Pharmacognostic, Phytochemical & Psychopharmacological Evaluation of *Oldenlandia corymbosa L. & Grangea maderaspatana L.*

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Content

Abstract

Oldenlandia corymbosa (L.) (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* (L.) Poir.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.

Brief description on the state of the art of the research topic

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health *viz*., Ayurveda, Unani, Siddha and Homoeopathy. These systems have rightfully existed side - by- side with Allopathy and are not 'in the domain of obscurity'[1]. Millions of Indians use herbal drugs regularly, as spices, home- remedies, health foods as well as over -the-counter (OTC) as self -medication or also as drugs prescribed in the non- allopathic systems[2].

The advancement 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[3]. A large number of plants used in the traditional practice have now become a part of the modern health care system either as a whole or product obtained from the plant resources[4].

There are two distinct phases in research on medicinal plants. In the first phase, the selection of plant is mainly based on their actual use and reputation in Indian traditional system of medicine, where as in second phase, more broad base, in which screening of large number of natural products for biological activity is undertaken, irrespective of the fact whether these plant are being used by the traditional system of medicine or not[5].

Oldenlandia corymbosa (L.) syn. *Hedyotis corymbosa* (L.) (Rubiaceae) is a weedy annual herb, found especially during rainy season in fields throughout India, Sri Lanka, tropical East Asia to Java and the Phillipines[6]. "Parppatakapullu" is its common name in traditional medicine of Kerala. The plant is known to clear heat and toxins, activate blood circulation, promote diuresis and relieve stranguria. It is also known to act against tumors of the digestive tract lymphosarcoma and carcinoma of the liver and larynx. It is also effective in appendicitis, hepatitis, pneumonia, cholecystesis, urinary infection, cellulites and snake bite. Chinese folk medicine describes the plant to treat skin lesions, ulcers, sore throat, bronchitis, gynecologic infections and pelvic inflammatory diseases[7,8,9,10]. It is given in jaundice, and other diseases of

the liver, heat eruptions, vitiated conditions of pitta, hyperdypsia, giddiness, dyspepsia, flatulence, colic, constipation, helminthiasis, leprosy, skin diseases, cough, bronchitis, necrosis and nervous depression. The important preparations of the drug are Amritarishtam, Candanasavam, Mahatiktaghrtam, Jatyaditailam, Aranyatulasyadi coconut oil etc[11].

Grangea maderaspatana (L.) Poir.is a weed commonly known as Madras carpet usually growing in sandy lands and waste places. It is reported to contain flavonoids, diterepenes, sesquiterpenoids, steroid, and essential oil. It is a medicinal plant widely used in Indian traditional system of medicine for curing various ailments. The herb is good for pain in the eyes and ears. The root is an appetizer; astringent to the bowels, diuretic, anthelmintic, emmenogogue, galactogogue, stimulant; 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[12].

Psychopharmacology is the scientific study of the effects drugs have on mood, sensation, thinking and behavior. It is distinguished from neuropsychopharmacology, which emphasizes drug-induced changes in the functioning of cells in the nervous system[13]. Psychiatry refers to a field of medicine focused specifically on the mind, aiming to study, prevent, and treat mental disorders in humans[14].

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[15].Two-thirds of the anxious, depressed or psychotic patients respond to the currently available treatments; but their clinical uses are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability. In the search for new therapeutics for the treatment of neurological disorders-medicinal plant research has also contributed by demonstrating pharmacological effectiveness of different herbs in various animal models[16,17].

Herbal medicines are gaining growing interest because of their cost- effective, eco- friendly attributes and true relief from disease condition. Since ancient time the herbal medicines are effective in the treatment of various ailments. Many plants have folklore claim in the treatment of several dreadful diseases but they are not scientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern medicine[18].

Definition of the Problem

As per the traditional claims the plant has been used in the treatment of depression. Literature review reveals that the Psychopharmacological activity is due to various chemical constituents like terpenoids, saponins, flavanoids, alkaloids etc. These constituents are already reported in *Oldenlandia corymbosa* & *Grangea maderaspatana*. *So,* Psychopharmacological activity of these plants can be explored by further research.

Objective

To explore the plant extract having less/no side effect for the treatment of psychopharmacological disorders, as the synthetic drugs used to treat these disorders have serious side effects.

Plan of work

- Procurement and Authentication of plants.
- Morphological, Microscopical and powder studies of plants parts.
- Standardization of plant material as per WHO guidelines
- Successive solvent extraction
- Phytochemical screening of extracts.
- Chromatographic analysis of phytoconstituents.
- Screening of Psychopharmacological activity of various extracts of plants in different animal models.

