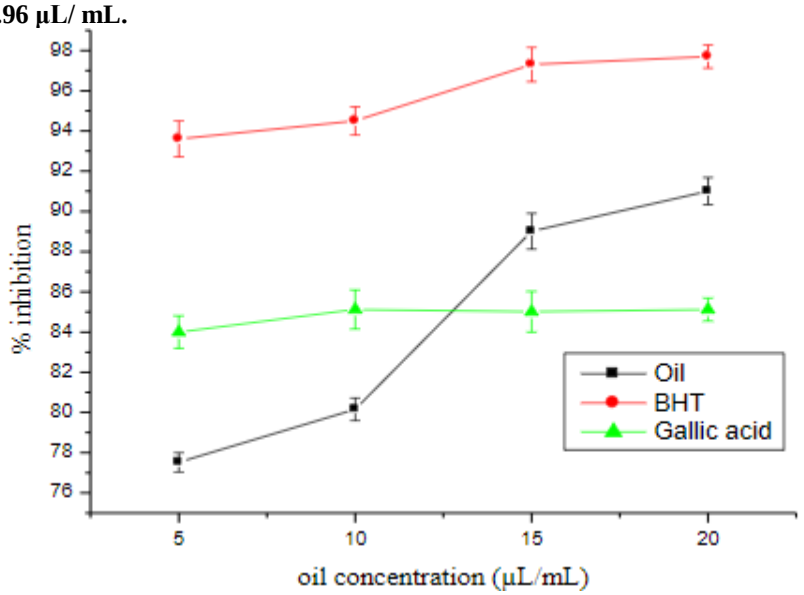
Mean absorbance values were significantly different at P<0.05.Reducing power of the essential oil was 2.01 ± 0.00 ASE/ mL.

The effect of antioxidants on DPPH radical scavenging is due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electronor hydrogen radical to become a stable diamagnetic molecule.

The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow colour with the result of which percent absorbance value increases.

The maximum absorption of a stable DPPH radical in ethanol is at 517 nm.The scavenging effect (% inhibition) of the oil and standards on the DPPHradical decreased in the order of BHT (97.7 %) > Essential oil (91.01 %) > Gallic acid (85.95 %) at 20µL/ mL.

Mean values were significantly different at P<0.05. I C50 value of the essential oil for DPPH radical scavenging activity was 2.90 ± 0.96 µL/ mL.



DPPH radical scavenging activity

Sample/standard	DPPH (IC_{50} µL/mL)	RP (ASE/mL)	Chelating (IC_{50} µL/mL)
GMO	2.90 ± 0.96	2.01 ± 0.00	1.80 ± 0.15
Gallic acid	1.60 ± 0.86	ND	0.71 ± 0.34
BHT	0.38 ± 0.47	1.48 ± 0.01	0.78 ± 0.46
Linoleic acid	ND	2.05 ± 0.24	ND

Antioxidant activity of maderas carpet

5.1 Total phenolic content

A group of compounds with similar chemical structures may show the same chemical interactions with a specific reagent during the reaction. The F– C assay is a reaction based on an ET from phenolic compounds and other oxidation substrates to the F–C reagent, phosphomolybdic/phosphotungstic acid complexes (H3PW12O40/H3PMo12O40), resulting in blue complexes (possibly (PMoW11O40)4–) which induce λmax at about 765 nm. Deviation from the λmax can result from oxy reduction responses of the F–C reagent and the complex structural differences of polyphenols (antioxidants) occurring in plants. This deviation could be capable of compromising the experimental responses by underestimating the quantitative data. Therefore, the absorption spectra of the G madraspatana extracts was compared with that of the reference compound, Gallic acid. All the crude extracts show an approximately similar absorption spectrum as the reference compound.

The optimal time for colour development was determined using the selected wavelength. According to the reaction kinetics, the absorbance increased from 5 to 30 min, for all samples tested, then remained steady for a while before progressively dropping.

TPC determination at steady-state improves the precision of analytical results. Conditions like high temperature and alkali levels, accelerate colour development and fading. Thus, studying reaction kinetics can provide useful information. Results of the current study demonstrated that 30 min of incubation time is required before measurement of the absorbance. The obtained findings were in agreement with previous reports on optimization of the F–C method for the determination of TPC from plant extracts.

