PHARMACOGNOSTIC, PHYTOCHEMICAL AND PSYCHOPHARMACOLOGICAL EVALUATION OF *OLDENLANDIA CORYMBOSA* AND *GRANGEA MADERASPATANA*

A Thesis submitted to Gujarat Technological University

for the Award of

Doctor of Philosophy

in

Pharmacy

By

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under supervision of

Dr. Vineet C. Jain

GUJARAT TECHNOLOGICAL UNIVERSITY

AHMEDABAD

January - 2018

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ABSTRACT

Oldenlandia corymbosa (Rubiaceae) is a weedy annual herb, found throughout India, commonly known as diamond flower. It is known to clear heat and toxins, activate blood circulation, promote diuresis and relieve stranguria. The plant contains flavonols, phenolic acids, anthocyanidins, irridoids and alkaloids. *Grangea maderaspatana* is a weed growing in sandy lands and waste places, belonging to the Asteraceae family and commonly known as Madras carpet. It is reported to contain flavonoids, diterepenes, sesquiterpenoids, steroid and essential oil.

Both the plants were evaluated for pharmacognostic study which includes macro and microscopic evaluation, determination of physicochemical parameters in a systematic way. HPTLC fingerprinting of both the plant for oleanolic acid and ursolic acid was done. Gallic acid in methanol extract of both plants was estimated by HPLC. The chloroform (200mg/kg, 400mg/kg) and methanol extract (200mg/kg, 400mg/kg) of *Oldenlandia corymbosa* and *Grangea maderaspatana* were evaluated for psychopharmacological activity using different animal models.

Both the plants showed correct taxonomy with specific morphological, microscopical and physico-chemical parameters which is helpful for the standardization of drugs. The extracts of *Oldenlandia corymbosa* and *Grangea maderaspatana* showed presence of terpenes, flavonoids, steroids, phenolics, saponin and carbohydrate. HPTLC fingerprinting confirmed the presence of oleanolic acid and ursolic acid in both the plant extracts. The content of Gallic acid in *O. corymbosa* and *G. maderaspatana* was found to be 2.45% w/w and 4.00% w/w respectively. The chloroform (200mg/kg, 400mg/kg) and methanol extract (200mg/kg, 400mg/kg) of *Oldenlandia corymbosa* and chloroform (400mg/kg) and methanol extract (400mg/kg) of *Grangea maderaspatana* showed psychopharmacological activity in different animal models *viz*, Forced swim test, Elevated plus maze, Hole board test and Spontaneous motor activity using Actophotometer.

The results of present study are encouraging and may be used for the correct botanical identification, authentication of the drug, standardization and also for the development of monograph. The chloroform and methanol extract of *Oldenlandia corymbosa* and *Grangea*

maderaspatana showed psychopharmacological activity due to presence of steroids, terpenes, saponins etc.

Key words: *Oldenlandia corymbosa*, *Grangea maderaspatana*, Psychopharmacological activity, Asteraceae, Rubiaceae.

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Pursuing a Dissertation project is a both afflictive and gratifying experience. It is just like climbing a high peak, step by step, accompanied with acrimony, asperities, frustration, encouragement and trust and with so many people's kind help. When I found myself at the top enjoying beautiful scenery, I realized that it was, in fact, teamwork that got me there. Though it will not be enough to express my gratitude in words to all those people who help me, I would still like to give my many, many thanks to all those people. Words are always poor approximation of what one intends to say.

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Tanvi D. Patel

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List of Abbreviation

ADHD:	Attention deficit hyperactivity disorder
ALP:	Alkaline phosphatase
ALT:	Alanine Aminotransferase
ANOVA:	Analysis of Variance
AST:	Aspartate Aminotransferase
CCl_4:	Carbon tetrachloride
CNS:	Central Nervous System
CPCSEA:	Committee for the Purpose of Control and Supervision of Experiments on Animals
ED50:	The "median effective dose" that produces a quantal effect in 50% of the population that takes it.
ELISA:	Enzyme Linked Immunosorbent Assay
GABA:	Gamma-Aminobutyric acid
GLC:	Gas Liquid Chromatography
HPLC:	High Performance Liquid Chromatography
HPTLC:	High Performance Thin Layer Chromatography
i.p.:	intra peritoneal route of administration
IAEC:	Institutional Animal Ethics Committee
IC50:	50 percentage inhibitory concentration
LDH:	Lactate dehydrogenase
MCF-7:	Michigan Cancer Foundation-7
SEM:	Standard Error of Mean
SGOT:	Serum Glutamic Oxaloacetic Transaminase
SGPT:	Serum Glutamate Pyruvate Transaminase
SSRIs:	Selective Serotonin Reuptake Inhibitors
TLC:	Thin Layer Chromatography
WHO:	World Health Organization
ZOI:	Zone of Inhibition

List of Symbols

% -	Percentage
µ -	Micro
µl -	Microliter
α -	Alpha
β -	Beta
A -	Absorbance
C -	Celsius
g -	Gram
h -	Hour
IU -	International Unit
Kg -	Kilogram
mg -	Milligram
min -	Minute
ml -	Milliliter
nm -	Nanometer
º -	Degree
ppm -	Parts per million
w/v -	Weight by volume
w/w -	Weight by weight

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CHAPTER - 1

INTRODUCTION

CHAPTER 1

Introduction

1.1 HERBAL MEDICINE

Ever since the birth of humanity, there has been a relationship between life, disease and plants. Primitive men started studying diseases and treatments[1]. There is no reflect that people in ancient set interest synthetic medicament for their aliments but they tested to make interest of the things they could gently procure. The most ordinary clothes they could find was there in surrounding i.e. the sapling and animals[2]. They embarked on using plants and establish that the majority of plants were suitable as food, where as other were either poisonous or medicinally useful[3].

By their experience, this knowledge of herbal remedies was transferred to generation as family medicine. So the history of herbal medication is equally old as human history. Most of these plant-derived drugs were originally identified through the subject of traditional remedies and folk knowledge of indigenous people and some of these could not be substituted despite the tremendous progress in synthetic chemistry. Therefore, plants can be depicted as a major source of medicines, not merely as isolated active principles to be doled out in standardized dosage form but also as crude drugs for the population. Modern medicines and herbal medicines are complimentary being used in areas for health care program in various developing countries including India[4].

Herbs had been practiced by all cultures throughout history, but India has one of the oldest, most productive and most diverse cultural living traditions associated with the role of medicinal plants. In the present scenario, the demand for herbal products is growing exponentially throughout the globe and major pharmaceutical companies are currently carrying on extensive research on plant materials for their potential medicinal value[5, 6].

Nevertheless, the folkloric use of crude drugs has been often empirical and is founded on observation from clinical trials without experimental support. The need for exhaustive systemic research into indigenous drugs cannot be overemphasized[4].

Plants have always acted as a major part in the handling of human and animal diseases. World- wide interest in the use of medicinal and aromatic plants is increasing[7]. In spite of the great advances observed in modern medical specialty in recent decades, plants still make an significant donation to health care8. Natural products have been our single most successful source of medicines. Every plant is like a factory capable of blending an infinite number of highly complex and uncommon chemical substances[9].

India delivers very long, safe and continuous usage of many herbal drugs in the officially documented alternative systems of health viz., Ayurveda, Unani, Siddha and Homoeopathy. These systems have fairly existed side - by- side with Allopathy and are not 'in the field of obscurity'[10]. Herbal drugs are regularly used as spices, home- remedies and health foods as well as over -the-counter (OTC) as self -medication or prescribed in the non- allopathic systems[11].

The progression of high - throughput screening and the post - genomic era provided more than 80% of drug substances, which are obtained from natural products or inspired by a natural compound[12]. More than one hundred natural invention resulting compounds are presently enduring clinical trials and around 100 similar projects are in preclinical development[13].

A diffusive number of plants used in the traditional practice have now turn into a part of the modern health overhaul system either as a whole or a product obtained from the plant expedient[14]. A series of natural products isolated from higher plants became clinical agents and are still in use today. Quinine and quinidine from Cinchona bark, morphine and codeine from the latex of *Papaver somniferum*, digoxin from Digitalis leaves, atropine and hyoscine from plants of the Solanaceae family continues to be in clinical use[15].

1.2 Ethnopharmacology

The scientific contemplation of traditional plant medicament can be considered as a major part of ethnopharmacology, a condition introduced in 1967. Ethnopharmacology can be explain as the expert meditation of materials utility by heathen and cultural nest as elixir' and in most token this is synonymous with the meditation of old-fashioned medicines. The influence of traditional plant medicines to isolate active constituents have been significant and some evident instance are isolation of atropine (*Atropa belladonna*), caffeine (*Coffea arabica, Thea sinensis*), digoxin (*Digitalis purpurea*), ephedrine (various species of Ephedra), ergometrine (*Claviceps purpurea*), pilocarpine (*Pilocarpus jaborandi*), reserpine (*Rauwolfia serpentina*) etc[16].

1. 3 A research approach to develop products using ethnopharmacology

The revelation process of herbal products is composed of several steps. The first stage must be the stated use of a naturally occurring material for curative purpose. If there is a sign of a remedial effect, then the material necessarily to be recognized and characterized according to scientific nomenclature. It can then be composed for trial studies followed by biological study associated to the isolation and structure determination of any chemicals which might be amenable for some bioactivity. The active compounds are usually espy by several cycles of fractional process of the extract associated to testing for action of each fraction, until pure compounds are separated from the active fractions. After establishing molecular structure, active compounds serve as the leads for the progression of clinically useful products[17].

Reverse Pharmacology is explained as the science of integrating documented clinical/ experiential hits, into leads by transdisciplinary experimental studies and more emerging these into drug candidates by experimental and clinical examination. The identification of structures with unique biodynamic effects can also lead to an innovative chemical entity trail for drug development. The scope of Reverse Pharmacology is to understand the mechanisms of action at diverse stages of biological organization and to make optimal safeness, effectiveness and acceptableness of the leads in natural products, based on relevant science[17].

There are two discrete forms of research on medicinal plants. In the first segment, the choice of plant is mainly based on their genuine use and reputation in the Indian traditional system of medicine, although in second stage, more extensive base, in which screening of a large number of natural products for biological activity is commenced, irrespective of the circumstance whether these plants are being used by the traditional system of medicine or not[18].

Herbal medicines include herbs, herbal materials, herbal formulations and finished herbal products. Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In several countries, these materials may be treated by various local processes, such as steaming, roasting or stir-baking with honey, alcoholic beverages or other materials.

Herbal preparations are the basis for finished herbal products and may comprise powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are formed by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations prepared by steeping or heating herbal ingredients in alcoholic beverages and/or honey or in other materials. Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term "mixture herbal product" can likewise be applied. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients[19].

1.4 Standardization

As commercialization of the herbal medicine has occurred, certainty of safeness, peculiarity and potency of medicinal plants and herbal products has become an essential issue. The herbal raw material is susceptible to a lot of variation due to some issues, the important ones being the identity of the plants and periodic dissimilarity, the ecotypic, genotypic and chemotypic differences, drying and storage conditions and the existence of xenobiotic[20].

1.5 Guidelines for the standardization of herbal drugs

The guidelines set by WHO:

Botanical characters, sensory evaluation, foreign organic matter, microscopic, histological, histochemical assessment, quantitative measurements, physical and chemical identity, fingerprints chromatography, ash values, extractive values, moisture content, volatile oil and alkaloids tests, quantitative estimation protocols, Estimation of biological activity, the values of bitterness, hemolytic index, swelling index, foaming index, pesticides residues, heavy metals, microbial contamination as total viable count, pathogens such as E.coli, Salmonalla, P.aeroginosa, S.aureus, Enterobacteriaceae, Microbial contamination and radioactive contamination are evaluated[21].

1.6 Chromatographic Fingerprinting and Marker Compound Analysis

A chromatographic fingerprint of a Herbal Medicine is a chromatographic pattern of the extract of certain common chemical components of pharmacologically active and or chemical constituents. This chromatographic contour should be highlighted by the essential attributions of "reliability" and "fuzziness" or "similarity" and "differences" so as to chemically represent the herbal medicine explored. It is proposed that with the help of chromatographic fingerprints acquired, the confirmation and identification of herbal medicines can be precisely conducted (reliability) even if the amount and/or concentration of the chemically characteristic components are not exactly the identical for diverse samples of herbal medicine (hence, "fuzziness") or, the chromatographic fingerprints could validate both the "sameness" and "differences" among several samples magnificently. Hence, we should universally reflect various components in the herbal medicine extracts, and not independently consider only one and/or two marker components for estimating the quality of the herbal medicine products. However, in several herbal medicine and its extract, there are hundreds of anonymous constituents and many of them are in little amount. Furthermore, they are generally occurs variability inside the same herbal materials. Therefore it is very significant to achieve reliable chromatographic fingerprints that characterize pharmacologically active and chemically distinctive constituents of the herbal medicine.

1.7 TLC

Thin layer chromatography is one of the most acceptable and modest chromatographic technique used for separation of compounds. In the phytochemical appraisement of herbal drugs, TLC is being use widely for the following reasons:

1. It facilitates rapid analysis of herbal extracts with minimum sample requirement.
2. It offers qualitative and semi quantitative information of the resolved compounds.
3. It facilitates the quantification of chemical components.

In TLC fingerprinting, the data that can be recorded using a high-performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra and λ max of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample. The data thus generated has a potential application in the designation of an authentic drug, in excluding the adulterants and in upholding the tone and consistency of the drug. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and the absorption spectra with different mobile phases.

Likewise, GLC is used for producing the fingerprint profiles of volatile oils and fixed oils of herbal drugs. Furthermore, the modern methodologies of applying hyphenated chromatography and spectrometry such as High-Performance Liquid Chromatography–Diode Array Detection (HPLC–DAD), Gas Chromatography–Mass Spectroscopy (GC–MS), Capillary Electrophoresis- Diode Array Detection (CE-DAD), High-Performance Liquid Chromatography–Mass Spectroscopy (HPLC–MS) and High-Performance Liquid Chromatography–Nuclear Magnetic Resonance Spectroscopy (HPLC–NMR) could provide the supplementary spectral information, which will be very useful for the qualitative analysis and even for the on-line structural elucidation[22,23].

1.8 HPTLC

HPTLC method is extensively used in the pharmaceutical industry in process development, recognition and detection of adulterants in herbal product and supports in identification of pesticide content, mycotoxins and in quality control of herbaceous plants and health foods[24]. It has been well designated that various samples can run simultaneously by use of a smaller quantity of mobile phase than in HPLC[25]. It has been stated that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated exposure of the chromatogram with the same or different conditions. Subsequently, HPTLC has been explored for simultaneous assay of several components in a multi-component formulation[26]. Through this technique, authentication of various species of plant is also possible[27].

1.9 HPLC

Preparative and analytical HPLC are extensively useful in the pharmaceutical industry for separating and purifying of herbal compounds. There are essentially two types of preparative HPLC: low pressure HPLC (typically under 5 bars) and high pressure HPLC (pressure >20 bar)[28]. The essential factors to be considered are resolution, sensitivity and fast analysis time in analytical HPLC however both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. throughput or recovery in preparative HPLC[29]. In preparative HPLC (pressure >20 bar), larger stainless steel columns and packing materials (particle size 10-30 μm) are required. The examples of normal phase silica columns are Kromasil 10 μm, Kromasil 16 μm, Chiralcel AS 20 μm while for reverse phase are Chromasil C18, Chromasil C8. The objective is to separate or purify compounds, however in analytical work the aim is to acquire information about the sample. This is certainly significant in the pharmaceutical industry of nowadays because fresh products (Natural, Synthetic) have to be taken out to the marketplace as quickly as possible. Because of such a great purification technique, it is possible to save time on the synthesis condition[30, 31,32].

1.10 *Oldenlandia corymbosa*

Oldenlandia corymbosa syn. *Hedyotis corymbosa* (Rubiaceae) is a weedy annual herb, found specifically during rainy season in fields throughout India, Sri Lanka, tropical East Asia to Java and the Phillipines[33]. It is usually identified as "Parppatakapullu" in traditional medicine in Kerala. The plant is known to clear heat and toxins, activate blood circulation, promote diuresis and relieve strangury. It is also known to act on lymphosarcoma and carcinoma of the liver and larynx. It is also active against appendicitis, hepatitis, pneumonia, cholecystesis, urinary infection, cellulites and snake bite. Chinese folk medicine describes the plant to treat skin sores, ulcers, sore throat, bronchitis, gynecologic infections and pelvic inflammatory diseases[34,35,36,37].

It is given in jaundice and other diseases of the liver, heat eruptions, vitiated conditions of pitta, hyperdypsia, giddiness, indigestion, gas, constipation, helminthiasis, leprosy, skin diseases, cough, bronchitis, necrosis, nervous depression caused by deranged bile and hepatopathy. The important preparations of the drug are Amritarishtam, Candanasavam, Mahatiktaghrtam, Jatyadi tailam, Aranyatulasyadi coconut oil etc[38].

Taxonomic classification

Kingdom: Plantae

Phylum: Angiosperms

Class: Dicotyledonae

Subclass: Asteridae

Order: Gentianales

Family: Rubiaceae

Subfamily: Rubioideae

Genus: Oldenlandia

Species: corymbosa

Vernacular Name

In different parts of India *O. corymbosa* is known by different names[39].

Sanskrit: Parpata, Parpataka

English: Diamond flower

Hindi: Daman pappar, Pitpapra

Malayalam: Parpatakapullu, Parpatakam

Geographical distribution: *Oldenlandia corymbosa* is native to Africa and India, but also found throughout Malaysia[40].

Morphology:

It is an annual slender herb up to 40 cm tall. Stem is 4-angled to flat, glabrous and angles are thick to wing. Leaves are simple, sub-sessile or very short petiole. They are linear, narrowly lanceolate or narrowly elliptic. The size of leaf is 0.8-2 cm long and 0.1-0.5 cm wide. The base and apex is acute, margin is entire, secondary veins are not visible, stipules fused to petiole bases.

Inflorescence axillary, usually cymose and contains 2-5-flowers. Pedicels are slender and 2-12 mm long. Calyx is glabrous. Hypanthium portion is subglobose to narrowly ellipsoid. Corolla is white or pink, funnel form to rotate, tube 0.8-1 mm, inside pubescent or glabrous. Fruit capsular, subglobose, ovoid, 1.2-2×1.2-2.2 mm size, dehiscent through flat to broadly rounded apex, beak when present to 0.5 mm, peduncles and pedicels usually extending promptly and prominently as the fruit develops. Seeds are smooth and dark brown[41,42].

1.11 *Grangea maderaspatana*

Grangea maderaspatana is a weed usually known as Madras carpet commonly budding in sandy lands and waste places. It is reported to have flavonoids, diterepenes, sesquiterpenoids, steroid and essential oil. It is a medicinal plant extensively used in the Indian traditional system of medicine for curing several ailments. The herb is worthy for pain in the eyes and ears. The root is an appetizer, astringent to the bowels, diuretic, anthelmintic, emmenogogue, galactogogue, stimulant, beneficial in griping, in troubles of the chest and lungs, headache, paralysis, rheumatism in the knee joint, piles, pain in the muscles, diseases of the spleen and the liver, reduces sweating. The plant is stomachic and uterine stimulant[43].

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Taxonomy of *Grangea maderaspatana* (L.) Poir[44].

Kingdom: Plantae

Subkingdom: Planta Tracheophyta

Subdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida (Dicotyledons)

Subclass: Asteriidae

Order: Asterals

Family: Asteraceae

Synonyms:

Grangea maderaspatana, *G. adansonia* , *Artemesia maderaspatana*

Vernacular name:

Gujarati: Jhinkimundi, Nahanigora, Khamundi

Hindi: Mukhatari, Mustaru

Malayalam: Nelampala

Marathi: Mashipatri

Tamil: Mashipatri

Telugu: Machi-Patri

Urdu: Afsantin

Kannada: Dodda gaadaari

Occurrence and distribution:

It is a weed habitually growing in sandy lands and waste places. It is dispersed all over India, Baluchistan, Ceylon, tropical and subtropical Asia and Africa.

Macroscopical characters[43,45]

It is a prostrate, ascending to erect annual herb, which is up to 55 cm tall, split from base with a taproot. Stems are numerous, prostrate, spreading from the center, 10-30 cm long, haired with soft white hairs.

Leaves: Leaves are numerous, sessile, 2.5-6.3 cm long, sinuately pinnatifid with 2-4 pairs of opposite or subopposite lobes smaller towards the base and largest towards the terminal lobe, margins coarsely serrate-dentate, pubescent on both sides.

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.

Fruits: The fruit is turbinate and compacted while the truncate achene is about 2mm long, smooth and sparingly glandular. The pappus consists of a ciliate cup. The hypocotyls are 2-2.5 mm long. The cotyledons are subsessile and elliptical to widely elliptical while epicotyl is absent.

1.12 Psychopharmacological activity:

Psychopharmacology is the systematic study of the effects of drugs on mood, sensation, thinking and behavior[46]. Psychiatry denotes to a field of medicine focused specifically on the mind, aiming to study, prevent, and treat mental disorders in humans[47]. The condition often co-exists with other chronic ailments that amount to even greater morbidity and mortality rates. According to the WHO, disability due to mental illnesses is greater than cancer and heart disease in developed countries[48].

Public concern on mental health has noticeably increased given the high prevalence of neuropsychiatric disorders. WHO reports approximately 450 million of people suffer by mental or behavioral disorder[49]. Two-thirds of the anxious, depressed or psychotic patients react to the currently available treatments; but their clinical uses are limited by their side effects such as psychomotor injury, potentiation of other central depressant drugs and dependence liability. In the hunt for novel therapeutics for the management of neurological disorders, medicinal plant research has also contributed by demonstrating pharmacological effectiveness of different herbs in various animals models[50,51].

Herbal treatments are gaining emergent attention because of their cost- effective, eco- friendly features and true relief from illness. Since antique tense the herbal remedies are effective in the control of some complaints. Various plants have a folklore claim in the dealing of some dreadful syndromes, but they are not scientifically exploited and/or incorrectly used. Thus, these plant dose demerit particularised contemplation in the luster of neoteric cure[52].

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CHAPTER - 2

REVIEW OF LITERATURE

CHAPTER 2

Review of Literature

2.1 *Oldenlandia corymbosa*

Figure 2.1 – *O. corymbosa* Plant

Botanical name: *Oldenlandia coymbosa*

Synonyms: *Hedyotis corymbosa*

Family: Rubiaceae

Vernacular name:

Sanskrit: Parpata, parpataka, Kshetraparpata

English: Flat top mille grains, Diamond flower, Five leaved fumitory

Hindi: Daman pappar, pitpapra

Bengali: Khet-papra

Gujarati: Parpat, khet-papra

Marathi: Papti, Phapti, khet-papda, paripat

Kannada: Parpatahullu, Kallasabatrasige

Telugu: Verrinella- vemu

Malayalam: Parpatakapullu

Tamil: Parpatagam, kattucayaver, pappanpuntu

Habitat:

Oldenlandia coymbosa is an annual herb distributed in the tropical and subtropical region of the world.

Description:

Oldenlandia corymbosa is an annual, terrestrial, dichotomous, slender ascending herb growing up to 50 cm. The leaves are 1.3 – 2 cm by 0.8 -3 mm, the lower leaves are often broader than upper ones, linear, acute, glabrous, usually with recurved margins. Flowers are white in pairs or in threes, usually on solitary axillary peduncles longer than the calyx. Fruits are loculicidal capsules, globose and the seeds are minute, pale brown, angular, testa teticulate[1].

Taxonomy classification:

Kingdom : Plantae

Phyllum : Angiosperms

Class : Dicotyledonae

Subclass : Asteridae

Order : Gentianales

Family : Rubiaceae

Subfamily : Rubioideae

Genus : Oldenlandia

Species : corymbosa

Ethnopharmacological information:

- The plant is reported to have immunopotentiation activity and in China, it has been used to treat some tumors[2].
- It is considered as a cooling medicine in the treatment of fever caused by deranged air and bile and also treats remittent fever with gastric irritability and nervous depression.
- In Konkan, the juice is applied to cool the burning sensation felt in the palms of the hand and soles of the feet. Internally, the juice is given with a little milk and sugar to cool the burning pit of the stomach. The decoction is used in remittent fever, heat eruptions and also applied to the surface of the body. The plant extract is used in jaundice and as an anthelmintic. The plant is used as a febrifuge in Indo China[3].

Phytochemical review

The chemical constituents reported in different parts of *Oldenlandia corymbosa* are mentioned beneath.

- Different phytochemical studies shows the presence of proteins, carbohydrates, phenols, tannins, flavanoids, saponins, steroids, terpenoids and glycosides. Some of the isolated compounds from whole plants are Geniposide, iridoid glycosides, 6 alpha – hydroxygeniposide, scandoside methyl ester (6 beta - hydroxygeniposide), 10-o-benzoylscandoside methyl ester, asperulosidic acid, asperuloside, deacteylasperuloside, 10-o-p-hydroxy benzoylscandoside methyl ester, rutin and (+)- lyoniresinol-3-alpha -o-beta glucopyranoside[4].
- The plant also contains ursolic acid, oleanolic acid and γ-sitosterol. The air dried plant contains 0.12% of alkaloids – bifloron and biflorin, these two alkaloids are interconvertible. It also contains 13.55% of inorganic ash, which is mainly responsible for its cooling effect[5].
- An aqueous extract of the plant yielded a polysaccharide, composed of rhamnose, arabinose, zylose, mannose, galactose and glucose[2].
- The methanol extracts of *Oldenlandia corymbosa* showed the presence of flavonols such as Quercetin, 3‟-Methoxy quercetin and 3‟, 4‟-Dimethoxy quercetin. Phenolic acids like vanillic, syringic acid, melilotic acid, p-hydroxy benzoic, p-coumaric,

ferulic and caffeic acids are also present. Anthocyanidins like cyanidin and pelargonidin are present. Irridoids and alkaloids are also present[6].