Original contribution by the thesis.

The pharmacognostic and phytochemical parameters of *Oldenlandia corymbosa* and *Grangea maderaspatana* were studied and they may be proposed as parameters to establish the authenticity of both the plants and also will assist in standardization viz., quality, purity and sample identification which can possibly help to differentiate the drug from its other species. Phytomarkers like Oleanolic acid and Ursolic acid were identified by HPTLC fingerprinting. Quantification of Gallic acid content in methanol extracts of both the plants was done by HPLC method. Psychopharmacological activity of both the plants was assessed in different animal models. The results were encouraging to pursue further studies to propose the underlying pharmacological mechanism and also to isolate and characterize probable bioactive molecule responsible.

Methodology of Research and Results

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.

The plant of *Grangea maderaspatana* was collected in the month of December from Saputara, Gujarat, India. The plant was identified and authenticated by Botanical Survey of India, Jodhpur and a voucher specimen was deposited at Botanical Survey of India (voucher specimen sample No. BSI/AZRC/I.12012/Tech./2015-16/419).

Macroscopic and microscopic investigation

The macroscopic features of *Oldenlandia corymbosa* and *Grangea maderaspatana* leaf, stem and root was determined using prescribed method[7]. For microscopical examinations, free hand sections of the fresh leaf, stem and root 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.

Leaf constants and physicochemical analysis were performed according to the official methods[19, 20,21,22].

Preparation of extracts and their phytochemical screening

The coarsely powdered material of the plant of *O. corymbosa* and *G. maderaspatana* were subjected to successive solvent extraction in a Soxhlet apparatus using various solvents in their increasing order of polarity. Starting from petroleum ether, chloroform, ethyl acetate and methanol. Water extract was prepared by maceration. After completion of extraction, the solvent was distilled off and the residue was concentrated and finally dried. The marc left after extraction with each solvent was dried completely in air before subjecting to next solvent. The vacuum dried extracts were subjected to chemical test to detect the presence of various phytoconstituents using standard procedures[23,24]. It was found that both the plants contain terpenes, saponins, steroids, carohydrates, phenolics and tannins.

HPTLC Fingerprinting:

HPTLC fingerprinting of chloroform and methanol extracts of *O. corymbosa* and *G. maderaspatana* was performed for oleanolic acid and ursolic acid[25].

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

Quantification of Gallic acid by HPLC

Estimation of Gallic acid in methanol extracts of *O. corymbosa* and *G. maderaspatana* was performed by HPLC[26].

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
Injection Volume	20 µL
Temperature	Ambient
Mode of Operation	Isocratic elution

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.

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.

Acute toxicity study:

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).

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 upto 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.

Treatment:

Animals were divided into six (I-X) groups. 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 extractof *O. corymosa* at doses of 200 and 400 mg/kg, p.o respectively. Groups VII to VIII received chloroform extract of *G. maderasatana* at doses of 200 and 400 mg/kg p.o respectively; Group IX to X received methanolic extract of *G. maderasatana* at doses of 200 and 400 mg/kg, p.o respectively.

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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[27,28].

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[29,30]. 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[31,32].

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[33].

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[33].

Achievements with respect to objectives

The pants of *Oldenlandia corymbosa* and *Grangea maderaspatana* were procured and authenticated. The morphological, microscopic and physicochemical study was done. Phytochemical screening of extracts of both the plant was done. HPTLC fingerprinting of chloroform and methanol extract of *O. corymbosa* and *G. maderaspatana* was done for the presence of oleanolic acid and ursolic acid. Psychopharmacological activity of chloroform and methanol extract of both the plant was performed using different animal models.

Conclusion

The plants of *Oldenlandia corymbosa* and *Grangea maderaspatana* 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 and 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, phloem, 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. 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 2000mg/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 show better activity which may be due to presence of saponins, terpenes, flavanoids and phenolics.

List of Publications

- A paper title "*Oldenlandia corymbosa*: A Phytopharmacological review" published in International journal of Phytopharmacy. 2014: 4(3), 7-82.

- A paper title "Evaluation of anti-anxiety activity of *Grangeamaderaspatana*L. Poir. Extracts in experimental animals." published in International journal of Ayurveda and Pharmaceutical chemistry. 2017: 6(1), 53-60.

- A paper title "Pharmacognostic standardization of *Oldenlandia corymbosa*" has been accepted in International journal of Pharmaceutical Research.

- An abstract title "Pharmacognostic standardization of *Grangea maderaspatana*" has been accepted for the poster presentation at 4^{th} International congress of the Society for Ethnopharmacology at UkaTarsadia University, Bardoli, Surat, Gujarat.

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