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Phytochemical Analysis of Methanolic Extracts of Leaves of Some Selected Medicinal Plants of Jorhat District, Assam, India

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Abstract: ***Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i. e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti - inflammation activities. Phytochemical are the dependable sources for the treatment of different health problem. The present investigation deals with the phytochemical studies of leaves of diverse medicinal plants such as Oroxylum indicum, Litsea citrata, Carissa carandens, and Garcinia morella. Methanolic (90%) extracts of leaf powders have been screened for quantitative determination of different secondary metabolites like flavonoids, phenols and total flavonols etc. The highest amount of phenol was found in L. citrata (8.28± 0.15 mg of GAE/gm) while O. indicum exhibited the lowest amt of phenol content (2.05 ± 0.15 mg of GAE/gm). The flavonoids and total flavonol content also varied among the four plant species. Altogether the results reveal the presence of medicinally active constituents in the four plants studied. The phytochemical compounds identified in this study have earlier been proved to be bioactive. The presence of some of these compounds have been confirmed by previous workers to have medicinal as well as physiological activity and therefore could be said to be responsible for the efficacy of the leaves of the plants studied in treatment of different ailments. The plant extracts could therefore be seen as a potential source for useful drug. The continued traditional medicinal use of these plants is therefore encouraged while it is suggested that further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of these plants.***

Keywords: Medicinal plants, Methanolic extracts, Phytochemical study, Secondary metabolites

1. Introduction

Throughout history, plants have been a significant source of ethnomedicine; between 14% and 18% of higher plants have been shown to have therapeutic properties. It's interesting to note that new details regarding the ethnomedical applications of 74% of pharmacologically active plants have been uncovered by research into these usage. In plants, primary and secondary metabolites are distinct from one another. Vital compounds such polysaccharides, proteins, carbohydrates, amino acids, and nucleic acids are examples of primary metabolites. However, a variety of chemical compounds referred to as secondary plant metabolites are generated by distinct metabolic pathways that deviate from primary ones. These secondary metabolites encompass a broad range of chemical compounds, including glycosides, flavonoids, phenolics, alkaloids, saponins, terpenes, lipids, and carbohydrates. These substances have antimicrobial, antifungal, and antiviral qualities that help shield plants against infections. For example, among the subset of secondary metabolites, phenolic compounds exhibit a wide variety of bioactivities (Wawrosch, 2021; Sunil et al., 2023). Phytochemicals are chemical compounds that occur naturally in plants. Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. This term is generally used to refer to those chemicals that may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients" (G. T. Opande, 2022).
The most commonly known phytochemical content is divided into several compounds such as alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, and phenols. Alkaloids are derived from amino acids and can be synthesized as secondary metabolites by plants and some animals. This compound is considered to play an important role in living organisms including humans for centuries. Apart from being a secondary metabolite, this compound also has biological effects on animals and humans in small doses (Kurek, 2019). Alkaloids are a class of nitrogen - containing organic compounds found in the plant kingdom. Many alkaloids are used as valuable medicinal agents which can be utilized to treat various diseases such as malaria, diabetes, cancer, cardiac dysfunction etc (Ain et al., 2016).

Compounds with one or more aromatic rings and one or more hydroxyl groups are known as phenolics. With over 8, 000 known phenolic structures, it is the most prevalent secondary metabolite found in plants and is found in a wide variety of plants. Plant phenolics play a role in plant colors and are mostly involved in defense against UV radiation and aggression from diseases, parasites, and predators. All plant organs contain phenol, which is also a common ingredient in human diets made from plants.

A technique for examining the elements of active substances present in materials, including their chemical structure, biosynthesis, natural distribution, biological activity, and isolation, is the phytochemical test. The chemical composition of different plant species is also compared through phytochemical testing. Plant samples in the form of leaves, stems, fruits, flowers, tubers, and roots may be utilized in the phytochemical test. The purpose of this study is to determine the phytochemical substances found in various kinds of therapeutic plants used in local medicines such as *Oroxylum indicum, Carissa carandas, Garcinia morella, Litsea citrata*.

Oroxylum indicum L. (Kurz.) belongs to the family Bignoniaceae (commonly known as bhatghila, trumpet flower, broken bone plant, bhut - vriksha etc.) is a small tree with purple to violet flowers as well as enormous seed pods that hang from the branches (Plate 1D). The plant is recognized for various medicinal properties such as anti - inflammatory, anti - microbial, anti - oxidant, anti - arthritic, anti - diabetic etc. (Deka et al., 2013). A flavonoid, baicalein, extracted from the methanolic extract of *Oroxylum indicum* have been recorded for its efficacy to inhibit proliferation of cancer cell line in vitro (Roy et al., 2007). Bark juice of the plant is also effective drug in the treatment of jaundice (Sarma, 2012). Ayurvedic preparations such as Dasamula, Amartarista, Dantyadyarista, Brahma rasayana etc. also requires *Oroxylum indicum* as one of the active ingredients (Preety and Sharma, 2016, Nath et al., 2016).