To determine the TPC in the resin extracts, under the specified conditions, a calibration curve was constructed using Gallic acid calibration standards (0 to150 µg/mL).

The coefficient of determination (R2) of the resulting calibration curve (y = 0.0082x + 0.0108) was 0.9997, suggesting excellent linearity in the studied range of concentrations.

Extract	Total Phenolics content (mg GAE) /g extract			
	Whole plant	Leafy branches	Flowers	Roots
Hexane	12.113 ± 0.019^{cde}	7.506 ± 0.287^{bc}	4.536 ± 0.066^{ab}	4.442 ± 0.075^{ab}
DCM	0.063 ± 0.001^{a}	0.051 ± 0.001^{a}	8.996 ± 0.248^{bcd}	7.600 ± 0.646^{bcd}
EtOAc	59.198 ± 1.479^{g}	57.921 ± 0.075^{g}	93.455 ± 4.958^{h}	61.602 ± 3.261^{g}
MeOH	26.974 ± 2.155^{f}	12.260 ± 0.047^{cde}	12.798 ± 0.254^{de}	15.860 ± 0.707^{e}

The LOD and LOQ of the method were 2.41 and 7.29 µg/mL, respectively. The TPC of G madrespatana extracted by different solvents was calculated using the calibration curve equation and reported as mg GAE/g dry mass of the extract.

Phenolic content of madras carpet

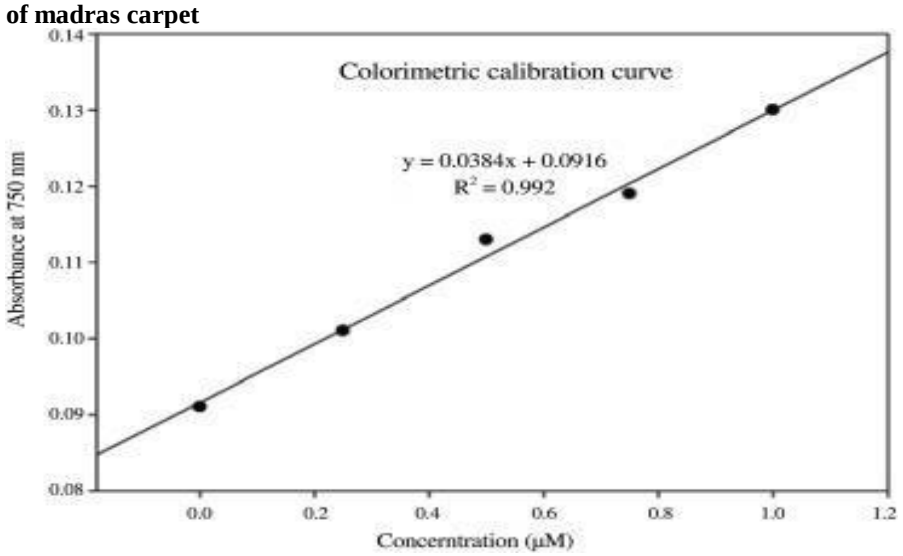


Fig. 11: Phenolic content of madras carpet.

5.2 Total flavonoid content

The TFC content of the G. Madraspatana extracts was also determined by extrapolation from the calibration curve (Y = 0.4638x+ 0.0778; R2 = 0.992) prepared from the quercetin concentrations and expressed in mg of quercetin equivalence (QE) per gram. The amounts of flavonoid compounds in the various extracts were obtained from regression equation and the values were expressed in quercetin equivalence.

Total flavonoid content (TFC) of different extracts of Madras carpet was determined by AlCl3 method. Determining the total flavonoids by using AlCl3 is based upon the formation of stable complex between AlCl3, keto and hydroxyl groups of flavones and flavonoids. The results for total flavonoid content in the studied plant extracts are presented in the graph (Figure 4).

The results show that MTE has the richest source of flavonoids, i.e. total content is 0.25 mg QE/g while on the contrary, ATE possess the lowest amount of flavonoid i.e. 0.164 mg QE/g. this is contrary to the findings of[34] where it was found that ethanolic extract has the highest flavonoid content.