Pharmacological activity

Pandey et al., 2012 demonstrated the anticancer activity of ethanol extract of the leaves of *Oldenlandia corymbosa* on k562 human leukemia cell lines. The cell viability was measured by SRB (sulforhodamine B) assay. The cell lines were grown under RPMI1640 medium containing 2 mML-glutamine, 10 % fetal bovine serum. The results were recorded on an ELISA plate reader at 540 nm to 690 nm wavelength. The nontoxic dose of *Oldenlandia corymbosa* showed anticancer activity as compared to the standard drug Adriamycin[7].

Endrini et al., 2011 also demonstrated the anticarcinogenic property of methanol extract of the whole plant by Microculture tetrazolium salt (MTT) assay on the MCF-7 human breast carcinoma dependent hormone cell lines. The highest anticancer activity on MCF-7 cell line observed with IC 50 value of 22.67 µg/ml. The anticancer activity of the plant extract is mainly due to its antioxidant activity[8].

Rathi et al., 2009 evaluated hepatoprotective activity against Perchloroethylene, CCl_4 and D-Galactosamine induced liver damage in experimental animals. Ethanol extract of *Oldenlandia corymbosa* was studied for hepatoprotective activity on perchloroethylene induced hepatic damage in Wistar albino female rats. The extract was administered orally at the dose of 400 mg/kg of body weight for ten days, showed significant reduction in liver marker enzymes (AST, ALT, LDH), lipid peroxidation and with a significant increase in antioxidant enzyme levels. The results show potent hepatoprotective activity upon perchloroethylene induced hepatic damage in rats and also have anti lipid peroxidative and free radical scavenging activities[9].

Chimkode et al., 2009 also assessed hepatoprotective activity of ether, ethanol, butanol, butanone, petroleum ether and ethyl acetate extract fraction of *Oldenlandia corymbosa* against CCl_4 induced hepatic damage in albino rats. An acute toxicity study was carried out in albino rats of either sex for determining LD 50 values for different extracts. The

petroleum ether and ethyl acetate extract does not show any significant hepatoprotective activity. The elevated levels of SGPT and SGOT were significantly decreased in ether and butanol extracts at $P < 0.001$ and in butanone and ethanol at $p < 0.005$. The enzymatic levels and histopathological studies showed that ether, butanol, ethanol, butanone extracts of *Oldenlandia corymbosa* have hepatoprotective activity in CCl_4 induced hepatic damage[10].

Gupta et al., 2012 reported antihepatotoxic activity of methanol extract of *Oldenlandia corymbosa* against D-Galactosamine induced hepatotoxicity in Wistar rats. The extract significantly reduced increased levels of marker enzymes with D-galactosamine (AST, ALT, ALP, γ-glutamyltransferase) and showed the significant reduction in lipid peroxidation at the dose of 200 mg/kg[11].

Agrawal et al., 2013 evaluated alcoholic and aqueous extract of whole plant of *Oldenlandia corymbosa* for antiulcer activity against aspirin in rats. The extracts were administered in two doses 200 mg/kg and 400 mg/kg by oral route 45 minutes prior to the administration of aspirin. The drug lansoprazole 8 mg / kg was used as standard. Both the extracts showed significant decrease in ulcer compared to control group which is characterized by reduction in ulcer index, gastric volume, free acidity, total acidity and pH. The percentage protection in alcoholic and aqueous extract at 200 mg/ kg, 400mg/kg showed 65.7%, 33% respectively in comparison with standard lansoprazole 88.89%[12].

Sasikumar et al., 2010 studied antioxidant activity of methanol extract of aerial parts of *Oldenlandia corymbosa* by different in vitro methods such as; 1,1 diphenyl-2-picryl hydroxyl (DPPH) assay, 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) cation decolorization test, ferric reducing power (FRP), scavenging capacity towards hydroxyl ion (OH.) radicals and nitric oxide (NO) radical inhibition assay. The extract showed high antioxidant activity against DPPH, ABTS, Nitric oxide and hydroxyl radical at 82, 130, 150, 170 µg/ml respectively. The study showed that methanol extract effectively attenuates the oxidative stress via antioxidant property[13].

Fatema et al., 2014 demonstrated analgesic activity of ethanol extract of *Oldenlandia corymbosa* in mice using three different models; hot plate reaction time, acetic acid writhing test and formalin induced pain method, with ketorolac as standard drug. The extract was administered two doses 200 mg/kg and 400 mg/kg by oral route. Formalin test procedure revealed the involvement of both peripheral and central mechanisms. The acetic acid writhing test involved the peripheral mechanism and the hot plate method involves the central mechanism. The extract showed a significant dose dependent anti nociceptive activity[14].

Mishra et al., 2009 evaluated Antimalarial activity of the methanol extract of *Oldenlandia corymbosa* by both in vitro and invivo methods. The extract showed significant antimalarial activity on chloroquine sensitive (MRC-pf20) and chloroquine sensitive (MRC-pf. 303) strains of Plasmodium falciparum. The in-vivo antimalarial activity of the extract was studied using mice. Drug treatment was initiated 1 day (24 hour) prior to the parasite treatment starting from 4th day post infection. Every alternate day, the blood was collected from tail to check the level of parasitemia. The combination of plant extract with curcumin showed more effective antimalarial activity[15].

Hussain et al., 2013 studied antibacterial activity of methanol extract of *Oldenlandia corymbosa* by disc diffusion method against gram positive and gram negative bacteria (Bacillus, Klebisella, Escherichia coli, Proteus, Staphylococcus aureus and Pseudomonas). The extract significantly inhibited the growth of both gram positive and gram negative bacteria and has a broad spectrum of antibacterial activity. The order of inhibition was found to be Proteus (22mm) < Pseudomonas (26mm) < Bacillus (27mm) < Staphylococcus aureus (28mm) < Escherichia coli (32mm) < Klebsiella (33mm)[16].

Hussain et al., 2013 assessed antifungal activity of whole plant extract of *Oldenlandia corymbosa* against Candida albicans and Aspergillus nigar. The maximum antifungal activity was found in Candida albicans. The activity was due to the presence of the constituents like steroids and glycosides[16].

Nikolajsen et al., 2011 evaluated the effect of ethanol extract of *Oldenlandia corymbosa* in the isolated uterine horn preparation of virgin female Sprague Dawley rat. The extracts were tested in different concentration 0.014, 0.14, 0.44 and 1.40 mg/ml. The De Jalon solution was used as the physiological solution and the response was compared against the standard (acetylcholine) and blank (ethanol). The extract showed significant uterine contraction[17].

2.2 *Grangea maderaspatana*

Fig. 2.2 *G. maderaspatana* plant

Botanical name: *Grangea maderaspatana*

Synonyms: *Grangea adansonia*, *Artemesia maderaspatana*

Family: Asteraceae

Vernacular name:

English: Madras carpet

Gujarati: Jhinkimundi, Nahanigora, Khamundi

Hindi: Mukhatari, Mustaru

Malayalam: Nelampala

Marathi: Mashipatri

Tamil: Mashipatri

Telugu: Machi-Patri

Urdu: Afsantin

Kannada: Dodda gaadaari

Habitat: Madras Carpet is an annual herb commonly seen in flat bunches in harvested fields, dry river and pond beds.

Description:

Grangea maderaspatana 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. The stems are prostrate, spreading from the centre, 10-30 cm long, hairy with soft white hairs. Leaves are 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 largest terminal lobe, all coarsely serrate-dentate, pubescent on both surfaces, oblong or oblanceolate[18,19,20].

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[18,20,21].

Taxonomy classification:

Kingdom: Plantae

Subkingdom: Planta Tracheophyta

Subdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida (Dicotyledons)

Subclass: Asteriidae

Order: Asterals

Family: Asteraceae

Ethnopharmacological information:

- The herb is antipyretic and good for pain in the eyes and ears.
- The root is an appetizer, astringent to the bowels, diuretic, anthelmintic, emmenogogue, galactogogue and stimulant. They are useful in griping, in troubles of the chest and lungs, headache, paralysis, rheumatism in the knee joint, piles, pain in the muscles, diseases of the spleen and the liver, troubles of the ear, the mouth and the nose; lessens perspiration (Unani).
- Plant is stomachic and uterine stimulant. Infusion of the leaves with ginger and sugar added is used in dyspepsia, hysteria and obstructed menses.
- Externally it is useful as an anodyne and antiseptic for inflamed and painful parts. The powdered leaves are applied to wounds and ulcers as an antiseptic. Juice of the fresh leaves is instilled into the ear for earache[21].

Phytochemical 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[22].
- Eight new clerodane diterpenes including five clerodane, a nor clerodane, a secoclerodane and a norseco clerodane derivatives along with auranamide were also isolated[23,24,25].
- A clerodane derivative, 15-hydroxy-16- oxo-15,16H-hardwickiic acid has been isolated from the aerial parts of *G. maderaspatana*[26].
- Three components viz., eudesmanolide, (-) frullanolide, (-) -7-alpha-hydroxyfrullanolide and a new eudesmanolide (+) -4 alpha, 13-dihydroxyfrullanolide have been isolated from the whole plant of *G. maderaspatana* . A new eudesmanolide was named (+) – Grangolide[27].
- 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*[28].

- Two new 5-deoxyflavones, 6-hydroxy-2′,4′,5′-trimethoxyflavone, 6-hydroxy-3′,4′,5′-tri -methoxyflavone and a known flavone, 7,2′,4′-trimethoxyflavone have been isolated from the whole plant of *Grangea maderaspatana*[29].
- 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, (-)-hy-droxyfrullanolide and (+)-grangolide[30].
- A new diterpenoid has been isolated as 8-hydroxy- 13 E -labdane-15yl-acetate from the acetone extract of *Grangea maderaspatana*[31].
- 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%)[32].

Pharmacological activities:

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 antinidational activity only if the drug was administered from days 1-3 and 7-9 post coitum[33].

Ahmed et al., 2001 evaluated Analgesic activity of methanol extract of *Grangea maderaspatana* (1 and 3 g/kg, p.o.) in acetic acid induced writhing in mice. The extract significantly and dose-dependently inhibited writhing in mice. The lower dose (1 mg/kg, p. o.) found to as effective as aminopyrine (50 mg/kg, p.o.) which was used as a reference[34].

Rachchh et al., 2013 also assessed Analgesic activity of methanol extract of the plant (500 mg and 1 g/kg, p.o.) by tail flick model. The plant extract in both dose significantly increased latency for tail flick indicated analgesic activity[35].

Ruangrungsi et al., 1989 evaluated cytotoxic activity of crude chloroform extract of Grangea maderaspatana in the KB cell culture assay. The extract exhibited strong cytotoxic activity (ED50=2 μg/ml)[27].

Patel et al., 2009 evaluated the antioxidant activity of the methanol extract 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 acivity depends on concentration and increased with increasing amount of the extract. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the extract[36].

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)[32].

Omhare et al., 2012 identified that the aqueous and ethanol extract (250 mg/kg, 500 mg/kg, p.o.) of *Grangea maderaspatana* Poir. effectively inhibited CCl_4 and paracetamol induced changes in the serum marker enzymes (SGOT, SGPT and ALP) in a dose-dependent manner as compared to the normal and the standard drug silymarin treated groups. Hepatic steatosis, hydropic degeneration and necrosis observed in CCl_4 and paracetamol treated groups were completely absent in histology of the liver sections of the animals treated with the extracts. The results suggest that the ethanol extract of *G. maderaspatana* possess significant hepatoprotective activity[37].

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 agar well diffusion method. The zone of inhibition (ZOI) values of the oil was in the range 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 Aeromonas hydrophila and Klebsiella pneumoniae[32].

Rachchh et al., 2013 evaluated Anti-inflammatory activity of methanol extract of *G. maderaspatana* (1000 mg/kg, p.o.) using acute model of carrageenan induced rat paw edema. Indomethacin was used as standard in this model. The extract showed significant protection against carrageenan induced rat paw edema indicating its anti-inflammatory activity[35].

Rachchh et al., 2013 also evaluated Antiarthritic activity of methanol extract of *G. maderaspatana* (1000 mg/kg, p.o.) using Complete Freund's Adjuvant (CFA) induced arthritis in rats. Dexamethasone was used as a standard in this model. The degree of arthritis was evaluated by hind paw swelling, body weight changes, erythrocyte sedimentation rate, rheumatoid factor, Creactive protein and arthritic index supported by histopathology of ankle joints. The extract treatment declined CFA induced rise of erythrocyte sedimentation rate, rheumatoid factor, Creactive protein significantly in rats. Histopathological study of ankle joint revealed that extract inhibited edema formation and cellular infiltration induced by CFA[35].

Ahmed et al., 2001 assessed diuretic activity of *Grangea maderaspatana* (L.) Poir[38].

Omhare et al., 2012 evaluated Acute oral toxicity by following Organization of Economic Co- operation and Development (OECD) guidelines 420- Fixed Dose Procedure (FDP). Results indicated that the aqueous and alcohol extract of *G. maderaspatana* up to a dose of 2000 mg/kg; p.o. did not produced any mortality[37,39].

2.3 Basics of Psychopharmacology

Psychopharmacology is the systematic study of the drugs effect and their effects on mood, sensation, thinking and behavior[40]. At the heart of Psychopharmacology lie two important things; psychoactive drugs and mental sickness as a clinically diagnosed disorder. Psychopharmacology refers to the study of drugs, pharmakon, that influence the human mental state, psyche, and behavior. It is a medical condition that disrupts a person's thinking, feeling, mood, ability to connect to others and daily operation[41].

Mood disarrangement are described by a distress in the regulation of mood, conduct and affect. They are segmented into Depressive disorders, Bipolar disorders and Depression in association with medical illness[42]. Major despair is a collective condition that lasts to result in extensive morbidity and mortality despite major advances in treatment[43].

The occurrence of sadness in common people is assessed to be around 5%. Currently 121 million individuals are expected to suffer from depression. As per assessed value, 5.8% of men and 9.5% of women are suffering by a depressive event in their lifespan. Suicide is one of the most common outcomes of depression[44]. According to the World Health report (WHO, 2001), roughly 450 million community suffer from a emotional or behavioral distemper, yet only a small minority of them get even the most fundamental treatment. This amounts to 12.3% of the broad overload of illness, and will increase to 15% by 2020[45].

Mental disorders have become highly prevalent due to ambitious lifestyle, urbanization, and stressful environment. Psychosis is a one of the most debilitating, complex, and costly illness. The meaning of "psyche" is mind or soul, and word "-osis" corresponds to an abnormal condition in Greek. Hence, psychosis is often described as involving a "loss of contact with reality." These illnesses alter a person's ability to think clearly, make good judgments, respond emotionally, communicate effectively, understand reality and behave appropriately. It is characterized by three general types of symptoms: Positive symptoms, negative symptoms and cognitive symptoms. Positive symptoms refer to a loss of contact with reality and comprise of hallucinations, delusions, bizarre behavior and positive formal thought disorders. Negative symptoms refer to a diminution in or absence of normal behaviors and include flat

affect, alogia, avolition, and anhedonia. Cognitive symptoms manifest as deficits in attention, learning, memory, concentration, and executive functions[46].

Overview of mechanisms of action

Psychopharmacology is very complex and extensive division of medicine with roots in the mechanisms of action of psychotropic drugs. Generally, the mechanism of action of drugs is largely due to pharmacodynamic factors. On the other hand, the onset, duration and magnitude of drug action are determined by pharmacokinetic factors.

Psychotropic drugs are amphiphilic in nature i.e. they possess both hydrophilic and hydrophobic properties. Because of this physical property, psychotropic drugs rapidly reach their sites of action. Psychotropic drugs either permeate through plasma membrane (hydrophilic) or build up in the hydrophobic interior of lipid bilayer of cell membranes[47].

Neurotransmitters

Neurotransmitters are endogenous chemicals in the human body that are responsible for the transmission of nerve impulses between neurons and target cells across a synapse. For a signal to get transmitted across, an optimum amount of neurotransmitters in the synaptic space must be present[48]. In mentally healthy individuals, there is a balance between the amount of neurotransmitters in the synaptic space and in the presynaptic neuron. It is the disruption of this balance that leads to mental and metabolic disorders affecting sleep, mood, weight, etc[49]. Some of the important neurotransmitters implicated in psychopharmacology are acetylcholine, serotonin, dopamine, norepinephrine, epinephrine, glutamate and GABA.

Acetylcholine

Acetylcholine is used to regulate muscle movement. Its cholinergic neurons are found all over the CNS, especially the brain, where it is involved in numerous functions such as pain perception, neuroendocrine regulation, REM regulation and memory and learning formation. Damage to the cholinergic system is an important pathology implicated in Alzheimer's disease.

Norepinephrine

Norepinephrine is the neurotransmitter that plays an important role in conditions related to stress. Along with epinephrine, it enables the body to "fight or flight" in emergencies by stimulating the heart rate, blood circulation and respiration to compensate for the increased oxygen requirement of the muscles.

Dopamine

Dopamine is synthesized from the amino acid, tyrosine. Tyrosine is converted to dopamine by the action of enzymes, tyrosine hydroxylase and L-amino acid decarboxylase, respectively. Deficiency of dopamine in the brain is implicated in the pathology of Parkinson's disease.Overactivity of same dopamine will cause Pyschosis (schizophrenia)[50].

Serotonin

The main function of serotonin is regulation of mood, appetite, sleep, cognition, and blood coagulation. The most widely prescribed and efficacious antidepressants, selective serotonin reuptake inhibitors (SSRIs), and older antidepressants such as tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), act on the serotonergic system by inhibiting serotonin reuptake into the presynaptic vesicle.

Glutamate

Glutamate is the primary excitatory neurotransmitter in the brain. An injury to a nerve (e.g. brain injury) results in its release and excessive concentration in the extracellular space, leading to excitotoxicity[51]. Excess extracellular glutamate may lead to excitotoxicity in vitro and in vivo in acute insults like ischemic stroke via the overactivation of ionotropic glutamate receptors. In addition, chronic excitotoxicity has been hypothesized to play a role in numerous neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer's disease and Huntington's disease[52]. Excessive glutamate levels in the brain is a precursor to psychosis in individuals at high risk for developing schizophrenia[53].

GABA

The inhibitory neurotransmitter GABA is synthesized from the amino acid, glutamate, by the enzyme glutamate decarboxylase in the GABAergic neurons[54].

Psychotropic drugs exert their pharmacologic action primarily by agonism or antagonism of neurotransmitter receptors, inhibition of regulatory enzymes or blockade of stimulators of neurotransmitter membrane transporters.

Table 2.1: General mechanisms of action of psychoactive drugs

General mechanism of actions of psychotropic drugs	**Examples**
Synthesis and storage of neurotransmitters	L-Dopa
Release of neurotransmitters from presynapse	Zolpidem, benzodiazepine
Blockade of receptors	Tricyclic antidepressants
Breakdown of neurotransmitters	MAO inhibitors, amphetamines
Reuptake of neurotransmitters	SSRIs
Transduction of G-proteins	Phenothiazines, butyrophenones
Effector system	Antidepressants

Pharmacological treatment of Psychosis

The anti-psychotic drugs are also termed as neuroleptic drugs, or neuroleptics, which is derived from Greek in which neuro refers to the nerves and lept means "to take hold of ". Thus, the word neuroleptic means "taking hold of one's nerves." Antipsychotic agents are the cornerstone of acute and maintenance treatment of schizophrenia and are effective in the treatment of hallucinations, delusions, and thought disorders. Antipsychotic medications are commonly classified into two categories: First generation (typical) and second generation (atypical)[55].

The typical antipsychotics are classified according to their chemical structure while the atypical antipsychotics are classified according to their pharmacological properties. The major difference between the two types of antipsychotics is that the first generation drugs block dopamine and the second generation drugs block dopamine and also affect serotonin levels. Although atypical antipsychotics are generally considered to be more effective and to have reduced side-effects compared to typical antipsychotics. Evidence suggests that some of the second generation drugs have milder movement related side effects than the first generation drugs[56].

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide medicinal activities, higher safety margins, and lesser costs. Dietary supplements along with herbal medicines may improve symptoms of psychosis.

However, the management and treatment plans may include one or more of these interventions[41]:

1. Psychopharmacologic treatment
2. Psychotherapy approaches
    - Brain stimulation
    - Institutionalization / rehabilitation programs
    - Psychodynamic therapy
    - Cognitive-behavioral therapy
    - Group therapy
    - Family intervention
    - Social rhythm therapy
3. Self-care

Other interventions and services may include[57]:

1. Employment assistance
2. Housing assistance
3. Reintegration measures into society
4. Psychosocial rehabilitation
5. Assertive community treatment

Additionally, several healthcare personnel may be involved in the execution of the management and treatment plans such as:

1. Family clinician
2. Psychotherapist
3. Psychiatrist
4. Pharmacist
5. Social worker
6. Family members

Classification of Psychotropic drugs

In a medical context, psychotropic drugs refer to a class of prescription medications that primarily exert their therapeutic effects on the central nervous system. Whether taken orally or administered intravenously, psychotropic drugs are absorbed by the blood and transported into the brain. They pass through the protective membrane, the blood brain barrier (BBB) and into the brain circulation.

Psychotropic drugs, on the other hand, are formulated especially to cross the BBB and act directly on the brain to alter perception and mood, induce behavioral changes and affect consciousness along with cognition[58]. The basic purpose of these drugs is to bring about the desired changes in mood and behavior to treat and manage psychiatric disorders.

Psychotropic medications are generally categorized into the following:

- Antipsychotics
- Antidepressants
- Anxiolytics
- Mood stabilizers
- Prescription stimulants
- Sedative-hypnotics
- Miscellaneous drugs (e.g. herbal supplements)

Antipsychotics

This subgroup contains a large number of medications that are used to treat psychosis. Psychosis is a generic term that encompasses disorders resulting from abnormal perception of reality accompanied by a defective insight. Psychotic patients primarily experience these two characteristics:

- Hallucinations: Sensory perceptions without an actual stimulus being present
- Delusions: False beliefs about reality

Antipsychotics are used in the treatment of mental illnesses such as schizophrenia, bipolar disorder, delusional disorders, and also wide range of non-psychotic disorders such as Tourette syndrome, autism, and dementia[59].

Antidepressants

Antidepressants comprise a wide variety of drugs that are basically indicated to treat the various symptoms of depressive disorders. However, many off label indications for using antidepressants also exist and conditions such as anxiety, sleep disorders, obsessive compulsive disorders, eating disorders, neuropathic pain, ADHD, migraines and substance abuse benefit from its use[60].

Anxiolytics and sedatives

Anxiolytics, as the name suggests, are medications that are used to curb anxiety. Tricyclic antidepressants and monoamine oxidase inhibitors also relieve anxiety but are rarely prescribed because of their extensive side effect profile. Barbiturates and benzodiazepines exhibit dose-dependent effects on the CNS, i.e. the higher the dose, the deeper the sedation-anxiolysis-anesthesia on the CNS. Benzodiazepines are primarily used for panic disorders and generalized anxiety disorder[61].

Mood stabilizers

Mood stabilizers are a group of antipsychotic medications that are primarily used to treat the symptoms associated with mood shifts in bipolar disorder, schizoaffective disorders, and sometimes even borderline personality disorders. The main purpose of the drug is to stabilize the intense mood shifts between depressive and manic episodes. The classic drug in this category is lithium carbonate[62].

Stimulants

Stimulants are psychoactive drugs that elevate mood and improve physical and mental functioning for a temporary period of time. They are used worldwide as prescription drugs and also have been widely abused as recreational substances. Essentially, stimulants increase brain activity within the central nervous system and peripheral nervous system. They are used to treat lethargy, obesity, excessive appetite, narcolepsy, and improve concentration in ADHD patients.

There are many type of stimulants i.e. ampakines, amphetamine related substances, eugeroics, norepinephrine reuptake inhibitors (NERIs), norepinephrine dopamine reuptake inhibitors (NDRIs), xanthine and caffeine-related drugs. Each type has a unique mechanism of action[63].

Sedatives / hypnotics

Sedatives or tranquilizers are a group of drugs that induces sleep by decreasing the excitatory mechanisms of the brain. Many of the drugs mentioned above have sedative effects, namely benzodiazepines. Barbiturates and antihistamines can all act as sedatives. Sedatives, when used prior to medical surgeries, are called sedative-hypnotics because their effects on the CNS are dose-dependent i.e. at lower doses; they may act as anxiolytics but at higher doses, can induce unconsciousness. They are used to induce sleep and are adjuncts to general anesthesia[64].

Miscellaneous drugs: complementary, herbal and over the counter

In the last 10 years, herbal formulations have been gaining popularity in the U.S. for the treatment of psychiatric disorders. These supplements are widely purported by their manufacturers to exhibit fewer and lighter side effects compared to their counterparts that require prescription. The herbal supplement, St. John's Wort, is one such example. It is obtained from the flowers and leaves of the herb, Hypericum perforatum. It is known by a number of other names including Tipton's weed, rosin rose, Amber, Amber Touch-and-Heal, goatweed, and Klamath weed.

Several studies propose the significant role of St. John's Wort in the treatment of mild to moderate depression [65]. Some users have also reported experiencing therapeutic benefits in the treatment of anxiety and related disorders.