Carissa carandas is a species of flowering plant in the dogbane family, Apocynaceae. It harvests berry - sized fruits that are commonly used as a condiment or preservative to Indian pickles and spices (Plate 1B). The shrub Commonly name karonda (Devanagari) karamardaka (Sanskrit), Koromcha (Bengali), Christ's thorn (South India), vakkay (Telugu), kilaakkaai (Tamil) and Karja tenga (Assam). Its botanical name was in recent years altered to *Carissa congesta* Wight (syn. *C. carandas* Auct formerly widely shown as *Carissa carandas*. The famous biological activities reported are analgesic, anti - inflammatory, anti - pyretic, Cardiotonic, and histamine - releasing. The plant is also an alternative source of oil, hydrocarbon, and phytochemicals. *Carissa carandas* Linn. Is a dichotomously pronged evergreen shrub with a short branch and strong thorns in pairs, *Carissa carandas* Linn is an evergreen diffuse and spiny shrub occurring through the country. The plant is very valued for the Indian System of medicine mainly Ayurveda. It is used for alleviating Vata and pitta disorders. Species is a rank - growing, usually growing to 10 or 15 ft (3 - 5 m) high, sometimes ascending to the tops of tall trees; and rich in white gummy latex. The branches, many and dispersal, forming dense masses, are set with sharp thorns, simple or forked, up to 2 in (5 cm) long, in pairs in the axils of the leaves. The leaves are evergreen, opposite, oval or egg shaped, 1 to 3 in (2.5 - 7.5 cm) long; dark - green, leathery, glossy on the upper surface, lighter green, and dull on the underside. The fragrant flowers are cylindrical with 5 hairy lobes that are twisted to the left in the bud instead of to the right as in other species. They are white, regularly tinged with pink, and borne in terminal clusters of 2 to 12. The fruit, in clusters of 3 to 10, is oblong, broad - ovoid or round, 1/2 to 1 in (1.25 - 2.5 cm) long; has fairly thin but tough, purplishred skin turning dark - purple or closely black when ripe; smooth, glossy; enclosing very acid to equally sweet, often bitter, juicy, red or pink, juicy pulp, radiating flecks of latex. There may be 2 to 8 small brown seeds. The *Karanda* is common throughout much of India, Burma, and Malacca and dry areas of Ceylon; is rather commonly cultivated in these areas as a hedge and for its fruit, and the fruit is marketed in villages. Fruits are rich source of iron and vitamin C, therefore, ethnomedically the fruits are used for curing anemia, as an astringent, antiscorbutic and as a remedy for biliousness. Its leaf decoction is used in contradiction of fever, diarrhea, and earache, whereas roots help as a stomachic, vermifuge, remedy for itches, and insect repellent (Bhosale et al., 2020)

Garcinia morella (Gaertn.) Desr., known as Indian gamboge, is a fruit - yielding tree belonging to the family Clusiaceae and is a close relative of mangosteen (*G. mangostana*). It is an evergreen tropical tree naturally distributed across the Indian subcontinent to Indochina and Sri Lanka. In India, it is commonly distributed in the Western Ghats and northeastern regions. The tree grows up to 12 m tall (Plate 1A); leaves are simple, opposite, and decussate; and bark is smooth and dark brown with white blaze, which oozes out a gum/resin that is bright yellow in color. Fruits are berries with a diameter of 3 cm that contain four seeds. Fruits are esteemed as a dessert fruit and are preserved by slicing and sun - drying. The yellow fat obtained from the seed is used in cooking and confectionery. It is also used as a substitute for ghee. Gamboge, the gum/resin obtained from the plant, is used as a yellow dye, as an illuminant, and in varnishes and watercolors. It is traditionally collected by cutting a thin slice off the bark of the tree about the size of the palm of the hand; the resin collects there and is scraped off when sufficiently dried. The plant is sometimes used as a root stock for mangosteen (*G. mangostana*) (Murthy et al., 2020).