The values from all the extracts were not significantly different (p > 0.05).

Extract	Total flavonoid content (mg QE / g of extract)			
	Whole plant	Leafy branches	Flowers	Roots
Hexane	196.672 ± 10.086^{er}	144.982 ± 12.607^{ca}	138.678 ± 5.043^{bc}	109.472 ± 5.685^{ab}
DCM	165.406 ± 6.051^{cde}	207.766 ± 20.171^{f}	191.629 ± 4.034^{ef}	156.665 ± 14.168^{ca}
EtOAc	474.701 ± 18.633^{g}	681.795 ± 8.068^{i}	753.337 ± 12.111^{j}	641.45 ± 8.068^{h}
MeOH	176.10 ± 3.557^{aer}	147.571 ± 11.521^{ca}	155.705 ± 8.703^{ca}	82.984 ± 1.824^{a}

Flavonoids display a crucial role in scavenging the free radicals and these are the phyto constituents that should be focused on for investigation of many biological activities. Phytochemicals especially polyphenols constitute a major group of compounds that act as primary antioxidants.

Flavonoid content of madras carpet

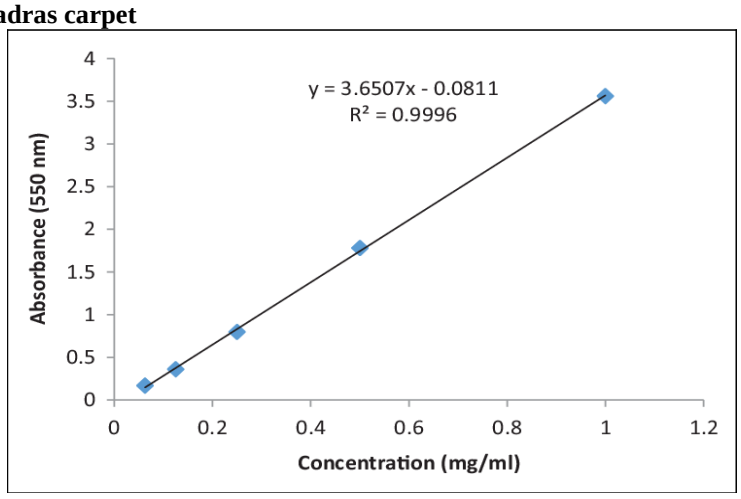


Fig. 12: Flavonoid content of madras carpet.

5.3 Antimicrobial activity

The bactericidal activity of the synthesized grangea madraspatana was tested against Bacillus subtilis [gram positive], Staphylococcus aureus (gram positive], Pseudomonas putrida (gram negative] and Escherichia coli [gram negative] as depicted Clear zones of inhibition were observed. The meandiameters of zones of inhibition of madras carpet against each bacterial strain are depicted. The Madras carpet synthesized from methanolic extract of maderaspatana have exhibited greater antimicrobial activity in comparison with the standard antibiotic, Ampicillin. The suppression of bacterial growth increased with increase in amount of Madras carpet This explained the dose- dependent antibacterial activity of Madras carpet. The growth of gram negative bacterial strains was more suppressed by madras carpet compared to the gram positive bacterial strains.

This phenomenon can be attributed to the structural organization of peptidoglycan (PG) in the membrane of the bacterial cell wall. Gram positive bacterial strains exhibit a thick PG layer whereas the gramnegative bacterial strains exhibit a thin PG layer. This results in differential inhibition of the bacterial strains. The probable mechanism for antibacterial activity of Madras

carpet may involve attachment of the Madras carpet to the surface of bacterial cell membrane, followed by penetration of the nanoparticles inside the bacteria and destruction of the cytoplasm (Scheme 1A) The Madras carpet produced significant changes in the morphology of bacterial cell membrane, thereby increasing their permeability. This disrupts the transport through cell membrane leaving the bacterial cells incapable of regulating transport through the plasma membrane, resulting in cell death.