Overview of newer vs. older psychotropic medications

The 19th and 20th centuries saw the emergence of psychotropic drugs that were initially used to treat other medical conditions. Bromides were introduced in 1857 as an anticonvulsant, and the oldest group of depressants, barbiturates, in 1912 for insomnia. These two groups of drugs were found to have sedative effects. Other drugs soon emerged such as amphetamines for depression and lithium for agitation in manic states. The first antipsychotic, chloropromazine, was first studied for its sedative properties in anesthesiology. Tricyclic antidepressants and monoamine oxidase inhibitors became the standard of treatment for depression in the 1950s. The most widely prescribed anxiolytics today, benzodiazepines, were introduced in the 1960s[66].

Animal model review

Forced swim test:

Purpose and rationale

Forced swim test was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression[67].

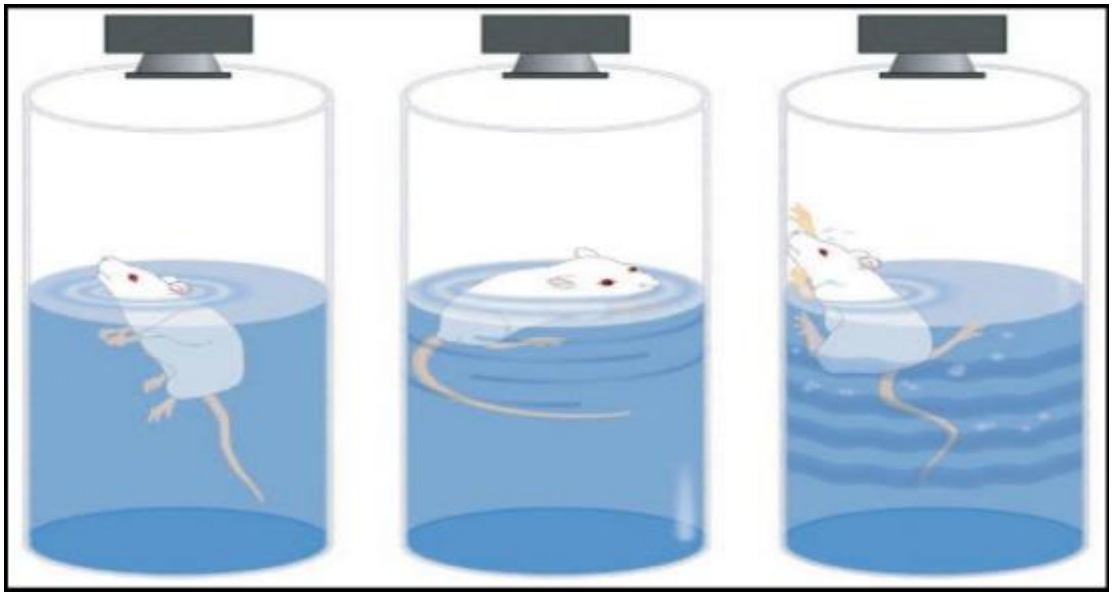

Figure 2.3 Forced Swim Test Instrument

Procedure

Rats of any sex were independently forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), comprising 19 cm of water at 25 ± 1º C. All the rats were divided in different groups. The total duration of immobility was recorded during the last 6 min of the 10-min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative off an antidepressant like effect[68].

Evaluation

Duration of calmness is assessed in different groups of animals. Antidepressant drugs, but also stimulants like amphetamine and caffeine, reduce duration of immobility[69].

Hole-board test

Purpose and rationale

The evaluation of certain components of behavior of mice such as curiosity or exploration has been attempted by Boissier et al. (1964)[69] and Boissier and Simon (1964)[70]. They used an open field with holes on the bottom into which the animals could poke their noses. The " hole-board" test has become very well accepted and has been altered and automatized by many authors.

Procedure

Mice of any sex having weight between 18 and 22 g are used. The dimension of hole-board is 40× 40 cm. There are 16 holes with a diameter of 3 cm each are scattered uniformly on the floor. The board is raised so that the mouse poking its nose into the hole does not see the bottom. Nose-poking is assumed to specify curiosity and is measured by visual observation. Generally, six animals are used for each group. After 30 min. the extract is administered and the first animal is placed on the hole-board and evaluated for 5 min.

Critical assessment of the method

The nose poking into a hole is a characteristic behavior of mice which specify certain degree of curiosity. Assessment of this type of activities has been demonstrated to be relatively beneficial. Benzodiazepines have a tendency to decrease nose-pocking at somewhat small doses.

Elevated plus maze test

Purpose and rationale

Out of various possibilities to alter maze tests (e.g. water maze (Danks et al. 1991)[71], the Y-maze, the radial maze (Di Cicco 1991)[72], and the elevated plus maze (Montgomery 1958)[73]; Pellow et al. 1985)[74]; Corbett et al. 1991)[75] have found recognition in various laboratories. The test has been recommended for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, decrease anxiety, increase the open arm exploration time while anxiogenic compounds have the reverse effect.

Procedure

The EPM consists of two open arms, 50× 10 × 40 cm, and two enclosed arms, 50 × 10 × 40 cm, with an open roof, organized so that the two open arms are opposite to each other. The maze is elevated to a height of 50 cm. The mice are divided into different groups having 6 mice for each dose. After 30 min. of administration of test or standard, the mouse is kept in the center of the maze, facing any of the enclosed arms. For the duration of a 5 min.the following measures are taken: the no. of entries into and time spent in the open and enclosed arms. If possible, the activity is assessed in a sound attenuated room.

Critical assessment of the method

The method is relatively time consuming, but can be considered as a reliable degree of anxiolytic activity. Currently computerized automatic systems are available to overcome these problems.

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CHAPTER – 3

MATERIAL & METHODS

CHAPTER 3

Material & Methods

3.1 Collection and Identification of Plant material

Plant collection and Identification

The plant of *Oldenlandia corymbosa* was collected in the month of September from the Botanical garden of M.S.U, Vadodara, Gujarat. The plant of *Grangea maderaspatana* was collected in the month of December from Saputara, Gujarat. Both the plants were identified and authenticated by the taxonomist of Botanical Survey of India, Jodhpur and a voucher specimens were deposited at BSI, Jodhpur.

Both the plants were dried under shade separately. Air dried plant material was ground to #10 powder and the plant materials were then used for further investigations.

3.2. Assessment of quality of plant materials

The plant materials were assessed as per WHO guideline[1, 2].

3.2.1 Determination of foreign matter

Both the plants were subjected to determination of any contamination by mould or insects and other animal contamination.

3.2.2 Macroscopic evaluation

Fresh plant parts of *Oldenlandia corymbosa* and *Grangea maderaspatana* were subjected to color, odor and taste, determination of shape, size, surface characteristics and appearance etc[3].

3.2.3 Microscopic evaluation

For microscopical examinations, free hand sections of the fresh leaf, stem and root of *Oldenlandia corymbosa* and *Grangea maderaspatana* were cut, cleared with chloral hydrate solution and water, and stained with a drop of hydrochloric acid and phloroglucinol. Photomicrographic images were taken by using Trino CXR camera[4,5,6].

3.2.3.1 Powder Microscopy

Dried powder of *Oldenlandia corymbosa* and *Grangea maderaspatana* were treated with chloral hydrate and were stained with phloroglucinol: HCl (1:1). On slide, a drop of glycerin was placed and covered it with cover slip and observed under microscope.

3.2.3.2 Quantitative microscopy[5]

Leaves of *Oldenlandia corymbosa* and *Grangea maderaspatana* were subjected to quantitative microscopy for the following values using reported method.

- Stomatal index
- Palisade ratio
- Vein islet number
- Vein termination number

3.2.4 Proximate analysis

Proximate analysis of powdered plant material of *Oldenlandia corymbosa* and *Grangea maderaspatana* was carried out using reported methods[1,2].

Following determinations were done

- Loss on drying
- Total ash

  Acid insoluble ash

  Water soluble ash
- Extractive value

  Alcohol soluble extractives

  Water soluble extractives
- Foaming index

3.2.4.1 Loss on drying:

Placed about 2 gm of the air dried plant material, accurately weighed in a previously tarred flat weighing bottle. Dried the sample by heating in an oven at 100-105º C for 5 hrs. Dried until two consecutive weighing was not differ by more than 5 mg, unless otherwise specified in the test procedure. Then calculate the loss of weight in mg/gm of the air dried material.

3.2.4.2 Determination of ash value:

The ash remaining following ignition of powder plant material was determined by three different methods which measures total ash, acid insoluble ash, water soluble ash.

3.2.4.2.1 Total ash

The total ash measures the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself and "non-physiological ash", which is residue of the extraneous matter adhering to the plant surface.

About 4 g of the ground air-dried powdered material was accurately weighed, in previously ignited and tarred silica crucible. The material was spreaded in an even layer and ignited by gradually increasing the heating to 500-600 ˚C until it was white, indicating the absence of carbon. It was allowed to cool in a desiccator and weighed. Content of total ash was calculated in form of mg per gm of air-dried material.

3.2.4.2.2 Acid insoluble ash:

Acid insoluble ash measures the amount of silica present, especially as sand and silicaceous earth.

In crucible containing the total ash, 25 ml of 2N hydrochloric acid was added, crucible was covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot distilled water and this liquid was added to the crucible. The insoluble matter on an ashless filter paper was washed with hot water until the filtrate was neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hotplate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and then weigh. Calculate the content of acid insoluble ash in mg per gm of air dried material.

3.2.4.2.3 Water soluble ash:

In crucible containing the total ash, 25 ml of water was added and boiled gently for 5 min. Insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate was neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite in a crucible for 15 min. at a temperature not exceeding 450 ˚C to constant weight. Allow the residue to cool in a suitable desiccator for 30 min. and then weigh. Weight of the residue was subtracted from weight of total ash. Calculate the content of water soluble ash in mg per gm of air dried material.

3.2.4.3 Extractive value

Extractive value determines the amount of active constituents extracted with solvents from given amount of herbal material. It is usually calculated as alcohol soluble extractive value and water soluble extractive value.

Alcohol soluble extractive value:

About 5.0 g of coarsely powdered air-dried material was weighed, in a glass-stoppered conical flask. Macerate with 100 ml of the alcohol for 6 hours, shaking frequently, and then allow standing for 18 hours. The content was filtered rapidly. Care was taken during filtration to avoid loss of solvent and 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water-bath. Dried the extract at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. Calculated the content of extractable matter in mg per gm of air-dried material.

Water soluble extractive value:

About 5 g. of the air-dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours shaken frequently during 6 hours and allowed to stand for 18 hours. The solution was filtered rapidly, 25 ml of filtrate was transferred to tarred flat bottom dish and evaporated to dryness on water bath. Extract was dried at 105 C for 6 hours, cooled in desiccator for 30 minutes and weighed without delay. Content of extractable matter was calculated in mg per g of air-dried material.

3.2.4.4 Determination of foaming index:

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of foaming index.

Procedure:

Accurately 1 gm of the powdered plant was transferred to a 500 ml conical flask containing 100 ml of boiling distilled water. It was boiled moderately for 30 minutes. The filtrate was cooled and filtered in a 100 ml volumetric flask and sufficient distilled water was added through the filter to dilute to volume. The decoction was poured into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and the volume of the liquid was adjusted in each tube with water to 10 ml. the tubes were

shaken in a lengthwise motion for 15 seconds, two shakes per second. Allowed to stand for 15 minutes and the height of the foam were measured.

Foaming index: 1000 / a

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

3.2.4.5 Determination of Total Tannin content

About 2gm of powdered sample was extracted for 20hrs with Petroleum ether. The residue was boiled for 2 hrs with 300 ml of double distilled water. The solution was cooled and diluted up to 500ml and filter. From the filtrate, 25ml of infusion was taken in to conical flask and 20 ml indigo carmine solution was added and diluted with 750 ml of double distilled water. Then the solution was titrated with standard $KMnO_4$ solution, 1ml at a time until blue color changed to green. Then few drops were added at a time till solution became golden yellow in color.

Similarly the blank reading was taken by omitting the sample. Multiply the difference between two titration by the factor to obtain value of total tannins.

$$\% \text{ Total Tannins} = \frac{(A-B) \times \text{Normality of } KMnO_4 \text{ solution} \times 0.004157 \times 1000}{\text{Weight of drug sample taken} \times 0.1}$$

3.2.5 Estimation of heavy metals

Elemental content of the plant material were estimated on atomic absorption spectrophotometer at Shree Dhanvantary Pharmaceutical Analysis & Research Centre, Kim.

3.3 Phytochemical screening

3.3.1 Preliminary phytoprofile

The powder of the air dried plant of *Oldenlandia corymbosa* and *Grangea maderaspatana* were extracted in soxhlet apparatus with solvents of increasing polarity as follows:

i) Petroleum ether
ii) Chloroform
iii) Ethyl acetate
iv) Methanol
v) Water

Each time before extracting with the next solvent, the material was dried. All the extracts were concentrated by distilling the solvent and the extracts were dried on water bath. Then consistency, color, appearance of the extracts and their percentage yield were noted.

3.3.2 Establishment of qualitative phytoprofile of successive solvent extracts. (chemical tests):[3,5,6]

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins and phytosterols using reported methods.

1. **Alkaloids:**

a. **Dragondorff's test:** To the extract add Dragondorff's reagent, reddish brown precipitate indicates the presence of alkaloids.
b. **Mayer's test:** To the extract add Mayer's reagent, cream colored precipitate indicates the presence of alkaloids.
c. **Wagner's test:** To the extract add Wagner's reagent, reddish brown precipitate indicates the presence of alkaloids.
d. **Hager's test:** To the extract add Hager's reagent, yellow precipitate indicates presence of alkaloids
e. **Tannic acid test:** To the extract add tannic acid solution, buff colored precipitate indicates presence of alkaloids.

2. Amino acids:

a. Millon's test: To the extract add about 2 ml of Millon's reagent, white precipitate indicates the presence of amino acids.

b. Ninhydrin test: To the extract add Ninhydrin solution, boil, violet color indicates the presence of amino acids.

3. Carbohydrates:

a. Molisch's test: To the extract add few drops of alcoholic α-napthol, then add few drops of concentrated sulphuric acid through sides of test tube; purple to violet ring appears at the junction.

b. Barfoed's test: 1 ml extract is heated with 1 ml of Barfoed's reagent, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min) may cause reduction, owing to partial hydrolysis to monosaccharides.

c. Selwinoff's test (Test for ketones): To the extract add crystals of resorcinol and equal volume of concentrated hydrochloric acid and heat on water bath, rose color is produced.

d. Test for Pentoses: To the extract add equal volume of hydrochloric acid containing a small amount of phloroglucinol and heat, red color is produced.

4. Flavanoids:

a. Shinoda test: To the extract add few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

b. Alkaline reagent test: To the extract add few drops of sodium hydroxide solution, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicates presence of flavonoids.

c. Zinc hydrochloride test: To the extract add a mixture of zinc dust and concentrated hydrochloric acid. It gives red color after few minutes.

5. Glycosides

General test:

Test A: Extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on water bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1 ml of Fehling's solution A and B until it becomes alkaline (test with pH paper) and heat on water

bath for 2 minutes. Note the quantity of red precipitate formed and compare with that of formed in Test B.

Test B: Extract 200 mg of drug using 5 ml water instead of sulphuric acid. After boiling add equal amount of water as used for sodium hydroxide in the above test. Add 0.1 ml Fehling's A and B until alkaline (test with pH paper) and heat on water bath for 2 minutes. Note the quantity of red precipitate formed.

Compare the quantity of precipitate formed in Test B with that of formed in Test A. If the precipitate in Test A is greater than in Test B then glycoside may be present. Since Test B represents the amount of free reducing sugar already present in the crude drug, whereas Test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

Chemical test for specific glycosides:

i. Anthraquinone glycosides

a) Borntrager's test: Boil the test material with 1 ml of sulphuric acid in a test tube for 5 minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of ammonia. A rose pink to red color is produced in the ammonical layer.

b) Modified Borntrager's test: Boil the test material with 2 ml of sulphuric acid. Treat it with 2 ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, shake it with equal volume of chloroform and continue the test as above. As some plants contain anthraceneaglyconein reduced form, if ferric chloride is used during extraction; oxidation to anthraquinones takes place, which shows response to Borntrager's test.

ii. Cardiac glycosides:

a) Kedde's test: Extract the drug with chloroform, evaporate to dryness. Add one drop of 90% alcohol and 2 drops of 2 % 3, 5-dinitro benzoic acid in 90% alcohol. Make alkaline with 20% sodium hydroxide solution, purple color is produced. The color reaction with 3, 5-dinito benzoic acid depends on the presence of α, β- unsaturated lactones in the aglycone.

b) Killer- Killiani test: Extract the drug with chloroform and evaporate it to dryness. Add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small

test tube; add carefully 0.5 ml of concentrated sulphuric acid by the side of test tube. Acetic acid layer shows blue color.

c) **Raymond's test:** Treat the extract with hot methanolic alkali, violet color is produced.

d) **Legal's test**: Treat the extract with pyridine and alkaline sodium nitroprusside solution, blood red color appears.

e) **Baljet's test:** Treat the extract with picric acid or sodium picrate, orange color is produced.

iii. Coumarin glycosides:

Place a small amount of sample in test tube and cover the test tube with a filter paper moistened with dilute sodium hydroxide solution. Place the covered test tube on water bath for several minutes. Remove the paper and expose it with ultraviolet (UV) light, the paper shows green fluorescence.

iv. Saponin glycosides:

a. **Froth formation test:** Place 2 ml solution of drug in water in a test tube, shake well, stable froth (foam) is formed.

b. **Haemolysis test:** Add 0.2 ml of extract to 0.2 ml of blood in normal saline and mix well. Centrifuge and note the red supernatant compare with control tube containing 0.2 ml of 10% blood in normal saline diluted with 0.2 ml of normal saline.

6. Phenolic compounds (Tannins):

a. **Ferric chloride test:** Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

b. **Phenazone test:** Add about 0.5 gm of sodium acid phosphate to 5 ml of extract warm it and filter. To the filtrate add 2 % phenazone solution, bulky precipitate is formed, which is often colored.

c. **Gelatin test:** To the extract add 1 % gelatin solution containing 10% sodium chloride. Precipitate is formed.

7. Proteins:

a. **Biuret test:** To the extract (2 ml) add Biuret reagent (2 ml), violet color indicates presence of proteins.

b. Xanthoproteic test: To the 5 ml of extract, add 1 ml of concentrated nitric acid and boil, yellow precipitate is formed. After cooling it, add 40 % sodium hydroxide solution, orange color is formed.

8. Steroids and Triterpenoids:

a. Libermann-Burchard test: Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of test tube, brown ring is formed at the junction. The layer turns green which shows presence of steroids and formation of deep red color indicates presence of triterpenoids.

b. Salkowski test: Treat the extract with few drops of concentrated sulphuric acid red color at lower layer indicates the presence of steroids and formation of yellow colored lower layer indicates presence of triterpenoids.

c. Sulfur powder test: Add small amount of sulfur powder to the extract, it sinks at the bottom.

3.3.3 TLC of successive solvent extracts

The various extracts obtained in the successive solvent extraction were then subjected to thin layer chromatographic studies using reported methods to confirm the presence of various phytoconstituents. These results were compared with the results obtained in qualitative tests[7,8].

3.3.4 HPTLC Fingerprinting

HPTLC fingerprinting of chloroform and methanol extracts of *O. corymbosa* and *G. maderaspatana* was performed for oleanolic acid and ursolic acid[9,10].

a) Sample preparation:

Accurately weighed 20 mg of each extracts individually into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1 and used for HPTLC profiling.

b) Standard preparation:

Accurately weighed 10 mg of each standard individually into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1 and used for HPTLC profiling.

Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 - Applicator
Application of sample	Automatic device "CAMAG LINOMAT – 5"
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets (10 x 10 cm)
Application Volume	10 µL
Mobile Phase	Toluene : Ethyl acetate : Formic acid (8 : 2 : 0.1)
Development Mode	CAMAG TLC Twin Trough Chamber
Spray reagent	Anisaldehyde sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Visualization	@ 510 nm after derivatization

3.3.5 Quantification of Gallic acid by HPLC

Estimation of Gallic acid in methanol extracts of *O. corymbosa* and *G. maderaspatana* was performed by HPLC[11].

a) Sample preparation:

Accurately weighed 1 mg of each extracts individually into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1 and used for HPLC profiling.

b) Standard preparation:

Accurately weighed 1 mg of Gallic acid was transferred into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1. From stock solution, different concentrations (10-70 µg/ml) were prepared and used for HPLC profiling.

Chromatographic Conditions:

Stationary Phase	Phenomenex Luna C18 (4.6 x 250mm, 5µ particle size)
Mobile Phase	Water : Acetonitrile (80 :20 %v/v)
Wavelength	272 nm
Flow Rate	1 mL/min
Total Run time	Max. up to 7 min.
Injection Volume	20 µL
Temperature	Ambient
Mode of Operation	Isocratic elution

4. Psychopharmacological studies

4.1 Acute toxicity study[12,13]

Acute toxicity study was performed for chloroform and methanol extracts of *O. corymbosa* and *G. maderaspatana* according to the acute toxic classic method as per guidelines prescribed by OECD (OECD, 1996). Acute toxicity test aims at establishing the therapeutic index, i.e. the ratio between pharmacological effective dose and lethal dose on the same strain and species (LD50/ED50). Greater the index, safer the compound.

2000 mg/kg of extract was administered as per OECD guidelines per orally to 6 mice. Effects were observed on behavior for 72 hours. Mice were examined for behavioral effects 45 minutes post administration of the extracts. No change in behavior or any abnormality in behavior was observed and no mortality was seen. Thus it was concluded that chloroform and methanol extract of *O. corymosa* and *G. maderaspatana* was nontoxic up to 2000 mg/kg doses. Then $1/5^{th}$ and $1/10^{th}$ of the administered dose was selected for future studies as per OECD guidelines.

Experimental Animals

Animals:

Swiss albino male mice weighing 25-30 gms, were used for all sets of experiments in groups of six animals. They were maintained at controlled room temperature (25±2˚C) on 12 hour light/dark cycle and allowed free access to food and water. The experiments were performed

after the experimental protocols approved by the Institutional Animal Ethics Committee of Babaria Institute of Pharmacy and care of animals was taken as per CPCSEA guidelines.
Animals were divided in to control group, standard group and extracts treated group. Each group consists of 6 animals.

Treatment:

Animals were divided into six (I-VI) groups for the assessment of both the plant extracts. Group I was a negative control; Group II was positive control; Groups III to IV received chloroform extract of *O. corymosa* at doses of 200 and 400 mg/kg p.o respectively. Group V to VI received methanolic extract of *O. corymosa* at doses of 200 and 400 mg/kg, p.o respectively.

For assessment of psychopharmacological activity of *Grangea maderaspatana* extracts, the animals were divided in to six (I-VI) groups. Group I was a negative control; Group II was positive control; Groups III to IV received chloroform extract of *G. maderasatana* at dose of 200 and 400 mg/kg p.o respectively. Groups V to VI received methanol extract of *G. maderasatana* at dose of 200 and 400 mg/kg p.o respectively.

Psychopharmacological activity was screened by:

a) Antidepressant activity was performed using forced swim test model.
b) Anxiolytic activity was done using Elevated plus maze model.
c) Exploratory behavior pattern was studied by Head dip test method.
d) CNS inhibitory activity was performed using Actophotometer**.**

4.2 Forced Swimming Test

The apparatus consisted of an opaque Plexiglas cylinder (50 cm high × 20 cm wide) filled with water at room temperature, to a depth of 30 cm. During the 6 min swimming test, immobility behavior was observed, defined as when the animal made no further attempts to escape except for the movements necessary to keep its head above the water. Reduction in immobility is considered as a behavioral profile consistent with an antidepressant like action[14,15].

4.3 Elevated Plus Maze

This apparatus consists of two open arms (50×10 cm) crossed with two closed arms (50×10×40cm). The arm was connected together with a central square (10×10 cm). The apparatus was elevated to a height of 70 cm in a dimly illuminated room. Each mouse was placed individually at the center of the elevated maze, 45 minutes post administration of the extracts and the standard. The number of entries in the open and closed arm of the elevated maze during a period of 5 minutes and the duration of stay in the open and closed arm were noted[16,17]. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution). Entry into the arms was defined as the point when the animal places all four paws in the arm. Subsequently, the percentage of open arm entries (100 × open/total entries) and the percentage of time spent in the open arms (100 × open/open + enclosed) were calculated for each animal[18,19].

4.4 Head dip test method

Exploratory behavior of mice in a novel environment was measured using a hole-board test (locally constructed). This method is used for measuring the response of the rat to an unfamiliar environment. The apparatus consisted of a grey cardboard box (50×50×50 cm) with 18 equidistant holes 3 cm in diameter in the floor. 30 minutes after proposed treatment with std/samples, head-dipping behaviors were checked for 20 minutes[20].

4.5 CNS Inhibitory Activity- Actophotometer

The actophotometer was switched on and the animals were placed individually in the activity cage for 10 min. Standard, test and vehicle were injected in each animal of proposed groups and after 30 min. each animal was tested for 10 min. The locomotor activity after treatment was noted[20].

Statistical Analysis

Results are represented as Mean ± SEM. The test extract, standard and control were analyzed with the help of one-way analysis of variance (ANOVA) followed by Dunnett's Test. P values < 0.05 were considered as statistically significant.

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CHAPTER 4

RESULTS & DISCUSSION

Chapter 4

Results & Discussion

In this chapter the various results obtained from different experiments carried out are compiled. An attempt has also been made to discuss these results in order to provide convincing reason for the studies performed.