Litsea cubeba Pers., Lauraceae, consists of more than 400 species and is predominant in tropical and subtropical regions of India, Southeast Asia, southern China, Taiwan, and Japan (Plate 1 (C). *Litsea* is evergreen, fast growing, and a rare deciduous tree or shrub that attains a height of about 8 m, growing spontaneously in the eastern Himalayas, Assam, Manipur, and Arunachal Pradesh up to an altitude of 2700 m from sea level. In the Assam state of India, the tree is known as "mejankari", while it is commonly called "May Chang" or "Chinese pepper" in China. *Litsea* plants are the primary source for traditional medicines but they also serve as a secondary source of food for muga silk worms (*Antheraea assama*). The muga silk ("mejankari pat") produced from the *Litsea* plant is very attractive and more expensive than the silk produced from other plants. The silk cocoons fed with *Litsea* produce high value silk, which is creamy, glossy, and five times more expensive than silk produced from a primary source of food plant, i. e., *Machilus bombycina* King.

Litsea cubeba is a pioneer herb traditionally utilized in medicine. Different extracts from its plant parts, such as bark, leaf, root, and fruits, have been utilized in traditional Chinese medicines for curing various diseases. The fresh green fruit is used for culinary purposes like salad preparation, chutneys, pickles, etc. . The *L. cubeba* essential oil (LEO) extracted from fresh fruits contains about 60–90% citral content, and is essential oil with volatile compounds having an intense lemon - like, fresh, sweet aroma, and insoluble in water. It was found effective against *Vicia faba*, and weevil (*Bruchus rufimanus*). China is the one of the largest producers and exporter of *L. cubeba* oil in the world. More than 4.4 million lb of LEO has been produced per year, and three quarters of that production is exported to England, United States, France, Germany, Holland, and other countries. LEO is highly aromatic in nature and extracted from the fresh fruits to exploit as an enhancer of

aroma in cosmetic products besides in foods. This is employed as raw material in the production of citral, vitamin A, E, and K, iodine and methylionine, and perfumes, and also to impart antimicrobial and insecticidal properties. Additionally, LEO is also used as an antifungal agent and bio - insecticides in the storage of grains, foods, archival documents, and/or clothing. The dried fruits are used for several medicinal purposes such as carminative (relieves flatulence), diuretic (aids urine passage), expectorant (aids secretion of sputum), stimulant, stomach ache, antiasthmatic, sedative, antidysentric, and antiseptic. Some recent studies described the functional properties of *L. cubeba*, such as its therapeutic, antimicrobial, antioxidant, anti - cancerous, anti - inflammatory, anti - diabetic, and anti - insecticidal activities (Kamle et al., 2019)

2. Materials and Methods

Collection of plant materials

Fresh leaves were collected from the Jorhat district of Assam, India. The plant material was authenticated and identified in the Department of Botany, J. B. University, Jorhat, Assam (Fig 1).

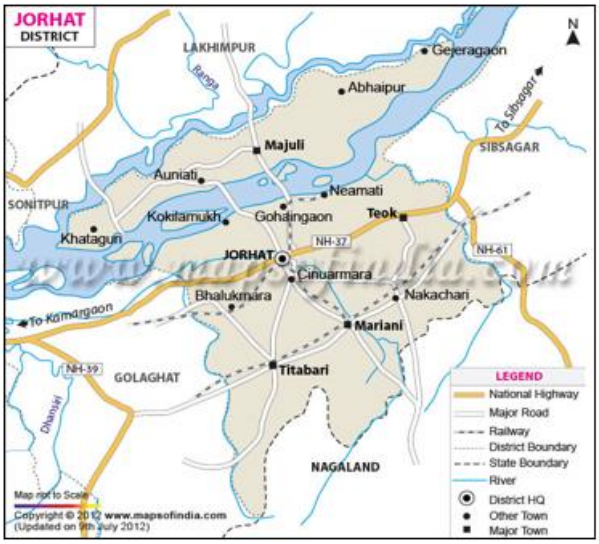

Figure 1: Map of Jorhat from where the plant parts were collected

Phytochemical analysis:

Preparation of Plant extract:

0.5mg of the plant leaves was measured using a weighing balance.50mL of 90% methanol was measured in a beaker. The leaves were macerated along with the methanol in a pestle mortar. The macerated leaves are then kept in a hot air oven for a period of 15 - 20 minutes to evaporate the solution. The solution was then filtered and the extract was obtained.

Test for phenol

The total soluble phenols were determined by the Singleton and Rossi (1965) method with slight modification. The methanolic extract of the plant in desired concentration (0.5mL) was mixed with 0.5mL of Folin Ciocalteu reagent (previously diluted 1: 1 with distilled water). The mixture was then incubated for 5 minutes at room temperature.1mL of 2% Na_2CO_3 solution was added to it. After incubation at room temperature for 10 minutes, the absorbance was measured at 730nm with a UV - vis - spectrophotometer.