The madras carpet due to their maximum surface area provide maximum contact with the environment. Hence, smaller the particle size more will be the ability of the particles to penetrate through the cell membrane Therefore, the binding of the Madras carpet to the bacterial membrane depends on the surface area available for the interaction. Hence, the increase in the concentration of Madras carpet is resulting in increased suppression of bacterial growth due to the increase in number of Madras carpet with large surface area for attachment to the bacterial cell membrane and penetration into the bacterial cell.

Methanol extracts grangea maderaspatana that showed maximum antimicrobial activity was taken for MIC assay.

Least efficacy was shown against S. epidermidis which was inhibited at 12 mg/ml concentration. Oliveira et al.[11] reported the antimicrobial activity of Syzygium cumini extract from leaves. The antimicrobial activity of grangea maderaspatana is due to tannins and other phenolic compounds. It is known to be very rich in gallic and ellagic acid polyphenol derivatives.[12,13]

Grangea maderaspatana showed inhibition at 3.0 mg/ml concentration against P. aeruginosa, S. aureus, and S. epidermidis followed by E. coli and K. pneumonia at a concentration of 6.0 mg/ml.
Grangea madereaspatana exhibited the highest antibacterial efficacy against S. aureus at 0.75 mg/ml concentration followed by E. coli and P. aeruginosa at 1.5 mg/ml concentration.

The antimicrobial activity of grangea maderaspatana extract may be due to the presence of phenolic constituents.

The antimicrobial activity of ethanolic extracts of grangea maderaspatana on four different human pathogenic organisms using agar diffusion method have showed **maximum zone of inhibition (23 mm)** against **Pseudomonas sp. followed by Staphylococcus sp. (22 mm), Klebsiella sp. (21 mm) and E. coli (21 mm).**In case of agar well diffusion method, the ethanolic extract of grangea maderaspatana showed the **maximum zone of inhibition (21 mm) against E. coli followed by Staphylococcus sp. (20 mm)** with the extract of Phyllanthus niruri.

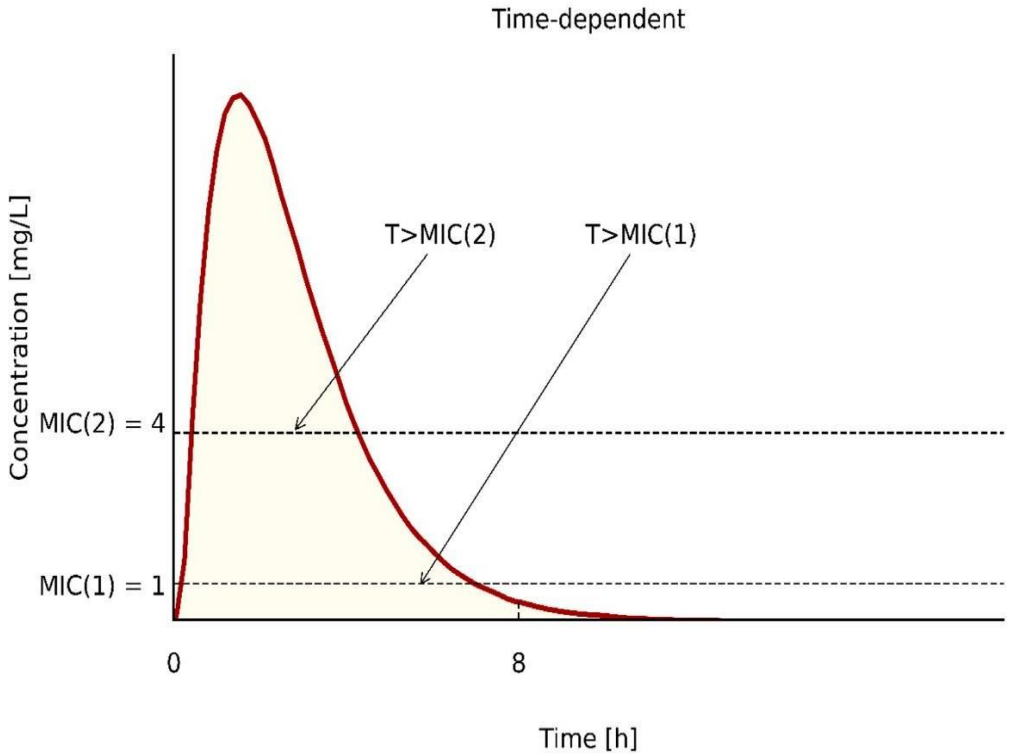


Fig. 13: Antimicrobial activity of grangea maderaspatana.