Oldenlandia corymbosa

4.1 Collection and Identification of Plant material

Plant collection and Identification

The plant of *Oldenlandia corymbosa* was collected in the month of September from the Botanical garden of M.S.U, Vadodara, Gujarat. Authentication was done by Taxonomist of the Botanical Survey of India, Jodhpur. A voucher specimen (No. BSI/AZRC/I.1202/Tech./2012-13/721) was deposited in the Herbarium of Botanical Survey of India, Jodhpur.

4.2. Assessment of quality of plant materials

4.2.1 Macroscopic evaluation

TABLE 4.1. Morphology of *O. corymbosa* leaf

Parameters	Observations
Type	Simple
Phyllotaxy	Opposite
Size	3.5 cm long , 5 mm wide
Shape	Linear – lanceolate or Elliptic
Margin	Entire
Apex	Acute
Base	Acute
Venation	Reticulate
Surface	Glabrous
Color	Dark green
Odour	Characteristic
Taste	Characteristic

Stem: Prostrate herb, Quadrangular, Glabrous, Stipules present. Stem is greenish purple colored.

Root: Primarily tap-rooted but form weak adventitious roots along the stolon nodes , White – brown colored

Flowers: Bisexual, Solitary or grouped together into an axillary cyme, Sessile, Petals – 4, White colored

Fruit: Capsule, Globose, contains minute brown seeds.

4.2.2 Microscopic evaluation

Leaf Microscopy:

The transverse section of leaf of *Oldenlandia corymbosa* shows dorsiventral nature. The section is broadly divided in to lamina and midrib region. The lamina of leaf shows three distinct regions viz., upper epidermis, lower epidermis and mesophyll. The epidermal cells were barrel shaped. The cells of upper epidermis were about double the size of lower epidermis. The mesophyll is differentiated in to palisade and spongy parenchyma. The palisade parenchyma has single layer narrow, cylindrical and compact cells. Rarely unicellular covering trichome is seen.

Midrib shows concave cavity on upper side and hemispherical bulge on lower side. The epidermal cells are polygonal in shape covered by thin cuticle. Few collenchymatous cells are present below the upper epidermis and above the lower epidermis. The ground tissue is parenchymatous. The vascular bundle is collateral type.

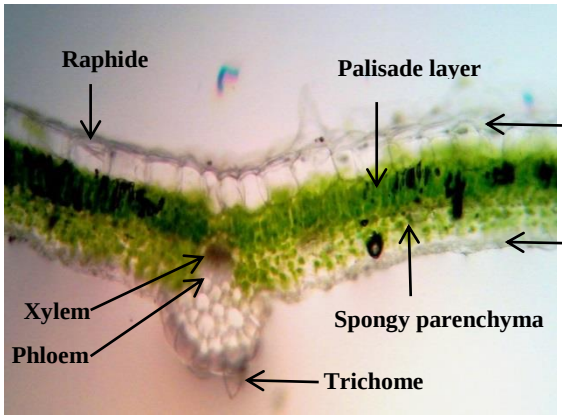


FIGURE 4.1, T.S. of *O. corymbosa* leaf (unstained) (10x10)

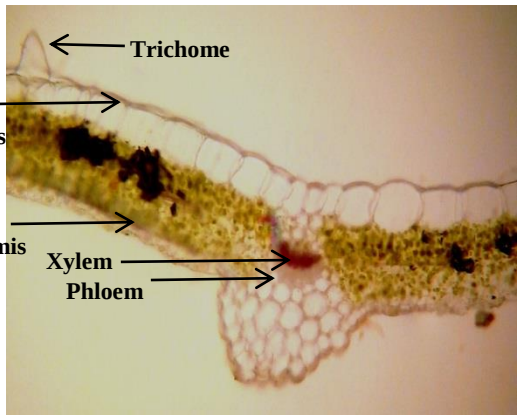


FIGURE 4.2, T.S. of *O. corymbosa* leaf (stained) (10x10)

Stem:

Transverse section of stem shows quadrangular outline. The epidermis consists of barrel shaped cells. Cortex is 6 to 8 layers thick and made up of thin walled parenchymatous cells. Some cells contain chlorophyll. Endodermis is indistinct. Phloem is narrow zone consisting of 4 to 6 layers of cells. The xylem consists of small vessels and parenchyma. The pith in the centre is large and made up of thin walled parenchymatous cells.

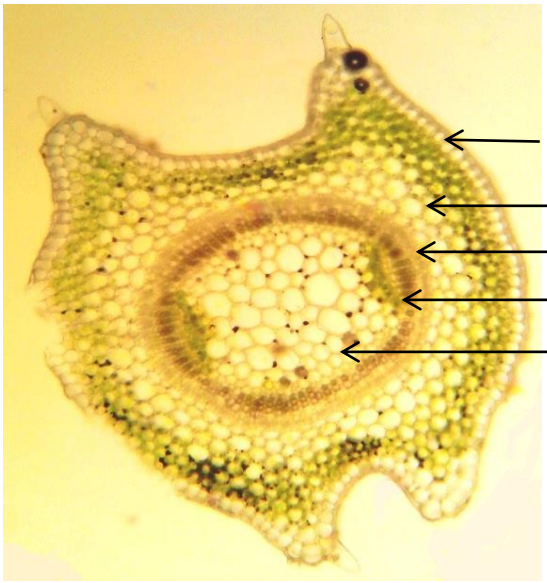


FIGURE 4.3, T.S. of *O. corymbosa* stem (unstained) (10x10)

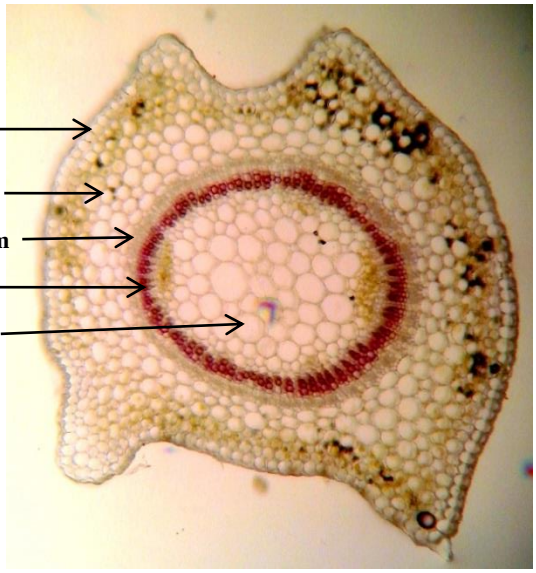


FIGURE 4.4, T.S. of *O. corymbosa* stem (stained) (10x10)

Root:

Transverse section of root shows circular outline. Cork is made up of 2-4 layers of thin walled cells. Cortex is made up of 6-9 layers of rectangular parenchymatous cells. Many cells of cortex contain raphides. Phloem is 5-7 layers thick. The xylem consists of small vessels, fibres, parenchyma and rays. The xylem rays are uniseriate and having pits on their wall.

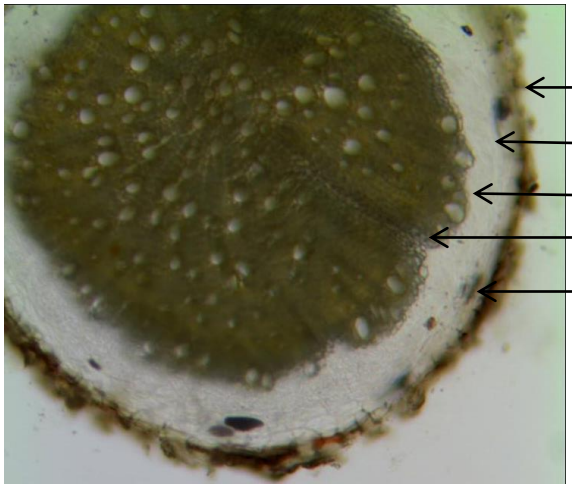


FIGURE4.5, T.S of *O. corymbosa* root (unstained) (10x10)

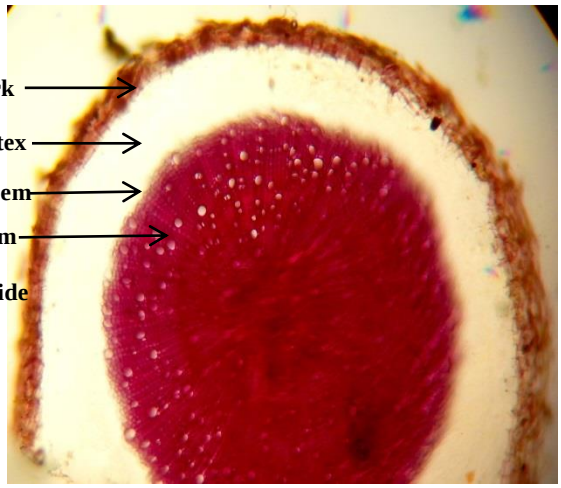


FIGURE4.6, T.S of *O. corymbosa* root (stained) (10x10)

4.2.2.1 Powder characteristics

The organoleptic evaluation of powder revealed the following characteristics. The powder is light green color with characteristic odor and taste. On microscopic examination, the powder showed lamina, paracytic stomata, raphides, phloem, cork, xylem.

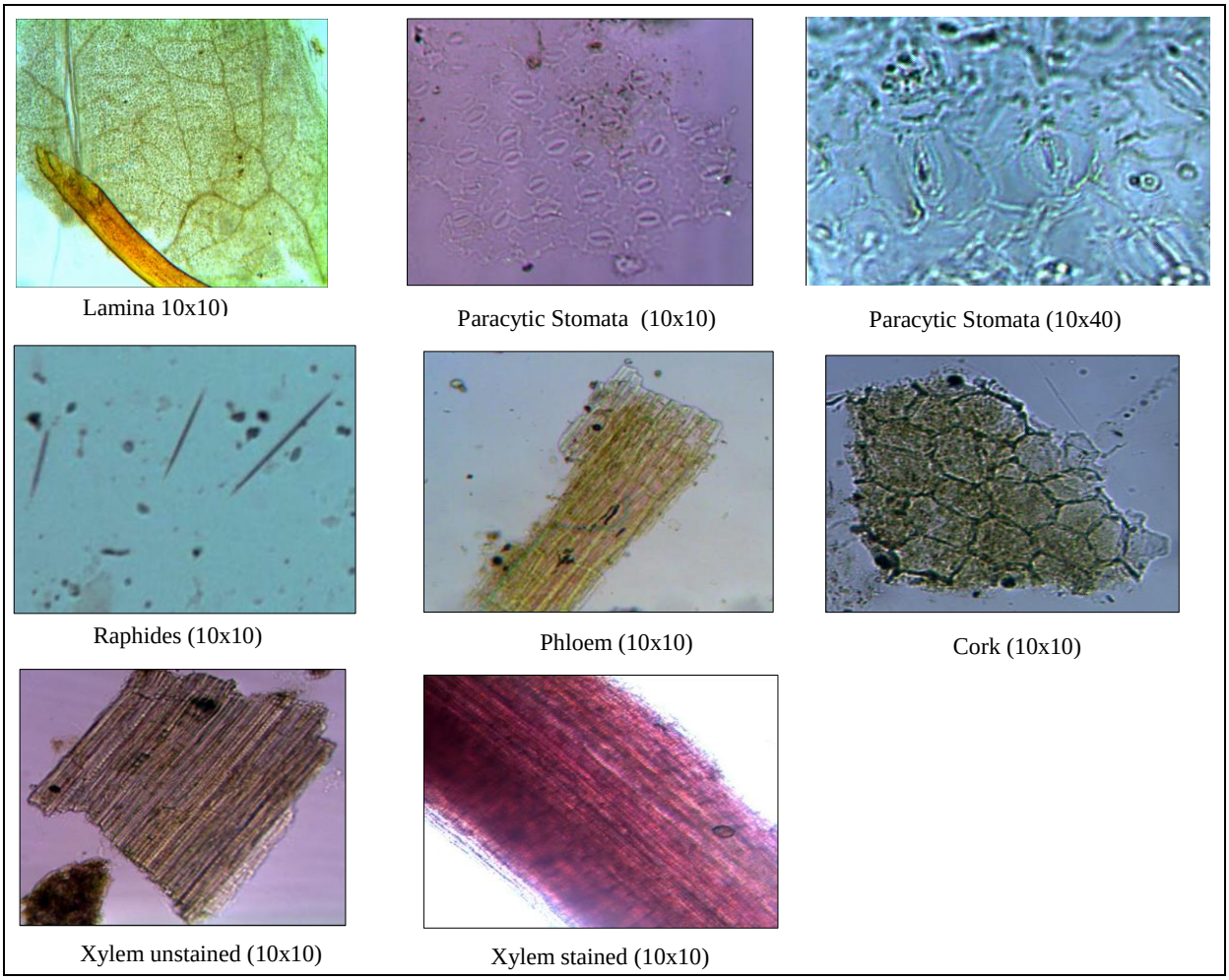


FIGURE 4.7, Powder characteristics of *O. corymbosa*

4.2.2.2 Quantitative microscopy of *Oldenlandia corymbosa* leaf

Leaf constants

The leaf constants viz., stomatal index, vein islet number, vein termination number and palisade ratio are presented in Table no. 4.2. Paracytic stomata are present in the leaf.

TABLE- 4.2. Quantitative microscopy of *O. corymbosa* leaf

Sr. no.	Parameters	Values
1	Stomatal index	23.07
3	Vein islet number	2.00
4	Vein termination number	00
5	Palisade ratio	6.00-8.00

4.3 Proximate analysis

The results obtained from various determinations are compiled in Table 4.3. The ash values of a drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The extractive values are primarily useful for the determination of exhausted or adulterated drug.

TABLE 4.3. Physico-chemical parameters of powder of *O. corymbosa*

Sr. No.	Parameters	Values (% w/w)
1	Loss on drying	8.00%
2	Ash value	
	Total ash	12.25%
	Acid insoluble ash	2.00%
	Water soluble ash	5.50%
3	Extractive value	
	Water soluble extractive	10.00%
	Alcohol soluble extractive	4.5%
4	Foaming Index	< 100

The values given here are expressed as percentage of air dried material. Each value is average of three determinations.

The morphological, microscopical and physico-chemical parameters of *Oldenlandia corymbosa* can possibly help to differentiate the drug from its other species and the pharmacognostic profile of the plant presented here will assist in standardization viz., quality, purity and sample identification.

4.3.1 Determination of total tannin content

The powder of *Oldenlandia corymbosa* plant contains 1.09% of total tannins.

4.5 Estimation of heavy metals

Contamination of the medicinal plant materials with heavy metals can cause chronic or acute poisoning. Therefore it has become necessary that all the starting materials should be ensured for their heavy metal content including other necessary inorganic elements. Elemental analysis was carried out to determine the presence of heavy metals and the results are presented in the table below.

TABLE-4.4. Content of heavy metals in powder of *O. corymbosa*

Sr. no.	Heavy Metal	Result (ppm)
1	Arsenic	Not detected
2	Cadmium	0.24 ppm
3	Lead	Not detected
4	Mercury	Not detected

4.3 Phytochemical studies

4.3.1 Preliminary phytoprofile

Oldenlandia corymbosa plant powder was subjected to successive solvent extraction (except water extract which was prepared by decoction). The different extracts obtained with their % yield, color, consistency are recorded in Table- 4.5.

TABLE 4.5. Preliminary phytoprofile of *O. corymbosa*

Sr. no.	Solvent	Color	Consistency	%Yield w/w
1	Petroleum Ether	Yellowish green	Slight Sticky	2.36
2	Chloroform	Dark green	Slight Sticky	2.02
3	Ethyl acetate	Green	Slight Sticky	1.78
4	Methanol	Green	Slight Sticky	3.80
5	Water	Brown	Slight Sticky	9.50

4.3.2 Qualitative chemical tests

The extracts obtained from successive solvent extraction process were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like steroids, carbohydrates, alkaloids, glycosides, phenolics and tannins etc.

TABLE 4.6. Phytochemical screening of extracts of *O. corymbosa*

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

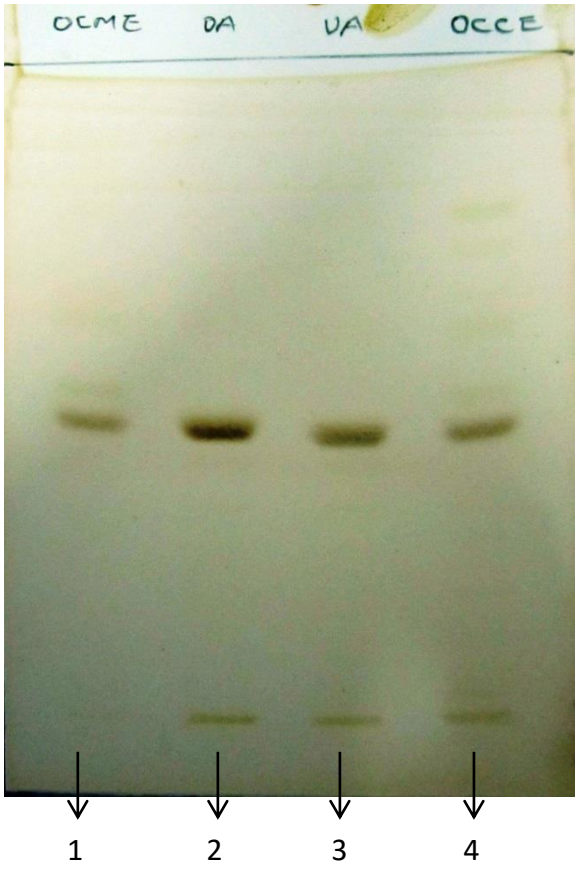
(+ - Positive test, - - Negative test)

4.3.3 HPTLC fingerprinting of *O. corymbosa* extracts

HPTLC fingerprinting of chloroform and methanol extracts of *O. corymbosa* for oleanolic acid and ursolic acid.

Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 - Applicator
Application of sample	Automatic device "CAMAG LINOMAT – 5"
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets (10 x 10 cm)
Application Volume	10 µL
Mobile Phase	Toluene : Ethyl acetate : Formic acid (8 : 2 : 0.1)
Development Mode	CAMAG TLC Twin Trough Chamber
Spray reagent	Anisaldehyde sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Visualization	@ 510 nm after derivatization



1. Oc. MeOH ext.
2. Std – Oleanolic acid
3. Std – Ursolic acid
4. Oc. $CHCl_3$ ext.

FIGURE 4.8, HPTLC plate of *O. corymbosa* extracts after derivatization

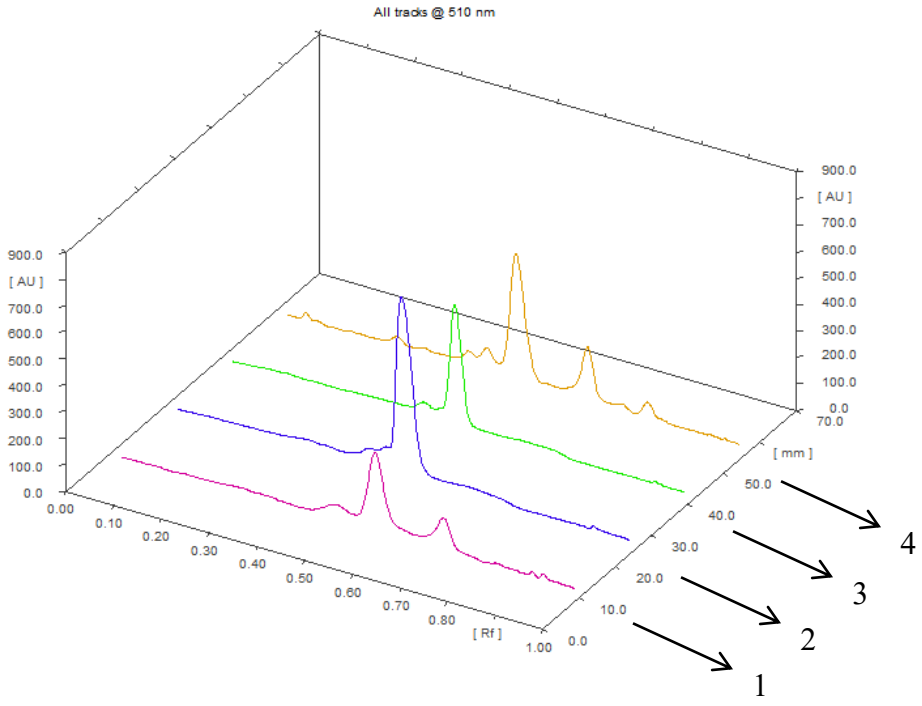


FIGURE 4.9, 3D Graph of standard and extracts of *O. corymbosa*

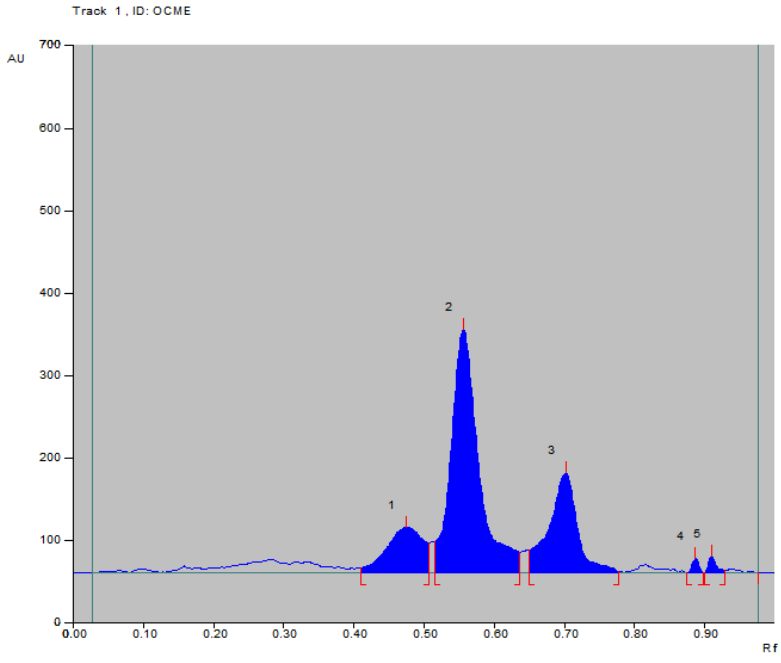


FIGURE 4.10, Chromatogram of methanol extract of *O. corymbosa*

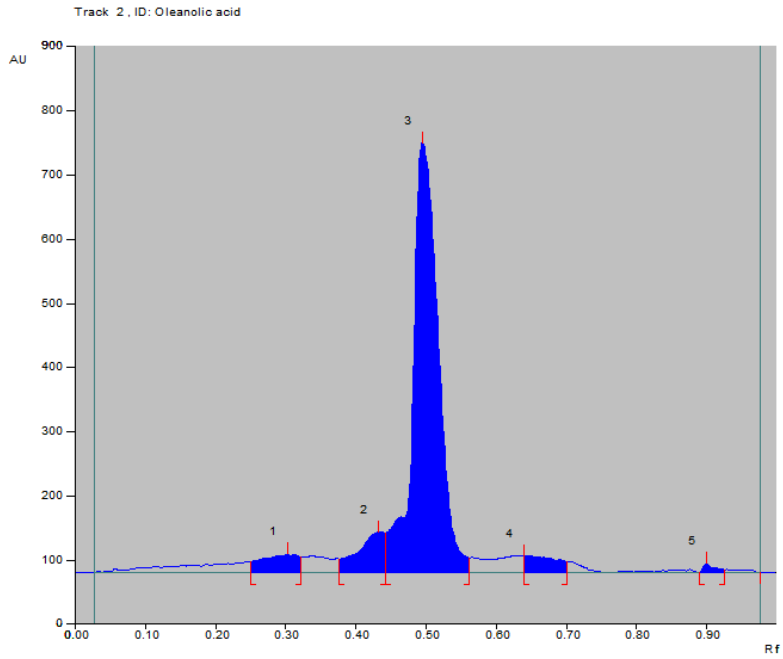


FIGURE 4.11, Chromatogram of standard Oleanolic acid

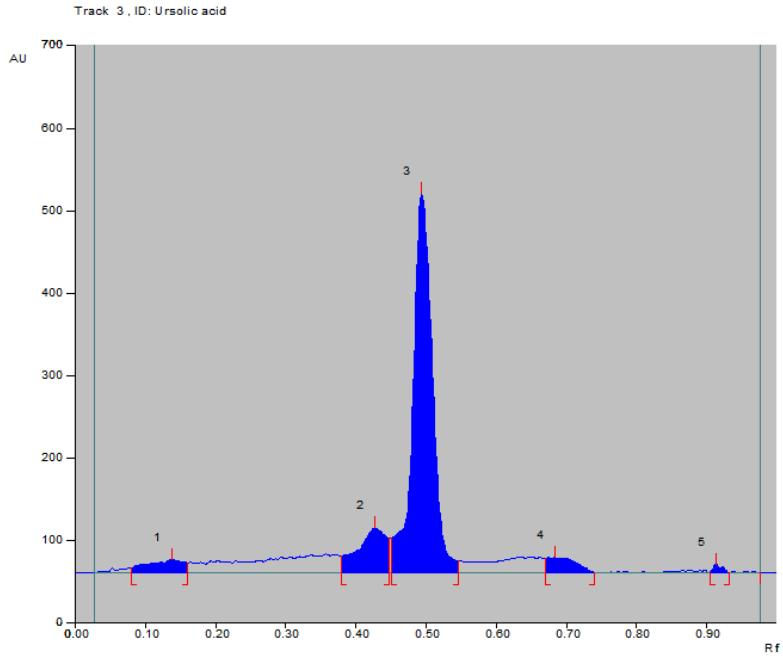


FIGURE 4.12, Chromatogram of standard Ursolic acid

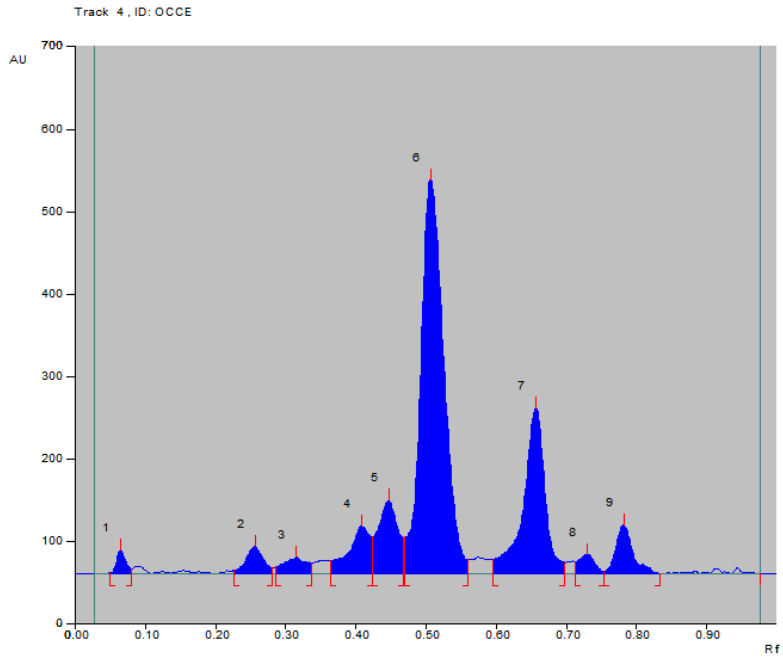


FIGURE 4.13, Chromatogram of chloroform extract of *O. corymbosa*

TABLE 4.7. Result of HPTLC analysis of extracts of *O. corymbosa*

	Sample			
	OCME	**Oleanolic acid**	**Ursolic acid**	**OCCE**
Rf value	**0.51**	**0.50**	**0.49**	**0.51**
AUC	**10747.1**	**22688.7**	**11885.7**	**14523.2**

The chloroform extract of *Oldenlandia corymbosa* contains 32.0% and 61.0% of oleanolic acid and ursolic acid respectively, whereas the methanol extract contains 23.5% and 45.0% of oleanolic acid and ursolic acid respectively.