Test for flavonoid

The flavonoid content was determined according to Zhishen et al (1999) with minor modifications (Goyal et al 2010) using quercitin as a standard. To 0.25mL of the methanolic extract of the plant was added to 1.25mL of double distilled water followed by 75µL of 5% $NaNO_2$. After 5 minutes of incubation at room temperature, 0.15mL of 10% $AlCl_3$ was added. After a further incubation of 6 minutes, the reaction mixture was treated with 0.5mL of 1mM NaOH solution. Finally, the reaction mixture was diluted with 275 µL of double distilled water. The absorbance was measured at 510nm after incubating the mixture for 20 minutes at room temperature.

Test for Total Flavonols

The method developed by Kumaran and Karunakaran (2006) was used to estimate the total flavonols using quercitin as a standard., 2mL of 2% $AlCl_3$ was added to 2mL of the methanolic plant extract.1mL of 1% CH_3COONa was added to it and then incubated for 2.5 hours at room temperature. Optical density was taken at 440nm.

3. Result

Phytochemical investigation of the methanolic extracts of leaves of the medicinal plants shows the presence of secondary metabolites – phenol, flavonoid and flavonol. The maximum phenol content was found in *L. cubeba* and minimum phenol content was observed in *O. indicum*. The flavonoids and total flavonol content also varied among the four plant species. Its maximum content is found in *G. morella* whereas *C. carandas* has the lowest flavonoid and total flavonol content. Altogether the results revealed the presence of medicinally active constituents in the four plants studied. The phytochemical compounds identified in this study have earlier been proved to be bioactive. The presence of some of these compounds have been confirmed by previous workers to have medicinal as well as physiological activity and therefore could be said to be responsible for the efficacy of the leaves of the plants studied in treatment of different ailments.

Our study revealed that the studied specimen medicinal plants is an amalgam of variety of important phytochemicals which contributes towards its multirole pharmacological properties and justifies its wide ethnomedicinal usages. The plant extracts could therefore be seen as a potential source for useful drug. The continued traditional medicinal use of these plants is therefore encouraged while it is suggested that further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of these plants.

In 2008, Muhammad *et al*. had worked on *Litsea sp.* and they found four different phenolic compounds from the methanolic extract. In several studies it was recommended that plant flavonoids, which showed antioxidant activity *in vitro*, also function as anti oxidants *in vivo.* Naturally occurring polyphenols and flavonoids can prevent lipid

peroxidation, low density lipoprotein oxidation, and the development of atherosclerosis and heart disease. In an earlier study, many medicinal plants contained high amounts of phenolic compounds and there was a positive linear correlation between the total phenolic content and antioxidant activity of the plants. This suggests that the genus *Litsea*, which contained higher levels of polyphenols might have high antioxidant properties.

Opande et al., (2022) did the phytochemical screening of *Carissa edulis* which showed the presence of some active phytochemicals which previous research has reported to produce a definite physiological action on the human body. As can be seen from the obtained results, the roots, barks and leaves contained Tannins and cardiac glycosides. The roots and barks tested positive for saponins, steroids and terpenoids. The leaves and barks tested positive for flavonoids. The leaves also tested positive for terpenoid and sterols.

Preliminary phytochemical screening and gas chromatography and mass spectrometry analysis of chloroform extract of *Garcinia cambogia* fruits revealed the presence of xanthones, flavonoids, phlobatannins, terpenoids steroids, phenolic compounds saponins, and anthraquinones. The GC - MS analysis provided peaks of 13 different bioactive compounds, namely 3, 7, 11, 15 - tetramethyl - 2 - hexadecenol (37.1%), pentadecanoic acid 14 - methyl - , methylester (100%), hexadecanoic acid 15 methyl - , methyl ester (100%), 9, 12 - octadecadienoic acid methyl ester (42.6%), docosanoic acid (100%), docosanoic acid methyl ester (37.3%), phenol, 2, 4, bis1, 1 - dimethylethyl) (100%), 9, hexadecanoic acid methyl ester (99.2%), 10 - octadecenoic acid methylester (100%), 11 eicosenoic acid methyl ester (53.1%), heptadecanoic acid 9 methyl - , methyl ester (100%), eicosenoic acid methyl ester (56.9%), and 5, 12d ethanofuro oxepino [2, 3, 4 - mn] (2, 3, 4 - ed) anthrace2 - one 9, 12 - dilol 6 methyl, 2a 3, 4, 4a, 5, 6, 7, 8a - octahydro (19%) were reported (**Priyadarshini J., 2019**).