Dilutions / Souches	Pure		1/2		1/4		1/8		1/16	
	D (mm)	S	D (mm)	S	D (mm)	S	D (mm)	S	D (mm)	S
Escherichia coli	9.5	+++	11.5	+++	7.15	+	7.7	+	7.1	+
Proteus sp.	11.9	+++	6.65	+	< 6	-	< 6	-	< 6	-
Serratia sp.	8.6	++	12.15	+++	12.3	+++	9.9	+++	11.45	+++
Klebsiella pneumoniae	8.75	++	7.55	+	11.05	++	8.55	++	11.05	+++
Staphylococcus aureus	18.75	+++	23.35	+++	9.65	+++	7.55	+	6.4	+
Streptococcus D	23.6	+++	12.25	+++	10	+++	11.5	+++	12.25	+++
Pseudomonas aeruginosa	23.6	+++	12.25	+++	10	+++	11.5	+++	12.25	+++
Acinetobacter baumanii	7.3	+	9.3	+++	9.9	+++	11.45	+++	8.1	++

D (mm): inhibition diameter; S: Signification.

Fig. 13: Antimicrobial activity of grangea maderaspatana.

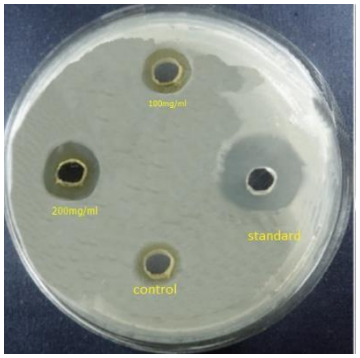
Antibacterial Activity of plant

Extracts at a concentration of 100 µg and 200 mg/ml per cup exhibited against one or other organisms in a dose-dependent manner. The extracts produced good antibacterial activity against gram +ve, gram -ve bacteria.

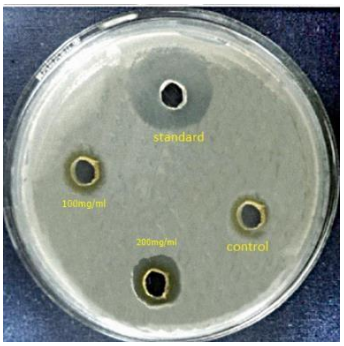
Extracts at the tested concentration have shown significant antibacterial activity compared to standard drugs. All the extracts have produced considerable inhibition against *E. coli* but not against *P. aeruginosa* at a dose of100 mg/ml. The above results demonstrate that the extracts had significant and substantial antimicrobial activity against various pathogens. The results of antimicrobial activity support the folkloric claims regarding the plants and theirmedicinal values.

Anti microbial activity of grangea maderaspatana report

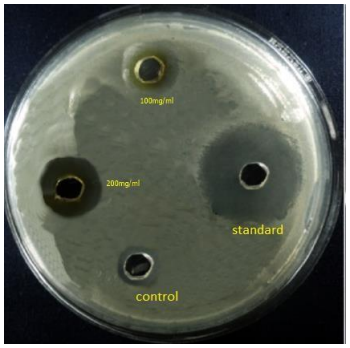
Bacteria	Extract 100mg/ml	Extract 200mg/ml	Negative Control (DMSO)	Standard (tetracycline) 10µg/ml)
B. subtilis	19	24	4	21
S. aureus	17	22	3	22
E. coli	22	25	4	20
P. aeruginosa	14	18	3	22