4.3.4 Estimation of Gallic acid in extracts of *O. corymbosa* by HPLC

Amount of Gallic acid was estimated in methanol extracts of *O. corymbosa* by HPLC.

Chromatographic Conditions:

Stationary Phase	Phenomenex Luna C18 (4.6 x 250mm, 5µ particle size)
Mobile Phase	Water : Acetonitrile (80 : 20 %v/v)
Wavelength	272 nm
Flow Rate	1 mL/min
Total Run Time	Max. 7 min.
Injection Volume	20 µL
Temperature	Ambient
Mode of Operation	Isocratic elution

TABLE 4.8. Linearity of Gallic acid

Concentration(µg/ml)	Peak Area
10	21.54767
20	58.50933
30	94.488
40	127.4617
50	163.156
60	190.9767
70	207.4273

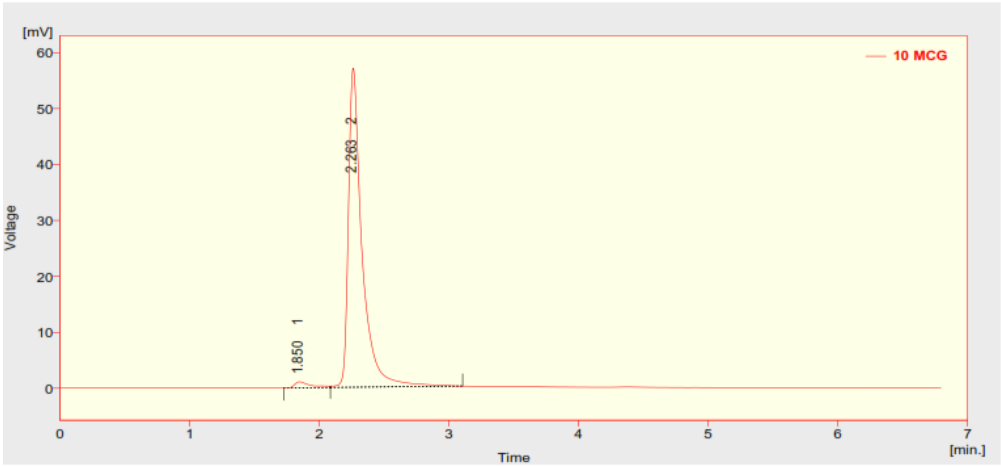


FIGURE 4.14, Chromatogram of Gallic acid (10µg/ml)

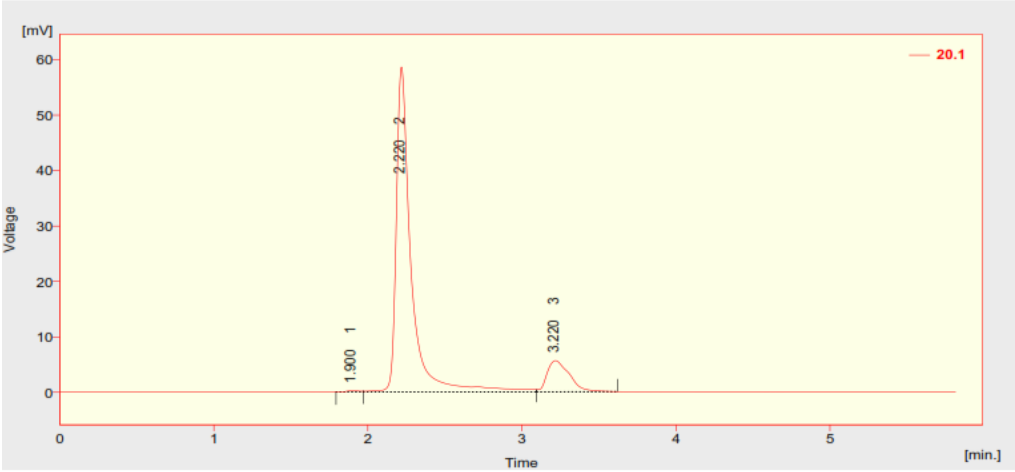


FIGURE 4.15, Chromatogram of Gallic acid (20µg/ml)

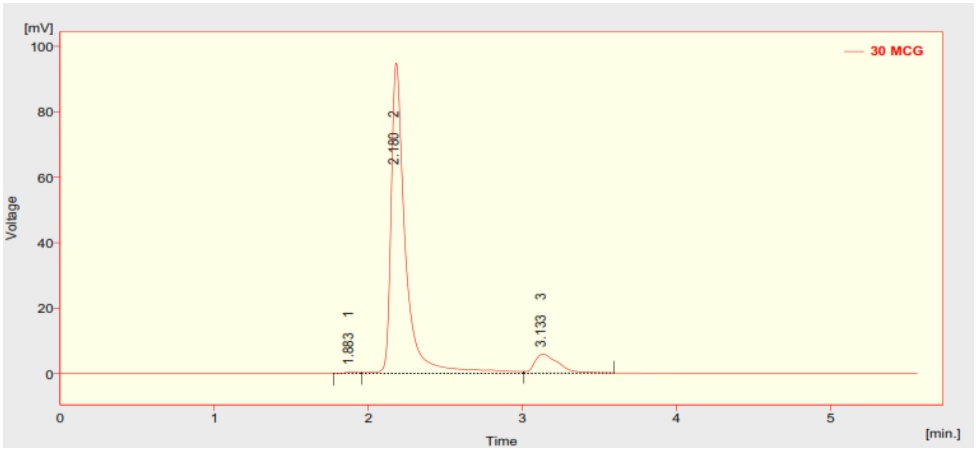


FIGURE 4.16, Chromatogram of Gallic acid (30µg/ml)

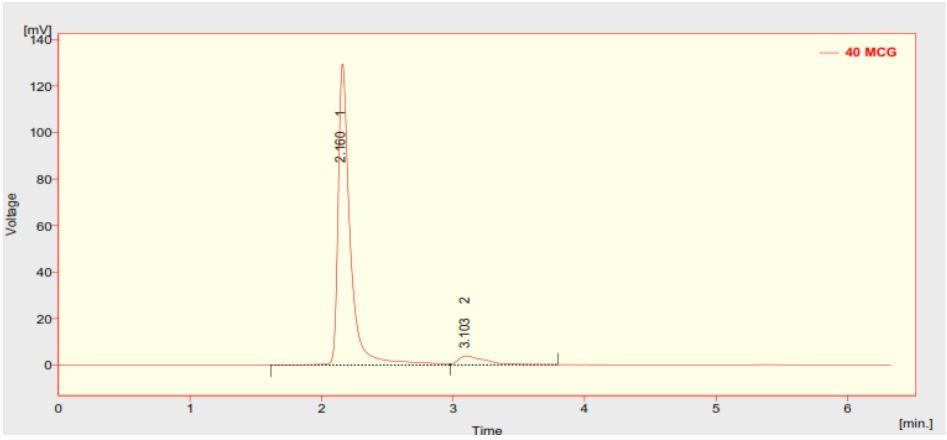


FIGURE 4.17, Chromatogram of Gallic acid (40µg/ml)

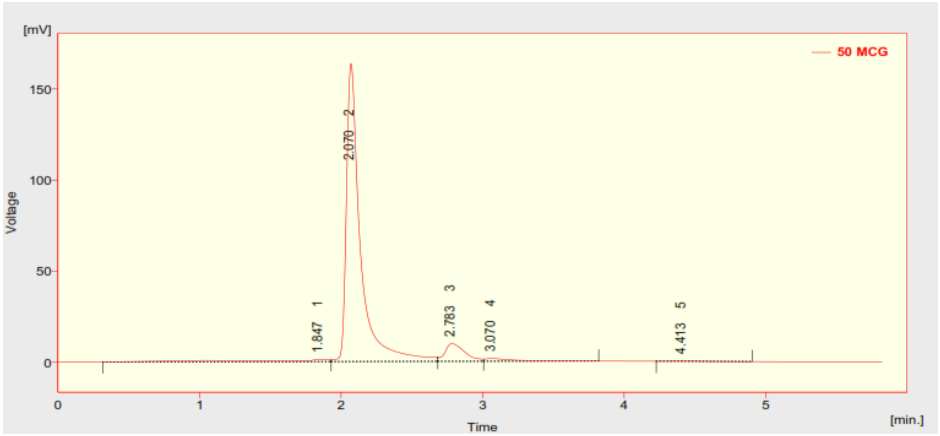


FIGURE 4.18, Chromatogram of Gallic acid (50µg/ml)

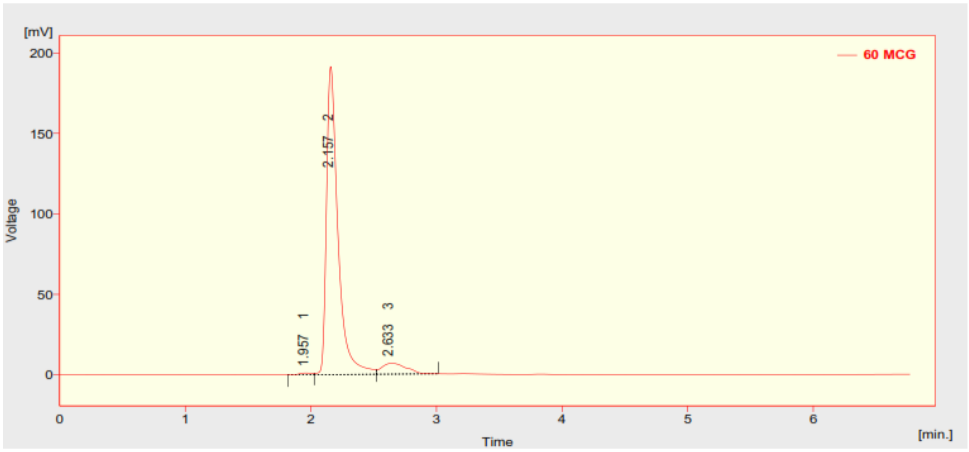


FIGURE 4.19, Chromatogram of Gallic acid (60µg/ml)

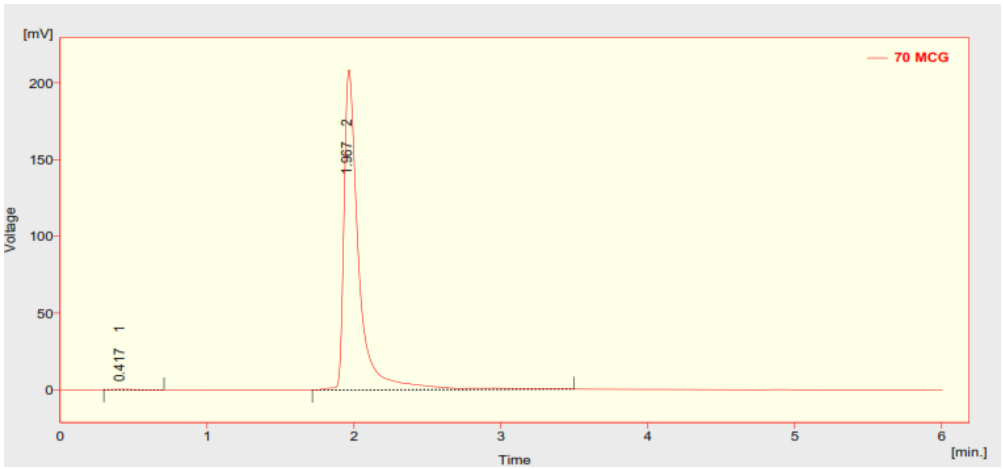


FIGURE 4.20, Chromatogram of Gallic acid (70µg/ml)

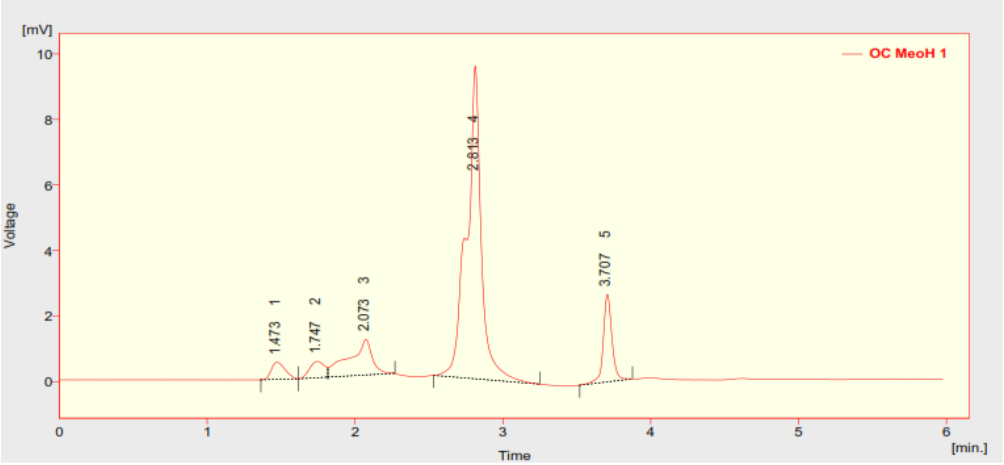


FIGURE 4.21, Chromatogram of methanol extract of *O. corymbosa* (1000µg/ml)

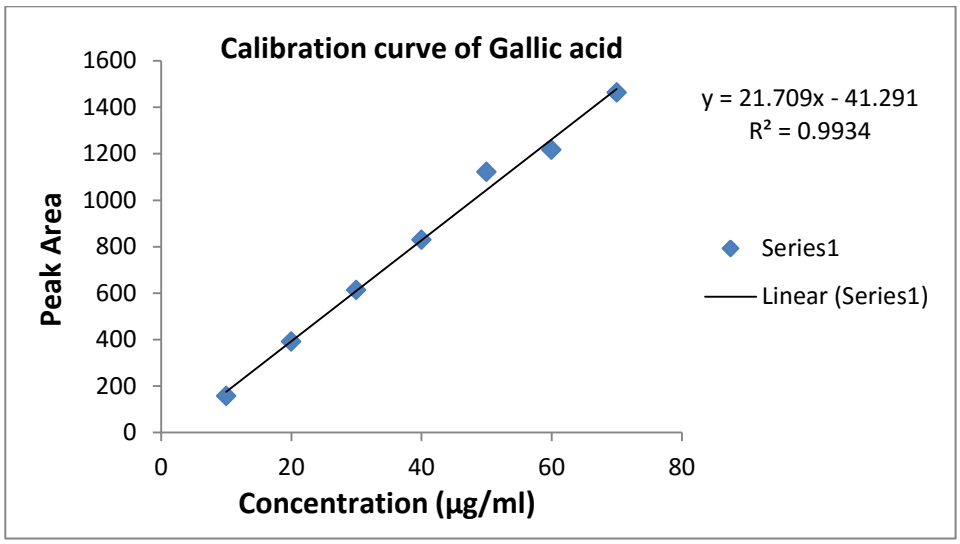


FIGURE 4.22, Calibration curve of Gallic acid

TABLE 4.9. Summary output (Regression statistics)

Multiple R Square	**0.978383**
R Square	**0.957232**
Adjusted R Square	**0.935849**
Equation of line	**y = 21.709x - 41.291**

The methanol extract of *Oldenlandia corymbosa* contains 2.45% of Gallic acid.

4.4 Psychopharmacological Activity

The results of the present investigation showed that the chloroform and methanol extracts of *Oldenlandia corymbosa* have some psychopharmacological activity.

4.4.1 Acute toxicity study

Assessment of acute toxicity is the first step in the toxicological investigation of an unknown substance. The chloroform and methanol extracts of *Oldenlandia corymbosa* were well tolerated by mice and there were no signs of acute or delayed toxicity after oral administration. Increasing doses up to 2000 mg/kg (p.o) were not lethal, the LD50 values for the extract was estimated to be higher than 2000 mg/kg for oral administration. Thus, suggesting that this administration route is adequate and secure to produce its psychopharmacological effects.

4.4.2 Antidepressant Activity by Forced swim test

The chloroform and methanol extracts of *O. corymbosa* (200 & 400 mg/kg) exerted increases in the immobility of mice following its administration to mice when compared with control group. These increases were significant ($p<0.001$) at lower dose (200 mg/kg) of the extract (Figure 4.15).

TABLE 4.10. Effect of *O. corymbosa* extracts on immobility time

Group	Treatment	Dose	Immobility period (Sec.)
I	Control	-	150 ± 0.843
II	Standard (Imipramine)	15 mg/kg	103 ± 1.78
III	Oc. $CHCl_3$ Ext.	200 mg/kg	109 ± 1.34**
IV	Oc. $CHCl_3$ Ext.	400 mg/kg	120 ± 1.19*
V	Oc. MeOH Ext.	200 mg/kg	112 ± 0.872**
VI	Oc. MeOH Ext.	400 mg/kg	128 ± 0.946*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

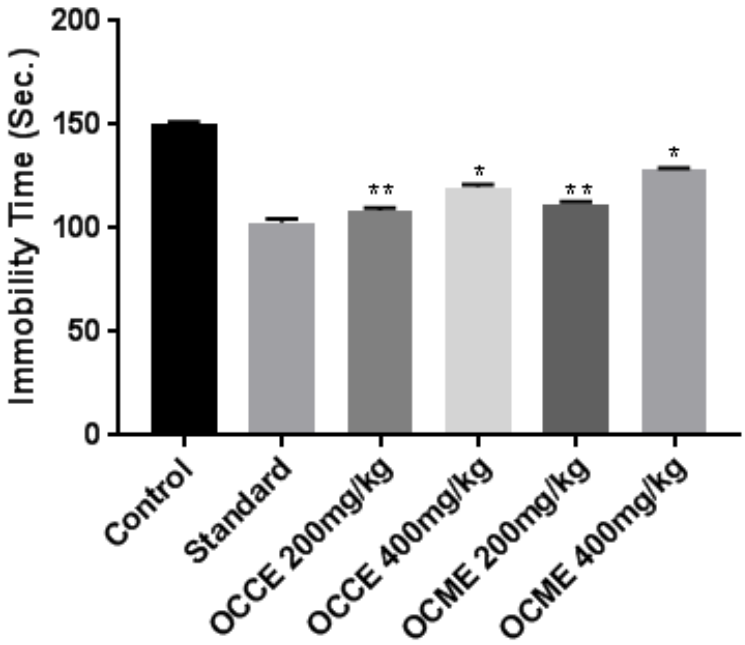


FIGURE 4.23, Effect of the chloroform and methanol extract of *O. corymbosa* on immobility time. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

4.4.3 Anxiolytic Activity by Elevated plus maze model

Anti-anxiety activity of chloroform and methanol extract of *O. corymbosa* were evaluated employing a widely used model, elevated plus-maze. The model was chosen as it is effective, inexpensive, simple, less time consuming, requires no preliminary training to the mice and does not cause much discomfort to the animals while handling. The model is principally based on the observations that the exposure of animals to an elevated and open maze results in approach–avoidance conflict which is manifested as an exploratory-cum-fear drive. The fear due to height induces anxiety in the animals when placed on the elevated plus-maze. The ultimate sign of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by the time spent by the animal in the open arms.

TABLE 4.11. Effect of *O. corymbosa* extracts on % Time spent in open arm of EPM

Group	Treatment	Dose	% Time spent in open arm
I	Control	-	21.5 ± 0.885
II	Standard (Diazepam)	1 mg/kg	66.7 ± 0.882
III	Oc. $CHCl_3$ Ext.	200 mg/kg	40.7 ± 0.882**
IV	Oc. $CHCl_3$ Ext.	400 mg/kg	51 ± 0.966**
V	Oc. MeOH Ext.	200 mg/kg	31.3 ± 1.15*
VI	Oc. MeOH Ext.	400 mg/kg	31.5 ± 1.12*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

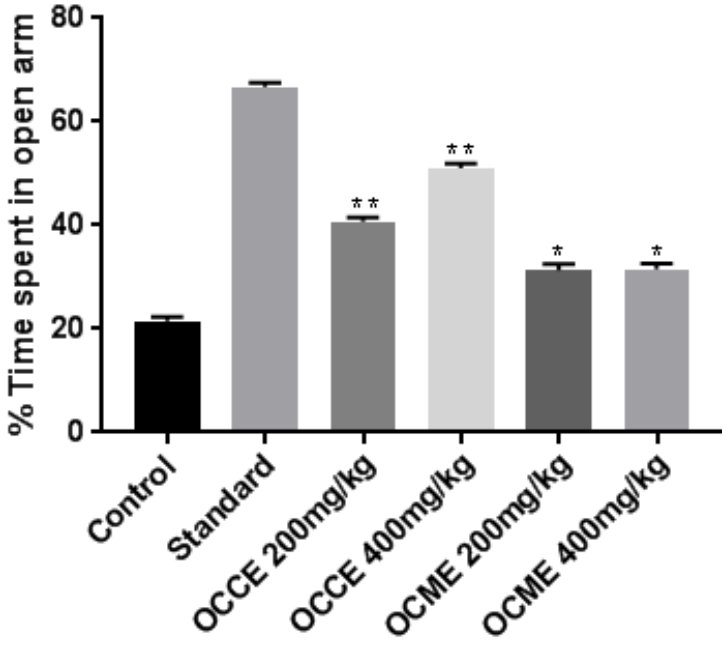


FIGURE 4.24, Effect of the chloroform and methanol extract of *O. corymbosa* on % time spent in open arm. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

The chloroform extract of *O. corymbosa* (200 and 400 mg/kg, p.o.) produced a significant (P<0.01) increase in % time spent in open arm of Elevated plus maze. The methanol extract of *O. corymbosa* (200 and 400 mg/kg, p.o.) produced a significant (P<0.05) increase in % time spent in open arm of Elevated plus maze**.** The chloroform extract of *O. corymbosa* (200 and 400 mg/kg, p.o.) showed better anxiolytic activity than methanol extract.