Chaudhary et al., (2020) did the preliminary phytochemical screening of the extract of *Oroxylum indicum* which showed the presence of alkaloids, flavonoids, phenols, tannins, resins, glycosides, steroids, fixed oils respectively. Result showed that *O. indicum* root was found complying LOD (126 mg/gm), total ash (87.5 mg/gm), acid insoluble ash (37.5mg/gm), water soluble ash (15 mg/gm), swelling index (1.2), phytochemical screening showed alkaloids, flavonoids etc was present. ethanolic extractive value 6.6% was found. In this study evaluate the standards for commonly used herbal formulation, which can be utilized in quality control of the formulation.

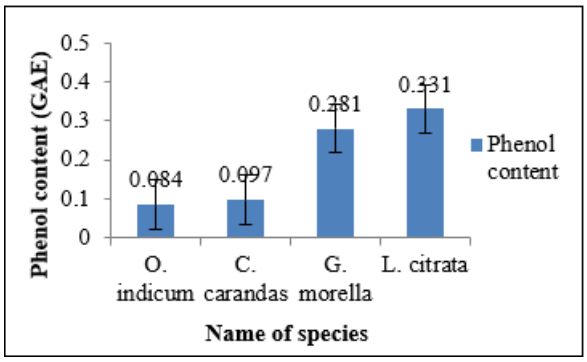

Figure 2: Phenol content in the four species

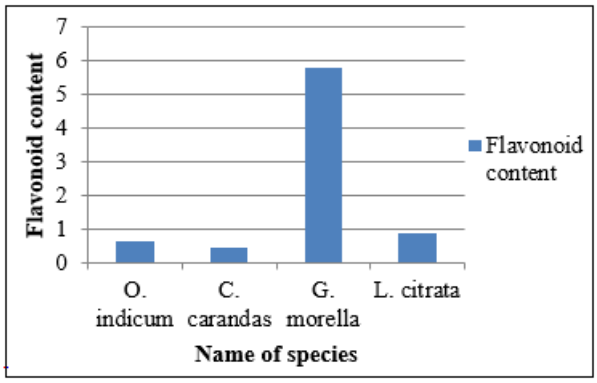

Figure 3: Flavonoid content in the four species

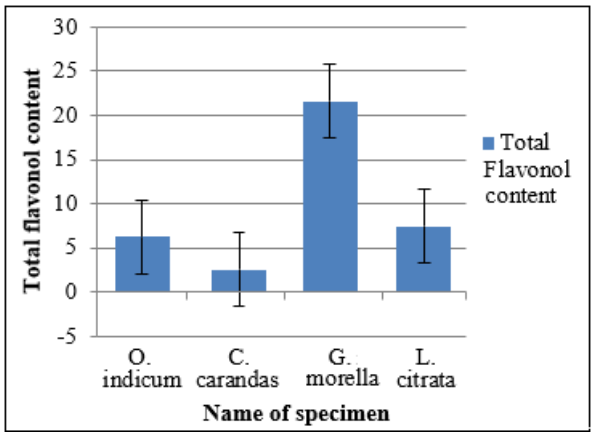

Figure 4: Total flavonol content in the four species

4. Conclusion

Herbal medicine is still the mainstay of maximum world population; mainly in the developing countries for primary healthcare not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. The identification of active principles and their molecular targets from traditional medicine provides an enormous opportunity for drug development. The current study takes us a step ahead in the process of drug development as well as new validated treatment of a traditionally used medicinal plant. Thus from the phytochemical analysis performed on the studied specimen it is found that they contain a high amount of phytochemicals. Thus the studied plants have a high prospect in the field of pharmacognosy which can be used for human welfare.

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Author Profile

Dr. Liza Handique has more than 10 years of teaching experience as a teacher in Jagannath Barooah University, Jorhat, Assam. She is an author of a book named "*Elaeagnus latifolia* L. conservation: Perspectives in land reclamation. " Which have been translated to 5 different languages viz. French, Italian, Portuguese, Russian and German. She had done her masters from Delhi University, Delhi, India in 2012 and Ph. D from Forest Research Institute (Deemed) University in Forest Botany in 2016. She has achieved the Best Graduate in Science from Dibrugarh University in 2010, followed by Manik Lal Phookan award in 2010 from J. B. College, Jorhat, She had also received InSc, Institute of Scholars" **Research Excellence Award** in Nov, 2020 **and** SADHNA "**Young achiever award**", 2020. She is also a permanent member of Society for advancement of Human and Nature since 2020 onwards and Institute of Scholars since 2020 onwards. She had published more than 10 research papers in Journals, 4 book chapters and a book.

Plate 1: A. *Garcinia morella*, B. *Carissa carandans*, C. *Litsea cubeba*, D. *Oroxylum indicum*.