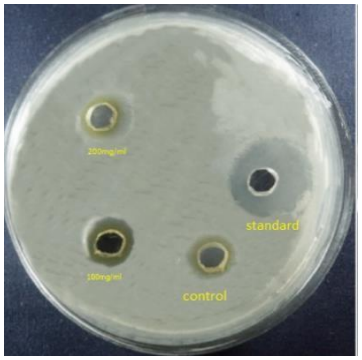
B. Subtulis



S. aureus



E. coli



P. aeruginosa

Agar diffusion method

Disk Diffusion Method 8mL of nutrient agar broth was added to 4 culture vials, respectively. S. aureus, E. Coli, B. subtilis and P. vulgaris were removed from stock plates in 4°C refrigerator using a sterile inoculating loop and subsequently added to individual culture vials. These were placed in 37°C shaking incubator overnight. 800mg of plant material was dissolved into 1mL of 5% DMSO through heating and shaking. It was subsequently diluted to the following concentrations: 100mg/ml, 1500, 200mg/ml,. 15uL of the desired plant sample and 5% DMSO was loaded onto respective 6mm Watmann AA Grade Discs on the day of testing. 5ug tetracycline Sensi Disc were ordered from Becton, Dickinson, and Company (Sparks, MD) and used as a positive control. Using the spectrometer set at 600nm, the bacteria samples were diluted to an absorbance of 0.4A. Nutrient agar plates were divide into four quadrants and inoculated with 500uL of the appropriate diluted bacteria stock. The inoculated Cravens 19 filter paper and positive control was placed in the center of each quadrant. The plates were placed in 30°C or 37°C incubator, depending on the preference of the bacteria samples. The zone of inhibition was measured after 24 hours.

Bacteria	Extract 100mg/ml	Extract 150mg/ml	Extract200mg/ml	Negative Control (DMSO)	Standard (tetracycline) 10µg/ml)
B. subtilis	19	24	28	4	21
S. aureus	17	22	25	3	22
E. coli	22	25	28	4	20
P.aeruginosa	14	18	22	3	22

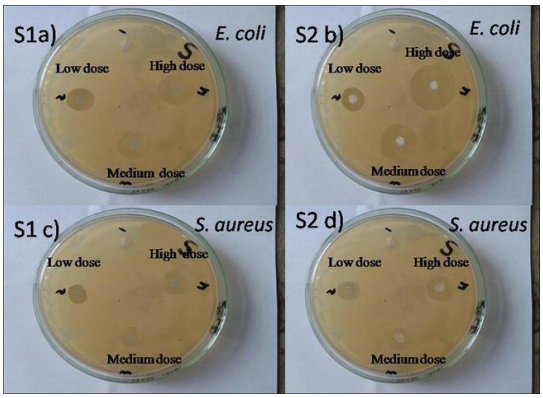


Fig. 15: Reports of antimicrobial activity.

SUMMARY AND CONCLUSION

Based on the reviewed discussions, it is observed that antioxidants total phenolic content and flavonoid content antimicrobial activity are present in the plant extract of madras carpet. This project provides the thesis about the study of antioxidants and total phenolic content and flavonoid content and antimicrobial activity of medicinal plant Madras Carpet.

The current research from the cited literature mainly focuses on the antioxidant activities and antimicrobial activities using different parts of the plant madras carpet. Based on the discussions it can be concluded that we can get the better knowledge of properties of the plant characterisation techniques focused in this study are XRD TEM SAED AND UV Spectroscopy. Different morphology. Crystalline functionality groups were used to get different analyses in terms of parts of plant and applicability as excellent reducing.

Grangea maderaspatana (L.) Poir is widely distributed throughout India. The plant appears to have a broad spectrum of activity on several elments. Various parts of the plant have been explored for antimicrobial activity, antifertility, analgesic, anti-inflammatory, antiarthritic, flavonoid, antioxidant, hepatoprotective, diuretic and antimicrobial activities. It is reported to contain flavonoids, diterepenes, sesquiterpenoids, steroid, and essential oil. The pharmacological studies reported in the present review confirm the therapeutic value of Grangea maderaspatana (L.) Poir. However, less information is available regarding the preclinical study, clinical study, toxicity study, phyto- analytical studies of this plant. With the availability of primary information, further studies can be carried out such as clinical evaluation, phyto analytical studies, toxicity evaluation. The plant is pre-clinically evaluated to some extent;if these claims are scientifically and clinically evaluated then it can provide good remedies and help mankind in various ailments.

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