TABLE 4.12. Effect of *O. corymbosa* extracts on % Open arm entries

Group	Treatment	Dose	% Open arm entries
I	Control	-	6.17 ± 0.401
II	Standard (Diazepam)	1 mg/kg	31.5 ± 0.428
III	Oc. $CHCl_3$ Ext.	200 mg/kg	21 ± 1.06**
IV	Oc. $CHCl_3$ Ext.	400 mg/kg	21.2 ± 0.946**
V	Oc. MeOH Ext.	200 mg/kg	7.67 ± 0.667
VI	Oc. MeOH Ext.	400 mg/kg	12 ± 0.577*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

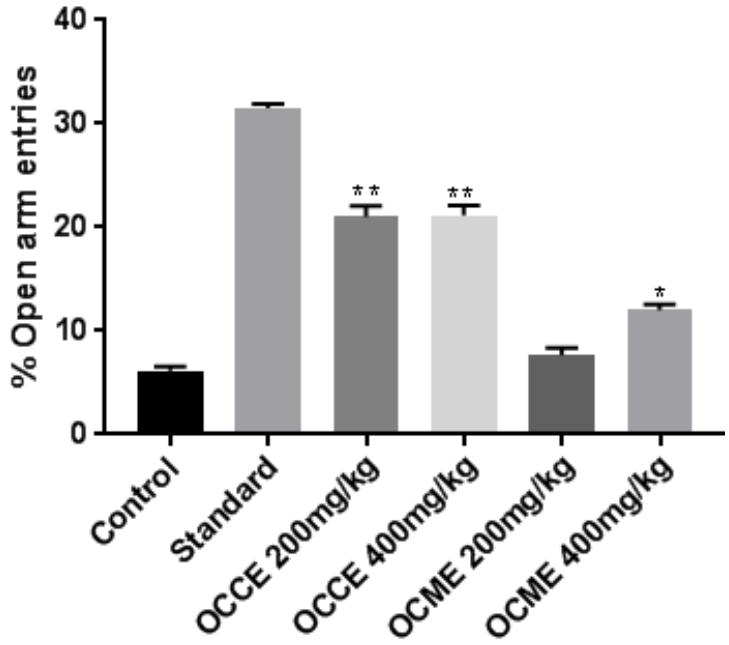


FIGURE 4.25, Effect of the chloroform and methanol extract of *O. corymbosa* on % open arm entries. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

The chloroform extract of *O. corymbosa* (200 and 400 mg/kg, p.o.) produced a significant (P<0.01) increase in % open arm entries in Elevated plus maze. The methanol extract of *O. corymbosa* (400 mg/kg, p.o.) produced a significant (P<0.05) increase in % open arm entries in Elevated plus maze**.** The chloroform extract of *O. corymbosa* (200 and 400 mg/kg, p.o.) showed better anxiolytic activity than methanol extract.

4.4.4 Exploratory behavior pattern by Hole board test

Hole-Board test is a measure of exploratory behavior and an agent that decreases this behavior reveals sedative activity.

TABLE 4.13. Effect of *O. corymbosa* extracts on no. of head dips

Group	Treatment	Dose	No. of head dip
I	Control	-	26.3 ± 0.989
II	Standard (Diazepam)	5 mg/kg	10.7 ± 0.558
III	Oc. $CHCl_3$ Ext.	200 mg/kg	20.8 ± 0.872*
IV	Oc. $CHCl_3$ Ext.	400 mg/kg	17.2 ± 0.703**
V	Oc. MeOH Ext.	200 mg/kg	24.2 ± 0.792
VI	Oc. MeOH Ext.	400 mg/kg	19.2 ± 0.703**

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

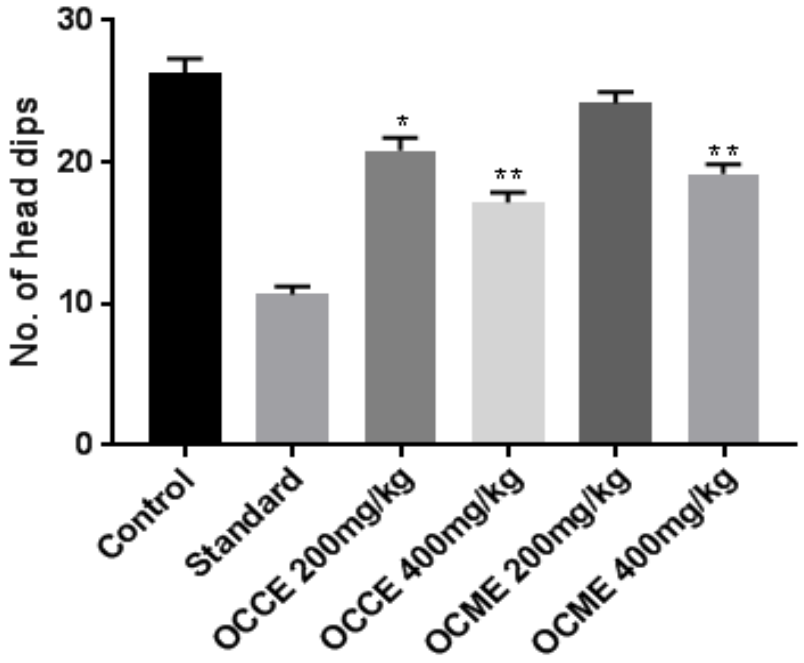


FIGURE 4.26, Effect of the chloroform and methanol extract of *O. corymbosa* on no. of head dips. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

The chloroform extract of *O. corymbosa* (200 and 400 mg/kg, p.o.) produced a significant (P<0.01) and dose-dependent reduction of exploratory behavior in the hole board test. The methanol extract of *O. corymbosa* (400 mg/kg, p.o.) produced a significant (P<0.01)

reduction of exploratory behavior in the hole board test. The methanol extract of *O. corymbosa* (200 mg/kg, p.o.) does not produced (P>0.05) any reduction of exploratory behavior in the hole board test.

4.4.5 Spontaneous motor activity

Monitoring of locomotor activity of animal has been an important step in assessing effects of drugs on the CNS. The movement is a measure of the level of excitability of the CNS and its decrease may be intimately related to sedation resulting from the depression of the CNS. The chloroform and methanol extracts of *O. corymbosa* (200 and 400 mg/kg, p.o.) produced a significant (P<0.05, P<0.01) and dose-dependent decrease in spontaneous motor activity. Likewise, positive control Imipramine (15 mg/kg, p.o.) also produced significant reduction in spontaneous motor activity. Decrease in the spontaneous motor activity leads to sedation as a result of reduced excitability of the central nervous system.

TABLE 4.14. Effect of *O. corymbosa* extracts on spontaneous locomotor activity

Group	Treatment	Dose	Locomotor activity
I	Control	-	140 ± 0.76
II	Standard (Imipramine)	15 mg/kg	31 ± 0.516
III	Oc. $CHCl_3$ Ext.	200 mg/kg	105 ± 0.833*
IV	Oc. $CHCl_3$ Ext.	400 mg/kg	82.3 ± 0.615**
V	Oc. MeOH Ext.	200 mg/kg	112 ± 0.577*
VI	Oc. MeOH Ext.	400 mg/kg	82.3 ± 0.882**

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

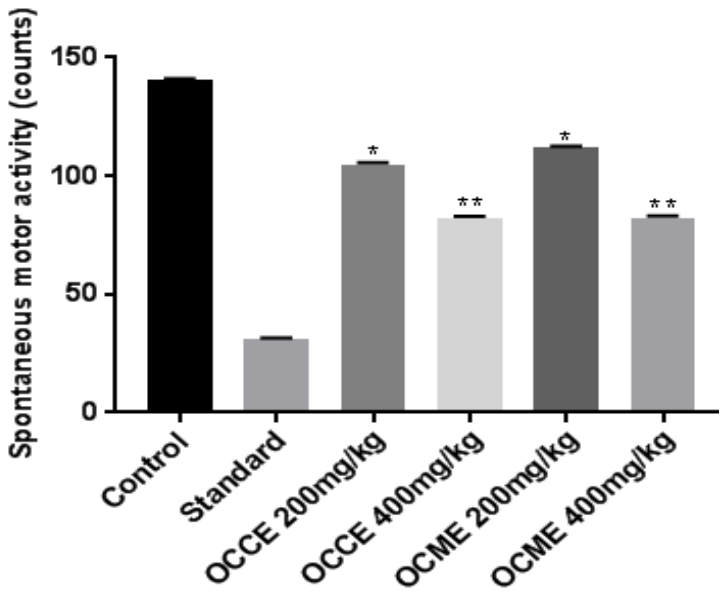


FIGURE 4.27, Effect of the chloroform and methanol extract of *O. corymbosa* on spontaneous locomotor activity. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

Grangea maderaspatana

4.5 Collection and Identification of Plant material

Plant collection and Identification

The plant of *Grangea maderaspatana* was collected in the month of December from Saputara.

Authentication was done by Taxonomist of the Botanical Survey of India, Jodhpur. A voucher specimen (No. BSI/AZRC/I.12012/Tech./2015-16/419) was deposited in the Herbarium of Botanical Survey of India, Jodhpur.

4.6 Assessment of quality of plant materials

4.6.1 Macroscopic evaluation

TABLE 4.15. Morphology of *G. maderasatana* leaf

Parameters	Observations
Type	Simple
Phyllotaxy	Alternate
Size	3.5-7.5 × 1.5-2.5 cm
Shape	Oblong-obovate
Margin	Sinuate, coarsely dentate
Apex	Obtuse
Base	Symmetric
Venation	Reticulate
Surface	Highly pubescent, hairs slightly longer on veins
Petiole	Absent (Sessile)
Color	Green
Odour	Characteristic
Taste	Characteristic

Stem: Prostrate, ascending to erect, which is up to 55 cm tall, branched from the base. Stem is green colored with numerous hairs on the surface.

Root: tap-root. White colored

Flowers: The inflorescence is a terminal, truncate-spherical head, 6-10 mm in diameter, solitary or 2-3 together, yellow colored. The peduncle is 1-4 cm long. The flowers are all tubular and about 1.5 mm long.

Fruit: The fruit is pale brown and compressed. Achenes are cylindric, glandular, and about 2 millimeters long

4.6.2 Microscopic evaluation

Leaf:

The transverse section of leaf of *Grangea maderaspatana* shows dorsiventral nature. The section is broadly divided in to lamina and midrib region. The lamina of leaf shows three distinct regions viz., upper epidermis, lower epidermis and mesophyll. The upper and lower epidermis is single layer of cells covered by cuticle. The mesophyll is differentiated in to palisade and spongy parenchyma. The palisade parenchyma are narrow, closely packed, elongated cylindrical cells. The cells of spongy parenchyma are compactly arranged. Multicellular covering trichomes and glandular trichomes are seen.

Midrib shows the epidermal cells are polygonal in shape covered by thin cuticle. Few collenchymatous cells are present below the upper epidermis and above the lower epidermis. The ground tissue is parenchymatous. The vascular bundle is bicollateral type.

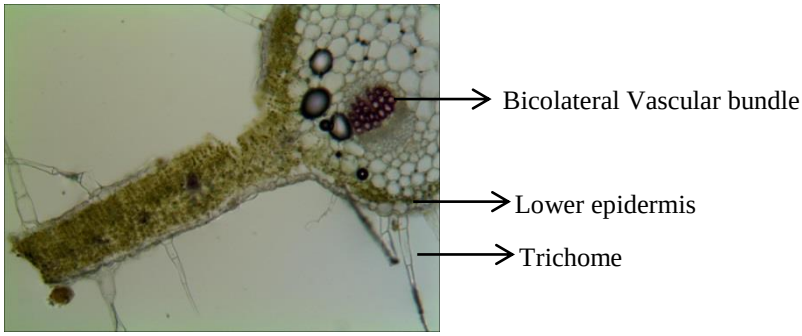


FIGURE 4.28, T.S of *G. maderaspatana* leaf (stained)

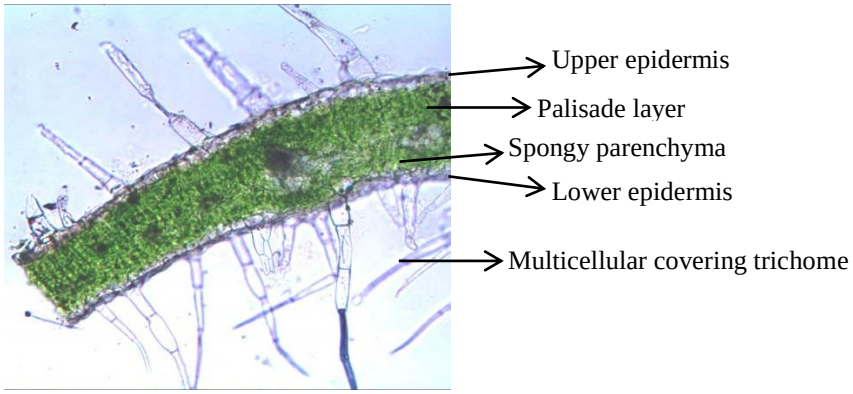


FIGURE 4.29, T. S of *G. maderaspatana* leaf showing lamina

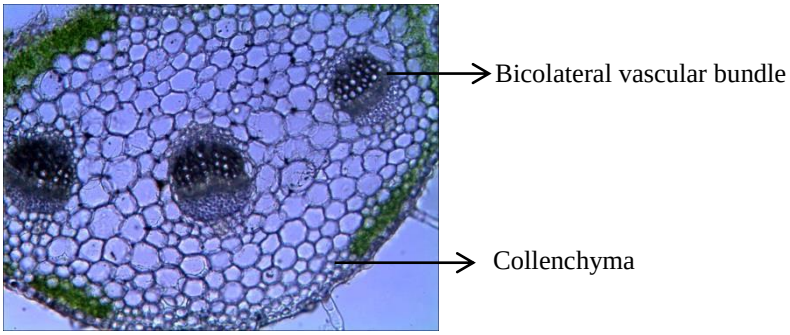


FIGURE 4.30, T.S of *G. maderaspatana* leaf showing midrib

Stem: Transverse section of stem shows circular outline. The epidermis is single layered covered with cuticle. Epidermis consists of multicellular covering trichomes. Cortex is 6 to 8 layers thick and made up of thin walled parenchymatous cells. Some cells contain microsphenoidal calcium oxalate crystals. Endodermis is indistinct. Phloem is narrow zone consisting of 4 to 6 layers of cells. The xylem consists of small vessels and parenchyma. The pith in the centre is large and made up of thin walled parenchymatous cells.

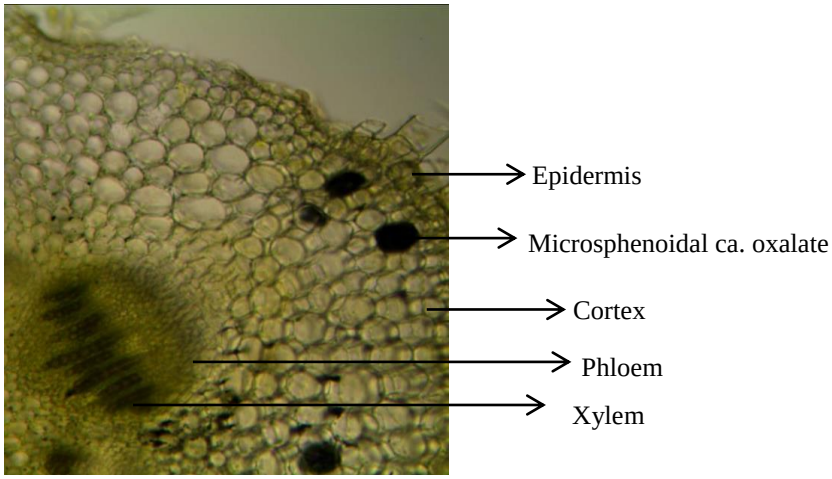


FIGURE 4.31, T.S. of *G. maderaspatana* stem (unstained)

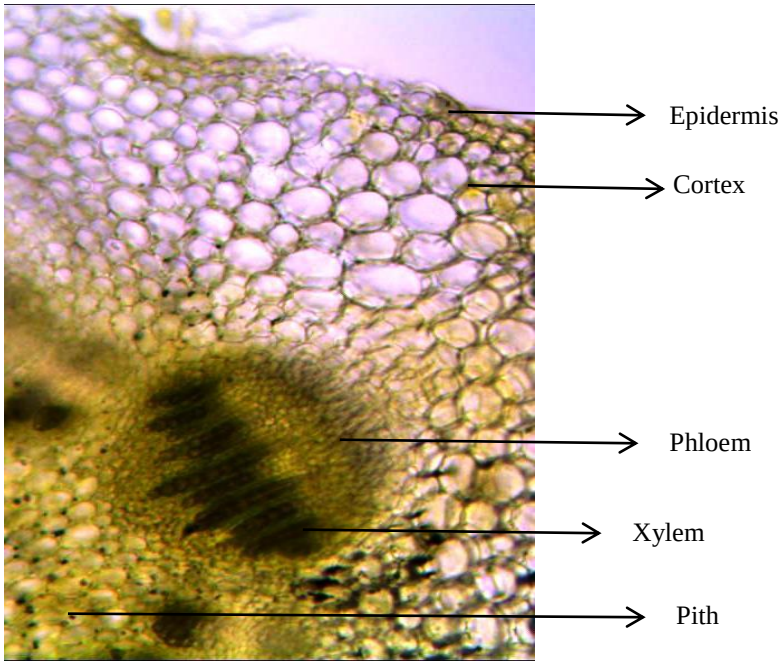


FIGURE 4.32, T.S. of *G. maderaspatana* stem (stained)

Root:

Transverse section of root shows circular outline. Cork is made up of 2-4 layers of thin walled cells. Cortex is made up of 5-7 layers of rectangular parenchymatous cells. The pericycle appers like semilunar patches of pericyclic fibres with parenchyma in between. The Phloem is 5-7 layers thick. The xylem consists of small vessels, fibres.

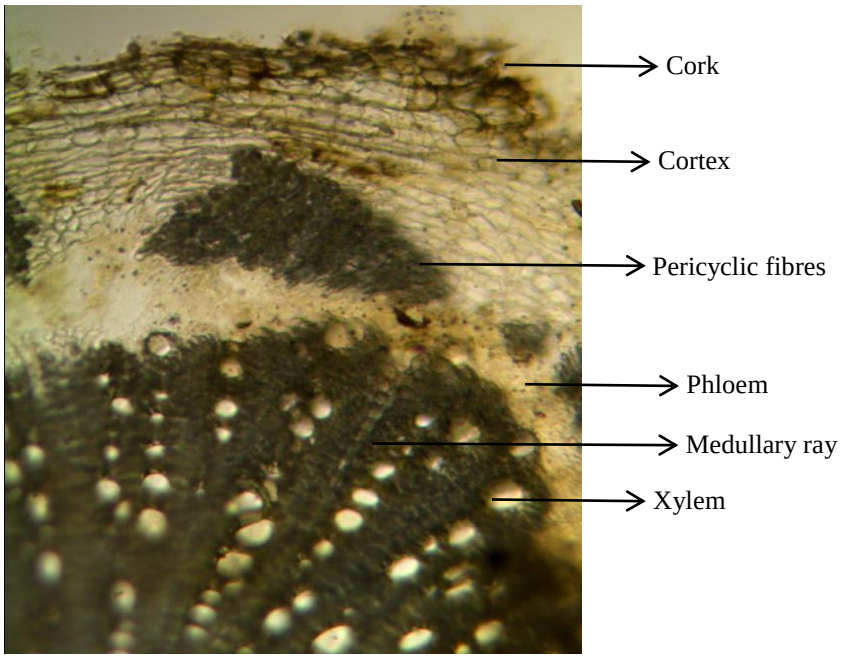


FIGURE 4.33, T.S. of *G. maderaspatana* root (unstained)

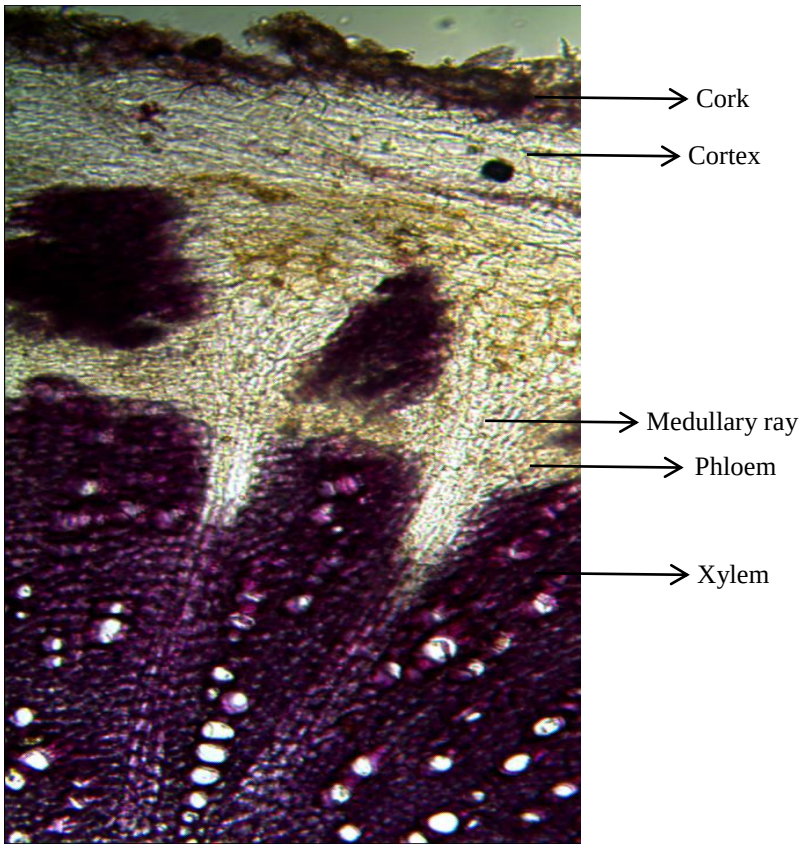
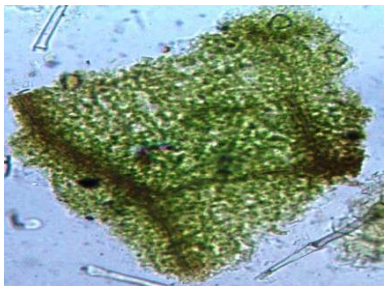


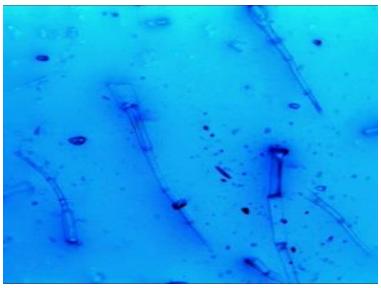
FIGURE 4.34, T.S. of *G. maderaspatana* root (stained)

4.6.2.1. Powder characteristics

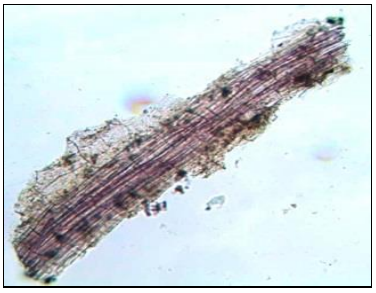
The organoleptic evaluation of powder revealed the following characteristics. The powder is light green color with characteristic odor and taste. On microscopic examination, the powder showed lamina, anisocytic and anomocytic stomata, multicellular covering trichomes, lignified fibres, phloem, cork, xylem.



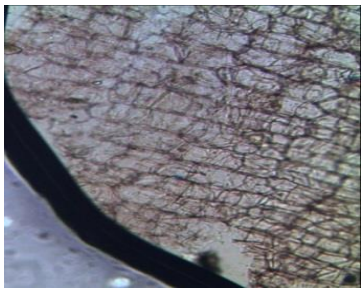
Lamina (10x10)



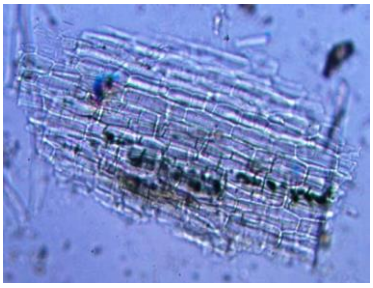
Multicellular covering trichomes (10x10)



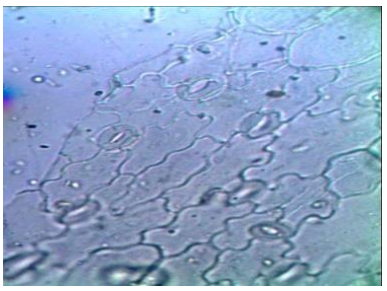
Lignified fibres (10x10)



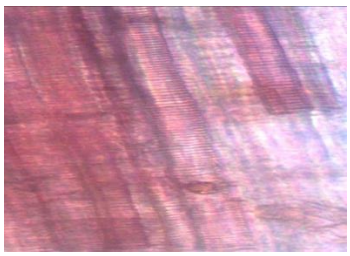
Cork (10x10)



Phloem (10x10)



Anisocytic and Anomocytic stomata (10x10)



Xylem (10x10)

FIGURE 4.35, Powder characteristics of *G. maderaspatana*

4.6.2.2 Quantitative microscopy of *G. maderaspatana* Leaf

Leaf constants

The leaf constants viz., stomatal index, vein islet number, vein termination number and palisade ratio are presented in Table 4.16.

TABLE 4.16. Quantitative microscopy of *G. maderaspatana* leaf

Sr. no.	Parameters	Values
1	Stomatal index	25-33
3	Vein islet number	2-5
4	Vein termination number	9-14
5	Palisade ratio	6.00-8.00

4.6.3 Proximate analysis

The results obtained from various determinations are compiled in Table 4.17.

TABLE 4.17. Physico-Chemical Parameters of powder of *G. maderaspatana*

Sr. No.	Parameters	Values (% w/w)
1	Loss on drying	8.00%
2	Ash value	
	Total ash	11.60%
	Acid insoluble ash	1.80%
	Water soluble ash	4.50%
3	Extractive value	
	Water soluble extractive	10.00%
	Alcohol soluble extractive	10.00%
4	Foaming Index	< 100

The values given here are expressed as percentage of air dried material. Each value is average of three determinations.

4.6.3.1 Determination of total tannin content

The content of total tannins in *Grangea maderaspatana* plant powder is 1.86%.

4.6.4 Estimation of heavy metals

Contamination of the medicinal plant materials with heavy metals can cause chronic or acute poisoning. Therefore it has become necessary that all the starting materials should be ensured for their heavy metal content including other necessary inorganic elements. Elemental analysis was carried out to determine the presence of heavy metals and the results are presented in the table below.

TABLE 4.18. Result of heavy metals content in *G. maderaspatana* plant powder

Sr. no.	Heavy Metal	Result (ppm)
1	Arsenic	Not detected
2	Cadmium	0.28 ppm
3	Lead	Not detected
4	Mercury	Not detected

4.7 Phytochemical studies

4.7.1 Preliminary phytoprofile

Powdered parts of *Grangea maderaspatana* were subjected to successive solvent extraction (except water extract which was prepared by decoction). The different extracts obtained with their % yield, color, consistency are recorded in Table-4.19.

TABLE 4.19. Preliminary phytoprofile of *G. maderaspatana*

Sr. no.	Solvent	Color	Consistency	%Yield w/w
1	Petroleum Ether	Light green	Slight Sticky	4.82
2	Chloroform	Dark green	Slight Sticky	1.37
3	Ethyl acetate	Brownish Green	Sticky	2.50
4	Methanol	Brown	Slight Sticky	6.20
5	Water	Dark brown	Slight Sticky	5.34

4.7.2 Qualitative chemical tests

The extracts obtained from successive solvent extraction process were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like steroids, carbohydrates, alkaloids, glycosides, phenolics and tannins etc.

TABLE 4.20. Phytochemical screening of extracts of *G. maderaspatana*

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)

4.7.3 HPTLC fingerprinting

HPTLC fingerprinting of chloroform and methanol extracts of *G. maderaspatana* for oleanolic acid and ursolic acid.

Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 – Applicator
Application of sample	Automatic device "CAMAG LINOMAT – 5"
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets (10 x 10 cm)
Application Volume	10 µL
Mobile Phase	Toluene : Ethyl acetate : Formic acid (8 : 2 : 0.1)
Development Mode	CAMAG TLC Twin Trough Chamber
Spray reagent	Anisaldehyde sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Visualization	@ 510 nm after derivatization

1. Gm. MeOH ext.
2. Std – Oleanolic acid
3. Std – Ursolic acid
4. Gm. $CHCl_3$ ext.

FIGURE 4.36, HPTLC plate of *G. maderaspatana* extracts after derivatization

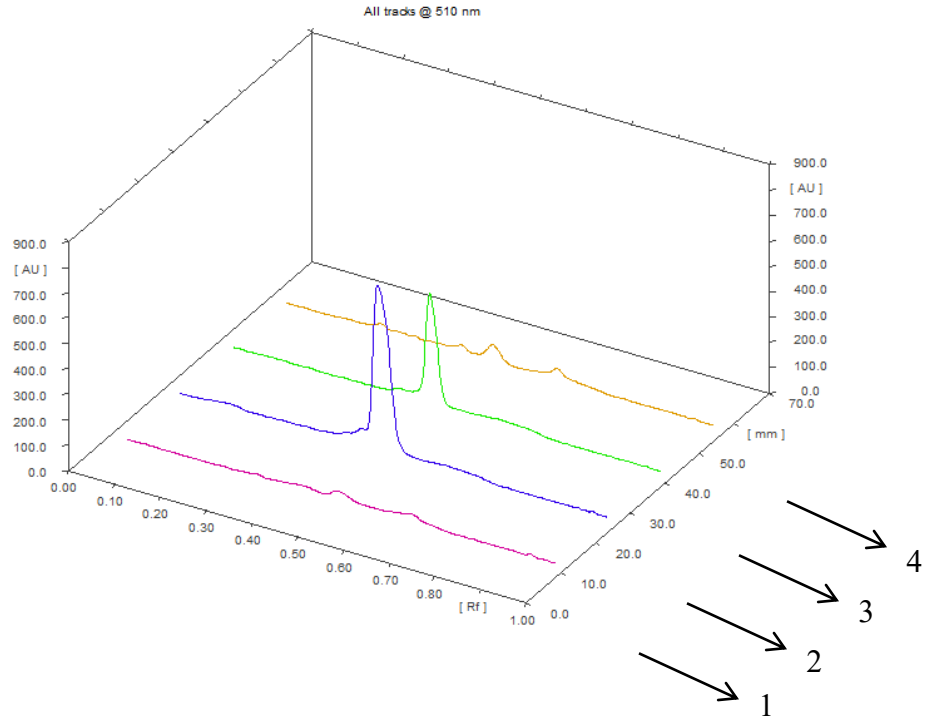


FIGURE 4.37, 3D Graph of standard and extracts of *Grangea maderaspatana*

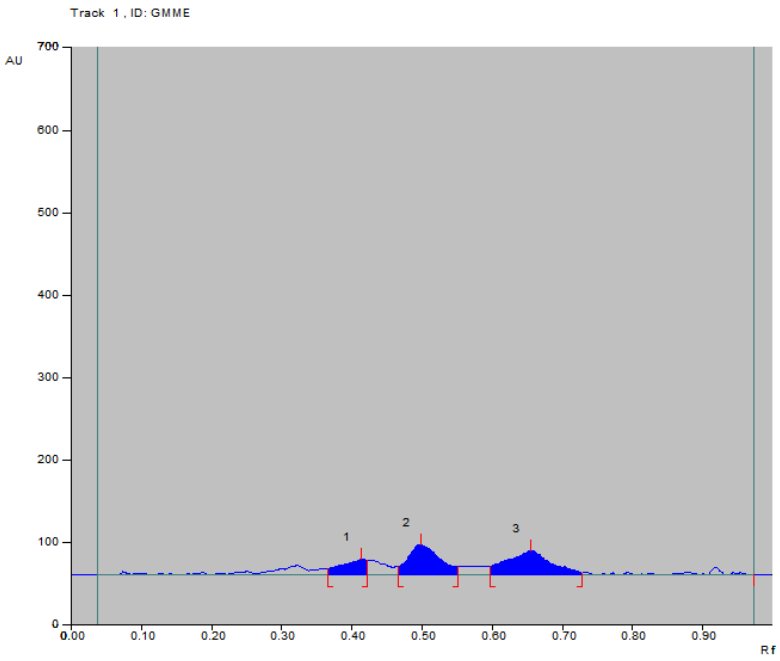


FIGURE 4.38, Chromatogram of methanol extract of *G. maderaspatana*

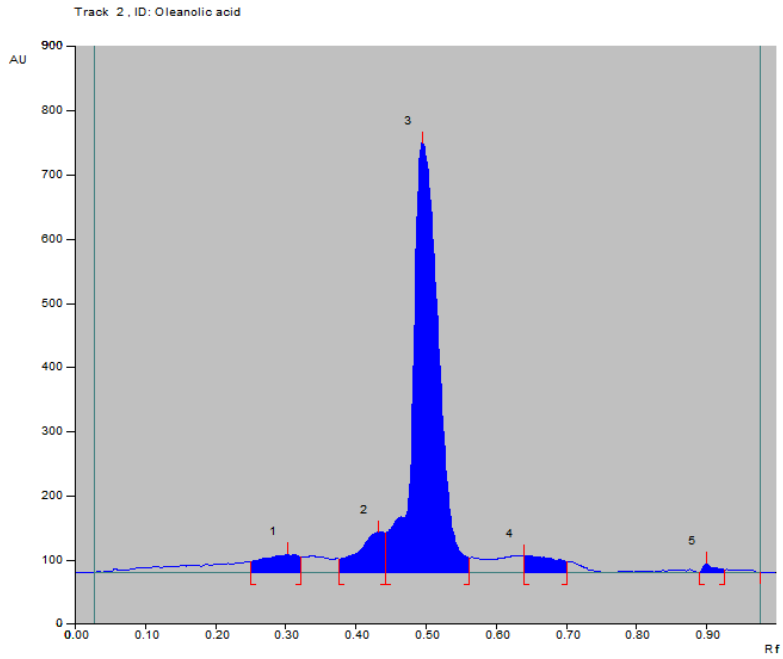


FIGURE 4.39, Chromatogram of standard Oleanolic acid

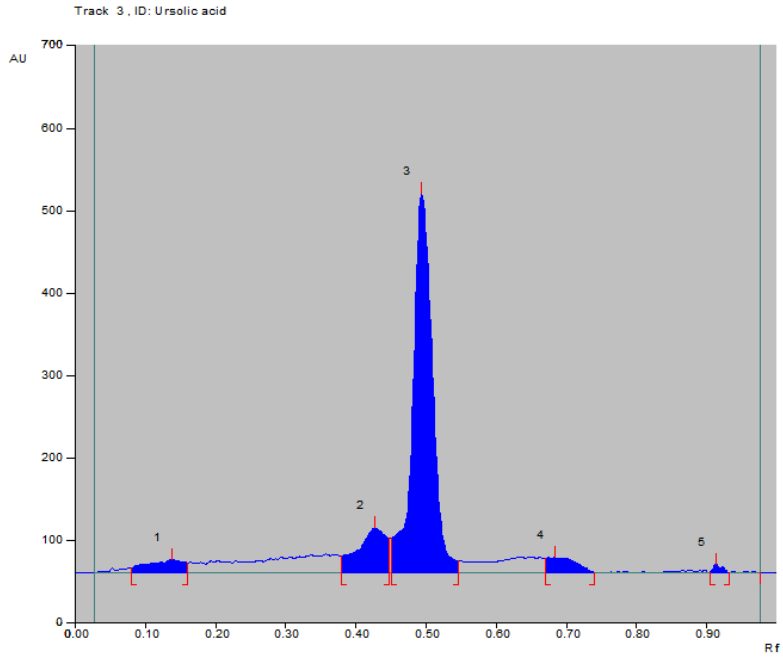


FIGURE 4.40, Chromatogram of standard Ursolic acid

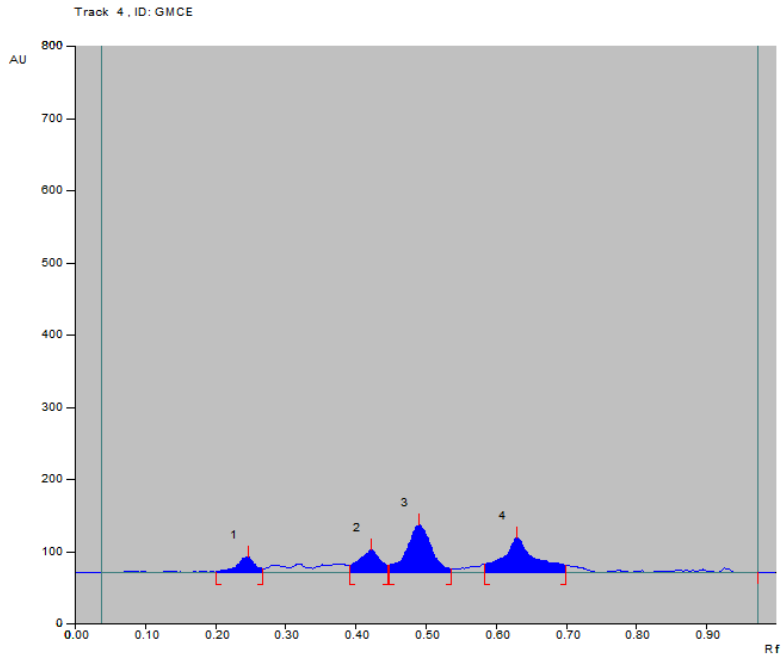


FIGURE 4.41, Chromatogram of chloroform extract of *G. maderaspatana*

TABLE 4.21. Result of HPTLC analysis of extracts of *G. maderaspatana*

	Sample			
	GMME	**Oleanolic acid**	**Ursolic acid**	**GMCE**
Rf value	**0.50**	**0.48**	**0.48**	**0.49**
AUC	**1517.5**	**24373.6**	**11179.7**	**2142.7**

The chloroform extract of *Grangea maderaspatana* contains 4.0% and 9.5% of oleanolic acid and ursolic acid respectively, although the methanol extract contains 3.0% and 6.5% of oleanolic acid and ursolic acid respectively.

4.7.4 HPLC

Estimation of Gallic acid in methanol extracts of *G. maderaspatana* by HPLC method.

Chromatographic Conditions:

Stationary Phase	Phenomenex Luna C18 (4.6 x 250mm, 5µ particle size)
Mobile Phase	Water : Acetonitrile (80 : 20 % v/v)
Wavelength	272 nm
Flow Rate	1 mL/min
Total Run time	Max. up to 7 min.
Injection Volume	20 µL
Temperature	Ambient
Mode of Operation	Isocratic elution

TABLE 4.22. Linearity of Gallic acid

Concentration(µg/ml)	Peak Area
10	21.54767
20	58.50933
30	94.488
40	127.4617
50	163.156
60	190.9767
70	207.4273

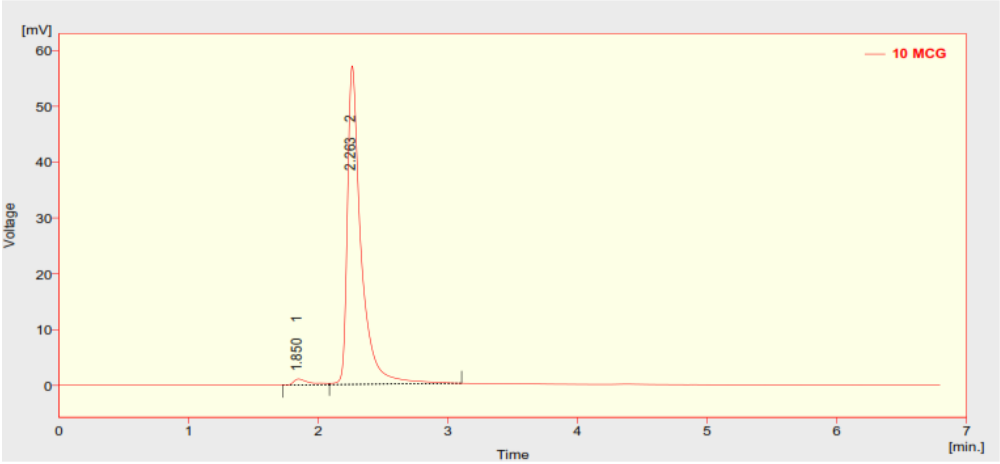


FIGURE 4.42, Chromatogram of Gallic acid (10µg/ml)

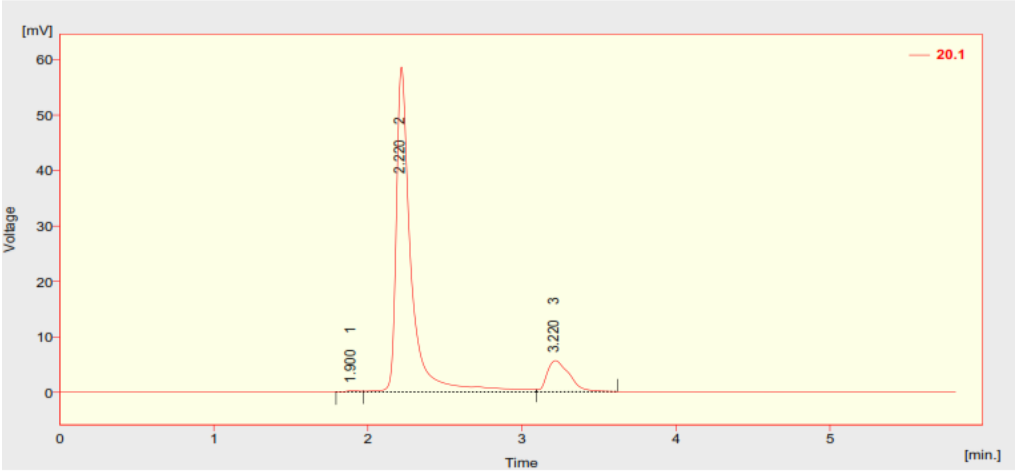


FIGURE 4.43, Chromatogram of Gallic acid (20µg/ml)

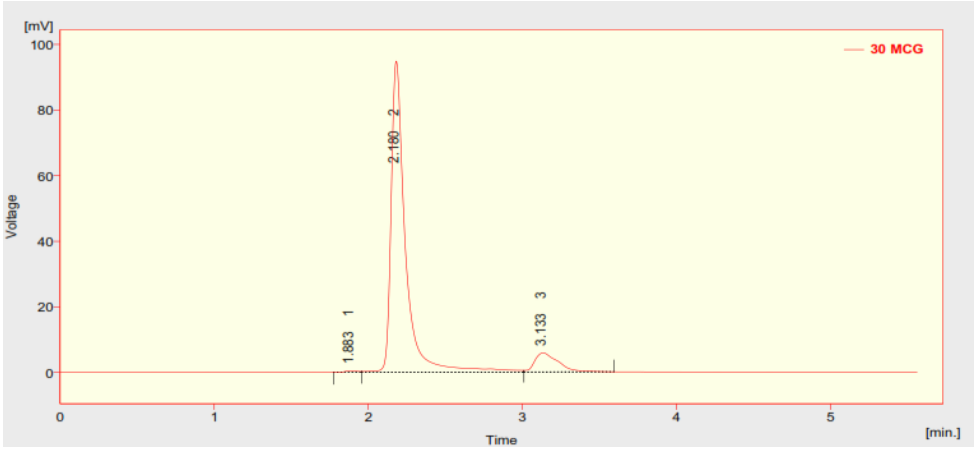


FIGURE 4.44, Chromatogram of Gallic acid (30µg/ml)

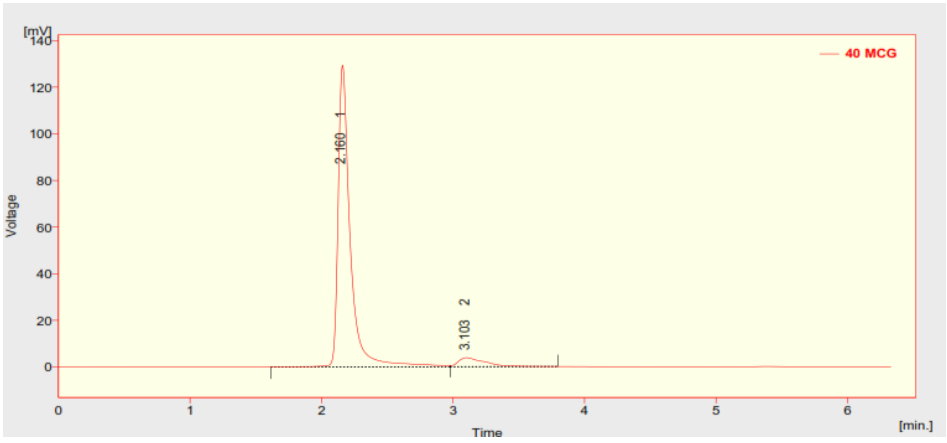


FIGURE 4.45, Chromatogram of Gallic acid (40µg/ml)

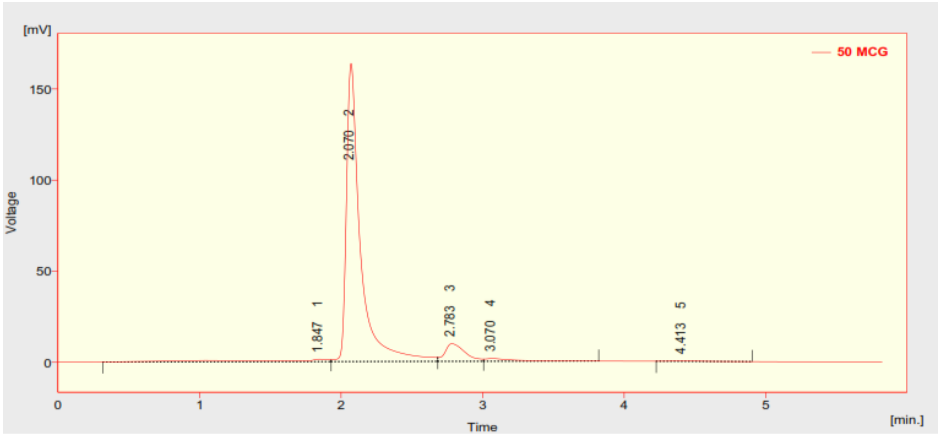


FIGURE 4.46, Chromatogram of Gallic acid (50µg/ml)

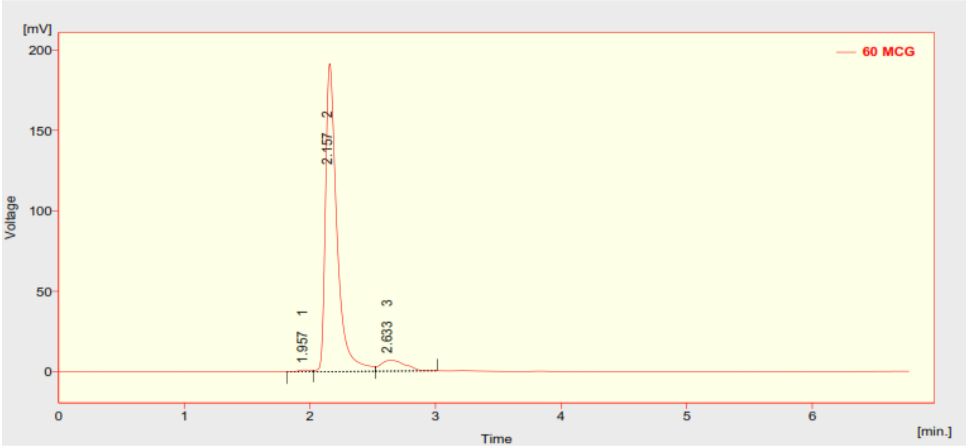


FIGURE 4.47, Chromatogram of Gallic acid (60µg/ml)

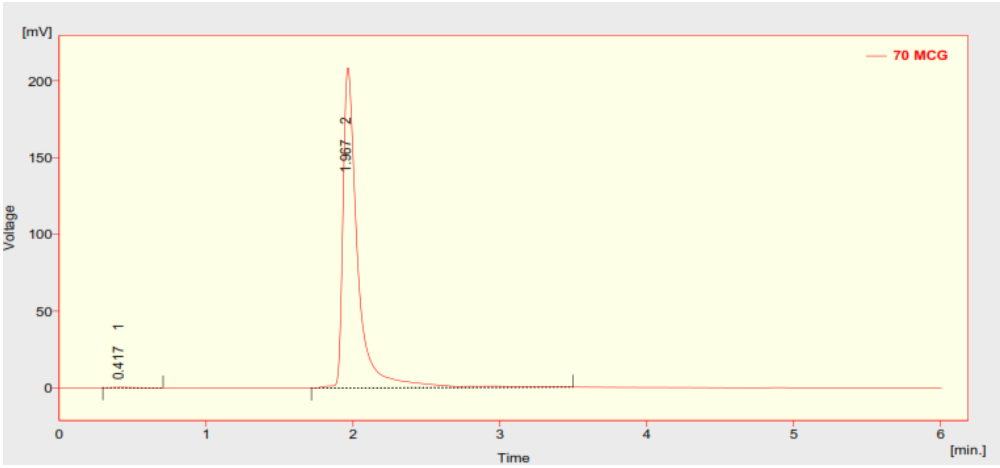


FIGURE 4.48, Chromatogram of Gallic acid (70µg/ml)

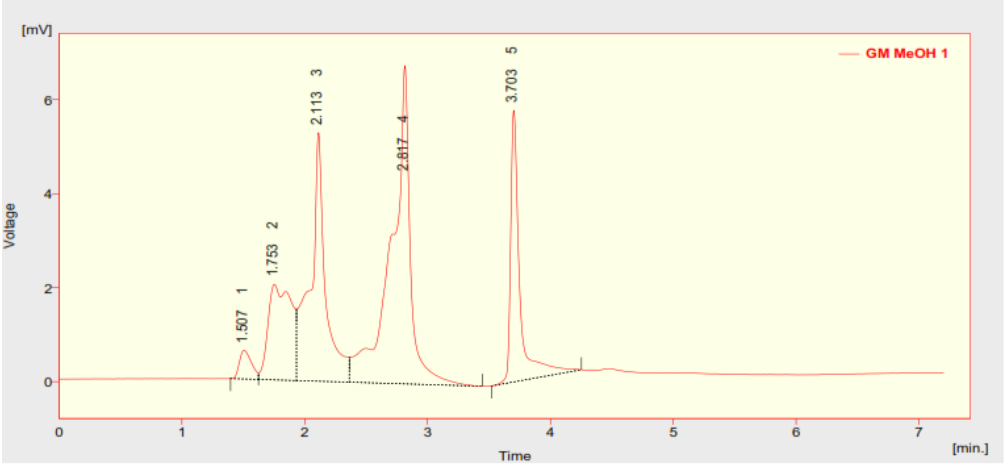


FIGURE 4.49, Chromatogram of methanol extract of *G. maderaspatana* (1000µg/ml)

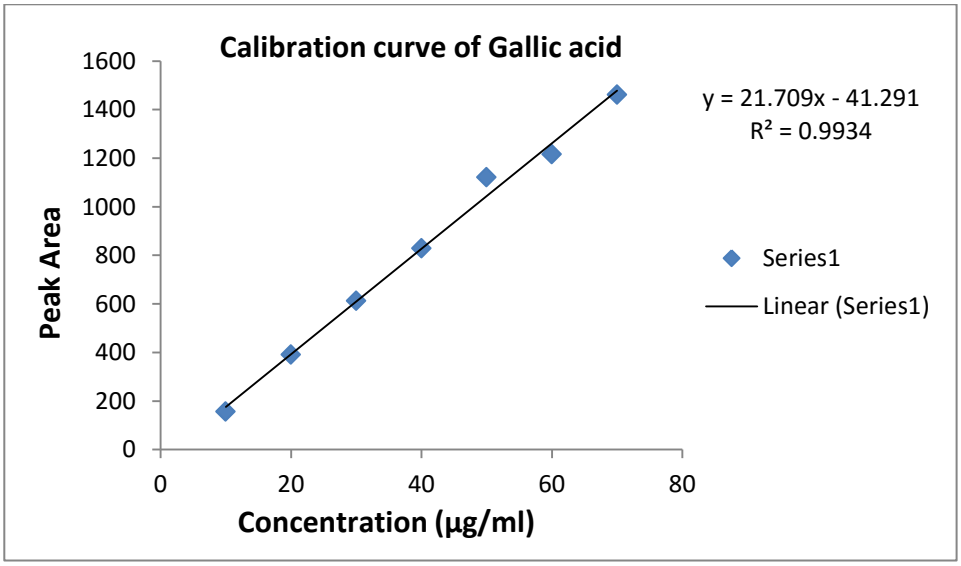


FIGURE 4.50, Calibration curve of Gallic acid

TABLE 4.23. Summary output (Regression statistics)

Multiple R Square	**0.999228834**
R Square	**0.998458263**
Adjusted R Square	**0.798458263**
Equation of line	**y = 21.709x - 41.291**

The methanol extract of *Grangea maderaspatana* contains 4.00 % of Gallic acid.

4.8 Psychopharmacological Activity

The results of the present investigation showed that the chloroform and methanol extracts of *Grangea maderaspatana* have some psychopharmacological activity.

4.8.1 Acute toxicity study

The chloroform and methanol extracts of *Grangea maderaspatana* were well tolerated by mice and there were no signs of acute or delayed toxicity after oral administration. Increasing doses up to 2000 mg/kg (p.o.) were not lethal, the LD50 values for the extract was estimated to be higher than 2000 mg/kg for oral administration. Thus, suggesting that this administration route is adequate and secure to produce its psychopharmacological effects.

4.8.2 Antidepressant Activity by Forced swim test model

The antidepressant activity of the chloroform and methanol extracts of *G. maderaspatana* (200 & 400 mg/kg) was evaluated by Forced swim test model. Both the extracts exerted antidepressant activity in mice. The chloroform extract (200 & 400 mg/kg) showed dose dependent increases in the immobility of mice when compared with control group. The methanol extract (200 & 400 mg/kg) showed increases in the immobility of mice ($p<0.05$) (Figure 4.51).

TABLE 4.24. Effect of *G. maderaspatana* extracts on immobility time

Group	Treatment	Dose	Immobility period (Sec.)
I	Control	-	150 ± 0.843
II	Standard (Imipramine)	15 mg/kg	103 ± 1.78
III	Gm. $CHCl_3$ Ext.	200 mg/kg	120 ± 0.872*
IV	Gm. $CHCl_3$ Ext.	400 mg/kg	111 ± 1.53**
V	Gm. MeOH Ext.	200 mg/kg	120 ± 1.05*
VI	Gm. MeOH Ext.	400 mg/kg	122 ± 1.08*

Values are expressed as mean ± SEM; n=6 in each group; *$p<0.05$; **$p<0.01$; ***$p<0.001$, significant as compared to control. Not significant – $p>0.05$.

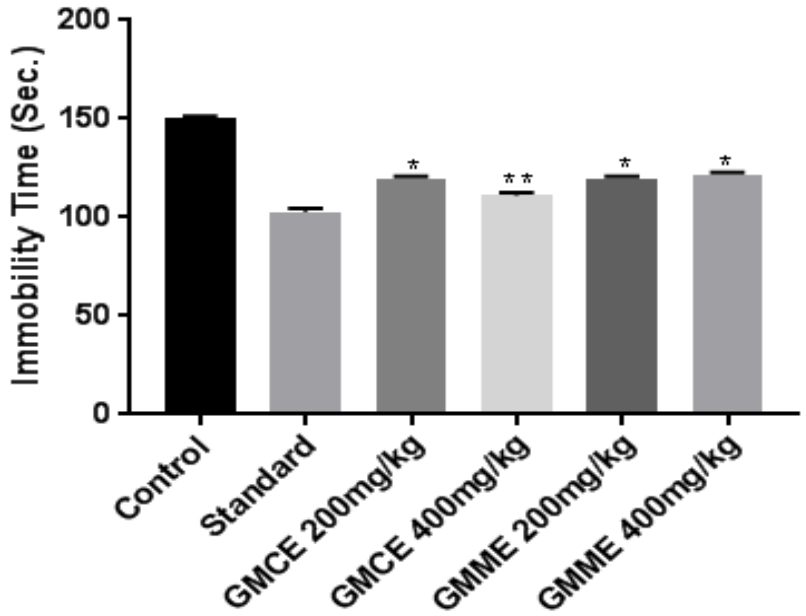


FIGURE 4.51, Effect of the chloroform and methanol extract of *G. maderaspatana* on immobility time. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *p<0.05, **p<0.01 when compared with control group

8.3 Anxiolytic Activity by Elevated plus maze model

Anti-anxiety activity of chloroform and methanol extract of *G. maderaspatana* was evaluated by elevated plus-maze model. The model was chosen as it is effective, inexpensive, modest, less time consuming, requires no preliminary training to the mice and does not cause much distress to the animals while handling. The model is principally based on the observations that the exposure of animals to an elevated and open maze results in approach–avoidance conflict which is manifested as an exploratory-cum-fear drive. The fear due to height induces anxiety in the animals when placed on the elevated plus-maze. The ultimate sign of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by the time spent by the animal in the open arms.

TABLE 4.25. Effect of *G. maderaspatana* extracts on % Time spent in open arm of EPM

Group	Treatment	Dose	% Time spent in open arm
I	Control	-	21.5 ± 0.885
II	Standard (Diazepam)	1 mg/kg	66.7 ± 0.882
III	Gm. $CHCl_3$ Ext.	200 mg/kg	35.7 ± 0.715*
IV	Gm. $CHCl_3$ Ext.	400 mg/kg	43.5 ± 1.18**
V	Gm. MeOH Ext.	200 mg/kg	28.5 ± 1.26
VI	Gm. MeOH Ext.	400 mg/kg	32.3 ± 1.48*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

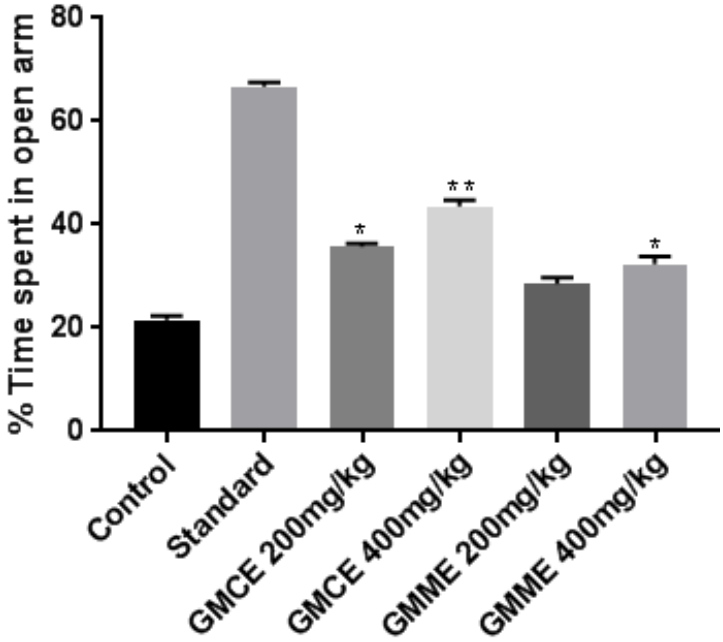


FIGURE 4.52, Effect of the chloroform and methanol extract of *G. maderaspatana* on % time spent in open arm. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

The chloroform extract of *G. maderaspatana* (200 and 400 mg/kg, p.o.) produced a significant (P<0.01) and dose dependent increase in % time spent in open arm of Elevated plus maze. The methanol extract of *G. maderaspatana* (400 mg/kg, p.o.) produced a significant (P<0.05) increase in % time spent in open arm of Elevated plus maze**.** The chloroform extract of *G. maderaspatana* (400 mg/kg, p.o.) showed better anxiolytic activity than methanol extract.

TABLE 4.26. Effect of *G. maderaspatana* extracts on % Open arm entries

Group	Treatment	Dose	% Open arm entries
I	Control	-	6.17 ± 0.401
II	Standard (Diazepam)	1 mg/kg	31.5 ± 0.428
III	Gm. $CHCl_3$ Ext.	200 mg/kg	16.8 ± 0.654**
IV	Gm. $CHCl_3$ Ext.	400 mg/kg	19.2 ± 0.703**
V	Gm. MeOH Ext.	200 mg/kg	5.5 ± 0.764
VI	Gm. MeOH Ext.	400 mg/kg	13.5 ± 1.12*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

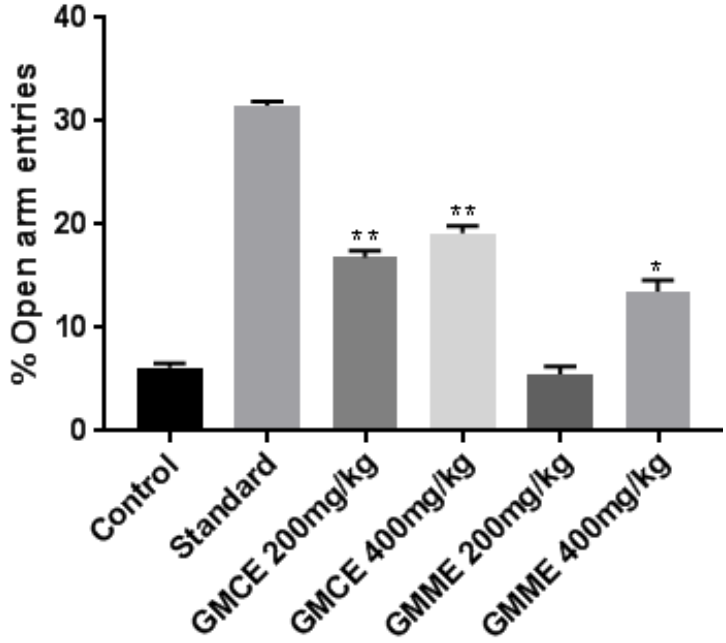


FIGURE 4.53, Effect of the chloroform and methanol extract of *G. maderaspatana* on % open arm entries. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group.

The chloroform extract of *G. maderaspatana* (200 and 400 mg/kg, p.o.) produced a significant (P<0.01) increase in % open arm entries in Elevated plus maze. The methanol extract of *O. corymbosa* (400 mg/kg, p.o.) produced a significant (P<0.05) increase in % open arm entries in Elevated plus maze**.** The chloroform extract of *O. corymbosa* (200 and 400 mg/kg, p.o.) showed better anxiolytic activity than methanol extract.

8.4 Exploratory behavior pattern by Hole board test

Hole-Board test is a measure of exploratory behavior and an agent that decreases this behavior reveals sedative activity. The chloroform and methanol extract of *G. maderaspatana* reduced exploratory behavior in the hole board test.

TABLE 4.27. Effect of *G. maderaspatana* extracts on no. of head dips

Group	Treatment	Dose	No. of head dip
I	Control	-	26.3 ± 0.989
II	Standard (Diazepam)	5 mg/kg	10.7 ± 0.558
III	Gm. $CHCl_3$ Ext.	200 mg/kg	23.8 ± 0.946
IV	Gm. $CHCl_3$ Ext.	400 mg/kg	19.3 ± 1.12**
V	Gm. MeOH Ext.	200 mg/kg	24.5 ± 0.992
VI	Gm. MeOH Ext.	400 mg/kg	21.5 ± 0.764*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

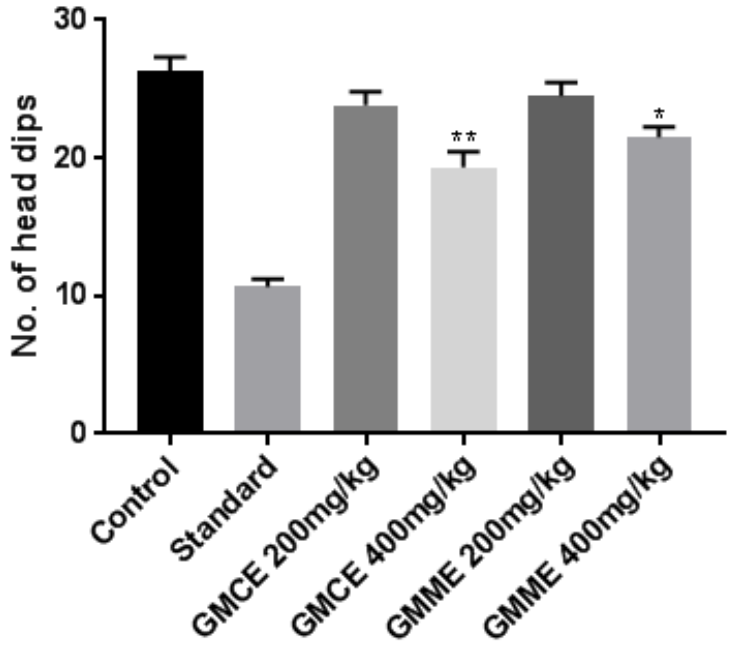


FIGURE 4.54, Effect of the chloroform and methanol extract of *G. maderaspatana* on no. of head dips. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

The chloroform extract of *G. maderaspatana* (200 and 400 mg/kg, p.o.) produced a significant (P<0.01) reduction of exploratory behavior in the hole board test. The methanol

extract of *G. maderaspatana* (400 mg/kg, p.o.) produced a significant (P<0.05) reduction of exploratory behavior in the hole board test. The methanol extract of *G. maderaspatana* (200 mg/kg, p.o.) does not produced (P>0.05) any reduction of exploratory behavior in the hole board test.

8.5 Spontaneous motor activity by Actophotometer

Monitoring of locomotor activity of animal has been an important step in assessing effects of drugs on the CNS. The movement is a measure of the level of excitability of the CNS and its decrease may be intimately related to sedation resulting from the depression of the CNS.

TABLE 4.28. Effect of *G. maderaspatana* extracts on spontaneous locomotor activity

Group	Treatment	Dose	Locomotor activity
I	Control	-	140 ± 0.76
II	Standard (Imipramine)	15 mg/kg	31 ± 0.516
III	Gm. $CHCl_3$ Ext.	200 mg/kg	120 ± 0.703*
IV	Gm. $CHCl_3$ Ext.	400 mg/kg	104 ± 0.703**
V	Gm. MeOH Ext.	200 mg/kg	123 ± 0.882*
VI	Gm. MeOH Ext.	400 mg/kg	105 ± 0.992**

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

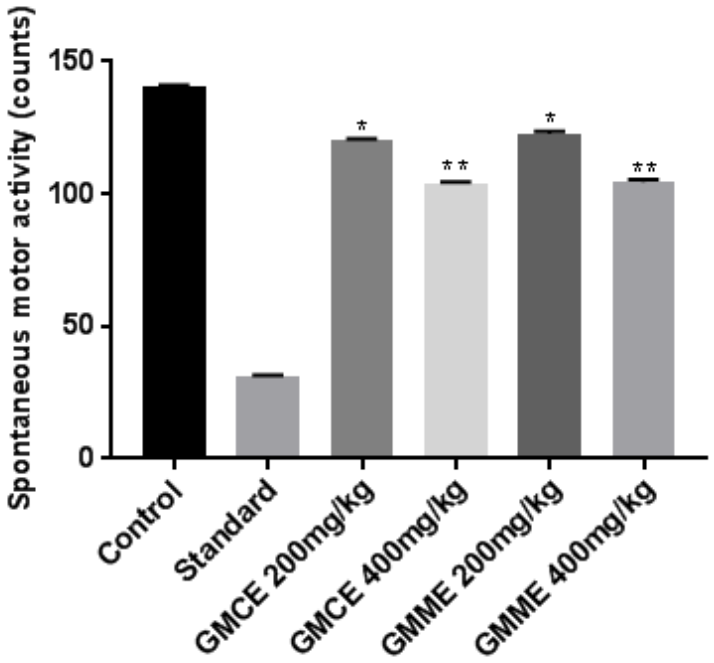


FIGURE 4.55, Effect of the chloroform and methanol extract of *G. maderaspatana* on spontaneous locomotor activity. Each bar represents the mean ± SEM (n = 6). One way ANOVA followed by Dunnett's test, *P<0.05, **p<0.01 when compared with control group

The chloroform and methanol extracts of *G. maderaspatana* (200 and 400 mg/kg, p.o.) produced a significant (P<0.05, p<0.01) and dose-dependent decrease in spontaneous motor activity. Likewise, positive control Imipramine (15 mg/kg, p.o) also produced significant reduction in spontaneous motor activity. Decrease in the spontaneous motor activity leads to sedation as a result of reduced excitability of the central nervous system.

Both the extracts (400 mg/kg) significantly decreased locomotor activity and increased immobility time as shown by the results of the above-mentioned tests suggesting depression and sedating potentials. Sedation may be due to interaction with benzodiazepines-like compounds.

CHAPTER 5

SUMMARY & CONCLUSION

Chapter 5

Conclusion

Present work is an attempt to compile pharmacognostic phytochemical and pharmacological work on *Oldenlandia corymbosa* and *Grangea maderaspatana.*

Both the plants were identified by Botanical survey of India, Jodhpur.

In morphological study it was found that *Oldenlandia corymbosa* leaf is simple, opposite, sessile, linear-lanceolate, acute; stem is green-purple, quadrangular; tap root is white colored; flower is sessile white colored; fruit is globose capsular. *Grangea maderaspatana* leaf is simple, alternate, oblong-ovate, obtuse, sinuate, highly pubescent; stem is prostrate, green colored, pubescent; tap root is white colored; flower is solitary yellow colored; fruit is cylindric, glandular.

In microscopic study it was found that *Oldenlandia corymbosa* leaf is dorsiventral, colateral vascular bundle, covering trichome; stem shows quadrangular, epidermis with covering trichomes, colateral vascular bundle, pith; root shows cork, cortex with raphides, phloem and xylem. *Grangea maderaspatana* leaf is dorsiventral, bicolateral vascular bundle, covering trichome; stem shows epidermis with covering trichomes, cortex with microsphenoidal calcium oxalate crystals, phlloem, xylem and pith; root shows cork, cortex,pericyclic fibres, phloem, xylem and medullary rays.

In powder microscopy, *Oldenlandia corymbosa* powder shows presence of paracytic stomata, raphides, cork, phloem and xylem. *Grangea maderaspatana* powder shows presence of Anisocytic and anomocytic stomata, fibres, cork, multicellular covering trichome, phloem and xylem.

The powdered drugs were subjected to phytochemical screening after successive solvent extraction. Qualitative chemical examination of extracts revealed presence of saponins, carbohyderates, triterpenes, phytosterols and phenolics/tannins.

The presence of Oleanolic acid and ursolic acid in chloroform and methanol extracts of both plants were confirmed by HPTLC fingerprinting and the content was calculated from AUC of oleanolic acid and ursolic acid. The chloroform extract of *O. corymbosa* contains 32.0% and 61.0% of oleanolic acid and ursolic acid respectively. The methanol extract of *O. corymbosa* contains 23.5% and 45.0% of oleanolic acid and ursolic acid respectively. The chloroform extract of *G. maderaspatana* contains 4.0% and 9.5% of oleanolic acid and ursolic acid respectively. The methanol extract of *G. maderaspatana* contains 3.0% and 6.5% of oleanolic acid and ursolic acid respectively.

Gallic acid was estimated by HPLC method, a linear relationship was observed within the range of 10-70 µg/ml and correlation coefficient was 0.9934. The content of Gallic acid by HPLC method in *O. corymbosa* and *G. maderaspatana* was 2.45% w/w and 4.00% w/w respectively.

Toxicity studies were performed for different extract to assess their safety in mice. Methanol, and chloroform extract of both plants were found safe and did not cause any mortality at the dose of 2000 mg/kg body weight.

Forced swim test, Elevated plus maze model, Head dip Test and Immobility test were used to evaluate Psychoharmacological activity of chloroform and methanol extracts of both plants.

Antidepressant activity was evaluated by Forced Swim Test in which immobility time was noted. Anxiolytic activity was performed using elevated plus maze model. This model itself induces anxiety. The % open arm entries and % time spent in open arm was noted. The exploratory behavior was performed using hole board test apparatus and no. of head dipping was noted. The

CNS inhibitory activity was done by using Actophotometer in which spontaneous motor activity count was noted.

The extracts significantly decreased locomotor activity and increased immobility time suggesting depression and sedating potentials. Sedation may be due to interaction with benzodiazepines-like compounds.

The chloroform extract of both the plants show better activity in all above mentioned model. The activity may be due to presence of terpenes, saponins, flavanoids and phenolics.

The morphological, microscopical and physico-chemical parameters of *Oldenlandia corymbosa* and *Grangea maderaspatana* can possibly help to differentiate the drug from its other species. The pharmacognostic profile of both the plants presented here may be useful to supplement information with regard to its identification and will be helpful in establishing standardization criteria.

The extracts of *Oldenlandia corymbosa* and *Grangea maderaspatana* possess significant psychopharmacological activity and hence may prove to be beneficial and an alternative in the treatment of anxiety like disorders. The outcomes are encouraging to pursue further studies to suggest the underlying pharmacological mechanism and also to isolate and characterize probable bioactive molecule responsible.

भारत सरकार / Government of India

पर्यावरण एवं वन मंत्रालय / Ministry of Environment & Forest
भारतीय वनस्पति सर्वेक्षण / Botanical Survey of India
शुष्क अंचल क्षेत्रीय केंद्र / Arid Zone Regional Centre
खेमे का कुआँ के पास, पाल–बासनी केनाल लिंक रोड / Near Kheme Ka Kuan, Pal-Basni Canal Link Road
सुभाष नगर–II, पी.ओ.–नंदनवन / Subhash Nagar-II, P.O.-Nandan Van
जोधपुर–342008 (राजस्थान) / Jodhpur-342008 (Raj.)

No.: BSI / AZRC / I. 1202/Tech./2012-13 (Pl. Id.) 721 Date: 23/ 01/ 2013

CERTIFICATE

This is to certify that the plant specimen sent to this Regional Centre by **Ms. Tanvi D. Patel** is identified as follows:

Hedyotis corymbosa (L.) Lam. [Family- Rubiaceae]
(= *Oldenlandia corymbosa* L.)

(Vinod Maina)
Scientist 'C' & HOO

Tel. / दूरभाष: 0291-2740415, 2747163, Fax No. : 91-291-2741736 E-mail ID: bsiazc@yahoo.com

भारत सरकार/Government of India
पर्यावरण एवं वन मंत्रालय/Ministry of Environment & Forest
भारतीय वनस्पति सर्वेक्षण/Botanical Survey of India
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जोधपुर-342008(राजस्थान) /JODHPUR-342008 (Rajasthan)

No.: BSI/AZRC/ I.12012 /Tech./2015-16/ (Pl. Id.)/ 419 Date: 15.09.2015

CERTIFICATE

This is to certify that plant specimen brought to this regional centre by Ms. **Tanvi Dodiya**, Assistant Professor, Babaria Institute of Pharmacy, Vadodara (Gujarat) identified as ***Grangea maderaspatana*** (L.) Poir. (belonging to family – Asteraceae (Compositae).

(Vinod Maina)
Scientist-D & Head of Office

BITS edu campus

B A B A R I A
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BABARIA INSTITUTE OF PHARMACY

CERTIFICATE

This is to certify that the experimental protocol titled **"PHARMACOGNOSTIC, PHYTOCHEMICAL & PSYCHOPHARMACOLOGICAL EVALUATION OF OLDENLANDIA CORYMBOSA & GRANGEA MADERASPATANA"** and bearing the proposal number **BIP/IAEC/2015/04** has been approved by the IAEC vide its meeting held on **3rd July 2015.**

Name of Chairman, IAEC:
Dr. Vandana B. Patel

03/7/2015
Signature with date

Name of CPCSEA Nominee, IAEC:
Dr. Ramtej Jayram Verma

3/7/2015
Signature with date

List of Publications

❖ **Poster Presentation:**

1. "Pharmacognostic standardization of *Grangea maderaspatana*". Dodiya TD, Jain VC at 4th International congress of the Society for Ethnopharmacology at UkaTarsadia University, Bardoli, Surat, Gujarat on 23rd – 25th February 2017.

2. "Screening of Pharmacological activity of *Grangea maderaspatana* extracts in experimental animals" Dodiya TD, Jain VC at 22nd National Convention of Society of Pharmacognosy and International Conference on "Integrating Ayurveda and Herbal Drugs for Next Generation Therapeutics and Supplements: Opportunities and Challenges" at Ganpat University, Mehsana, Gujarat on 20th & 21st January 2018. (Abstract accepted)

❖ **Publications:**

Review Article:

Patel TD, Jain VC, Dodia RA, 2014, *Oldenlandia corymbosa*: A Phytopharmacological review, International Journal of Phytopharmacy, 4, 79-82.

Research Article:

1. Dodiya TD, Jain VC, 2017, Evaluation of Anti-anxiety Activity of *Grangea maderaspatana* Extracts in Experimental Animals, Int J Ayu Pharm Chem, 6, 53-60.

2. Dodiya TD, Jain VC, 2017, Pharmacognostic Standardization of *Oldenlandia corymbosa*, International journal of Pharmaceutical Research, 9, 22-25.

3. Dodiya TD, Jain VC, 2017, Pharmacognostic, Physicochemical and Phytochemical Investigation of *Grangea maderaspatana*, The journal of Phytopharmacology. (Article accepted)

4. Dodiya TD, Jain VC, 2017, Screening of Behavioural and Antidepressant Activity of *Oldenlandia corymbosa*, International journal of Research in Ayurveda and Pharmacy. (Article accepted).